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Prospective Study of Coronary Heart Disease Incidence in Relation to Fasting Total Homocysteine, Related Genetic Polymorphisms, and B Vitamins

The Atherosclerosis Risk in Communities (ARIC) Study

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Background—Elevated plasma total homocysteine (tHcy), low B-vitamin intake, and genetic polymorphisms related to tHcy metabolism may play roles in coronary heart disease (CHD). More prospective studies are needed.

Methods and Results—We used a prospective case-cohort design to determine whether tHcy-related factors are associated with incidence of CHD over an average of 3.3 years of follow-up in a biracial sample of middle-aged men and women. Age-, race-, and field center-adjusted CHD incidence was associated positively ($P<0.05$) with tHcy in women but not men, and CHD was associated negatively ($P<0.05$) with plasma folate (women only), plasma pyridoxal 5'-phosphate (both sexes), and vitamin supplementation (women only). However, after accounting for other risk factors, only plasma pyridoxal 5'-phosphate was associated with CHD incidence; the relative risk for the highest versus lowest quintile of pyridoxal 5'-phosphate was 0.28 (95% CI=0.1 to 0.7). There was no association of CHD with the C₆₇₇T mutation of the methylenetetrahydrofolate reductase gene or with 3 mutations of the cystathionine β -synthase gene.

Conclusions—Our prospective findings add uncertainty to conclusions derived mostly from cross-sectional studies that tHcy is a major, independent, causative risk factor for CHD. Our findings point more strongly to the possibility that vitamin B₆ offers independent protection. Randomized trials, some of which are under way, are needed to better clarify the interrelationships of tHcy, B vitamins, and cardiovascular disease. (*Circulation*. 1998;98:204-210.)

Key Words: coronary disease ■ vitamins ■ homocysteine ■ polymorphism (genetics)

Homocystinuria is a rare autosomal recessive deficiency of CBS associated with an elevated blood tHcy concentration and a very high incidence of premature vascular disease. Moderately elevated tHcy also is believed to be a risk factor for CHD, atherothrombotic stroke, and peripheral vascular disease. A recent meta-analysis¹ estimated that each 5- μ mol/L tHcy increase is associated with a 60% (men) to 80% (women) greater risk of CHD. Although this evidence is compelling, only two^{2,3} of five²⁻⁶ published prospective studies have found tHcy concentration to be a CHD risk factor. One of these studies,² when extended by 2.5 years, no longer showed a statistically significant association between tHcy and CHD incidence,⁷ nor did it subsequently find a relation between tHcy and risk of angina pectoris.⁸

concentrations.¹ Prospective evidence relating these factors to incident CHD is limited.^{7,9-11} Two prospective epidemiological studies reported low dietary folate and B₆¹¹ or low serum folate⁹ to be associated significantly with increased incidence of CHD. Another study⁷ found low serum folate and vitamin B₆ associated, although not statistically significantly, with increased CHD incidence. This latter study also found the C₆₇₇T mutation of the *MTHFR* gene was not associated with CHD incidence.¹⁰

To provide additional prospective evidence on these topics, we used a nested case-cohort design within the ARIC study to determine the association of fasting serum tHcy with CHD incidence. In addition, we assessed the contributions to CHD of dietary and plasma B₁₂, PLP, and folate, as well as several genetic variants associated with tHcy concentration.

See p 196

Heterozygous *CBS* deficiency, homozygosity for the thermolabile variant of *MTHFR*, and low dietary folate, vitamin B₁₂, or vitamin B₆ (PLP) are among the factors that can elevate tHcy

Methods

Study Population

In 1987 through 1989, the ARIC study¹² recruited a population-based cohort of persons aged 45 to 64 years from 4 US communities. A

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Selected Abbreviations and Acronyms

ARIC	= Atherosclerosis Risk In Communities
CBS	= cystathionine β -synthase
CHD	= coronary heart disease
MI	= myocardial infarction
MTHFR	= methylenetetrahydrofolate reductase
PLP	= pyridoxal 5'-phosphate
tHcy	= total homocysteine

total of 15 792 participants completed a home interview and clinic examination. ARIC reexamined participants in 1990–1992 (93% return rate) and in 1993–1995 (86% return rate).

Baseline Measurements

We defined hypertension as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or current use of antihypertensive medication. We expressed physical activity as a sport index ranging from 0 (low) to 5 (high).¹³ Technicians measured waist (umbilical level) and hips (maximum) to compute waist/hip ratio. We computed body mass index (kg/m^2). Technicians measured average carotid intima-media thickness using standardized B-mode ultrasonography.^{14,15} We defined prevalent CHD at baseline, for exclusion, as a reported history of a physician-diagnosed heart attack, prior MI by ECG, prior cardiovascular surgery, or prior coronary angioplasty. ARIC also measured exertional angina by questionnaire¹⁶ and prior stroke or transient ischemic attack through a standardized interview.

Trained interviewers collected information on usual alcohol intake and on dietary intake using an adaptation of Willett's food frequency questionnaire.¹⁷ Interviewers asked about use of vitamin supplements in a medication interview. Because we did not obtain dose and frequency of vitamin supplementation, we coded vitamin supplement use as "any" versus "none" for analysis.

ARIC measured fasting plasma total cholesterol,¹⁸ triglycerides,¹⁹ HDL cholesterol,²⁰ and fibrinogen²¹ and calculated LDL cholesterol.²² We defined diabetes as fasting glucose ≥ 140 mg/dL, nonfasting glucose ≥ 200 mg/dL, or a physician diagnosis or pharmacological treatment for diabetes.

Ascertainment and Classification of Incident CHD Cases

ARIC ascertained all CHD events in the cohort.^{12,23} For the present study, we included CHD events occurring between ARIC visit 1 and December 31, 1991. The median follow-up time was 3.3 years (maximum of 5 years). We defined CHD incidence as (1) a definite or probable MI, (2) a silent MI between examinations by ECG, (3) a definite CHD death, or (4) a coronary revascularization.

Cohort Sample

We used a case-cohort design for the present study, in which information on plasma tHcy, B vitamins, and related genotypes was determined only for CHD cases and a stratified random sample of the ARIC cohort. For this reference cohort, we oversampled participants with thin average carotid intima-media thickness measurements at baseline (<30 th percentile) and also stratified the sampling by age and sex.

Processing of Stored Baseline Samples

In 1995, after ARIC had identified the incident cases and cohort sample, technicians pulled these participants' baseline samples, frozen in 1987–1989. The technicians thawed the frozen buffy coat, extracted genomic DNA,²⁴ treated it with a proteinase K (1.9 $\mu\text{g}/50$ to 500 ng DNA), placed the DNA in multiple aliquots, and froze them at -70°C . Approximately 90% of the sera for tHcy had been thawed once or twice previously, and 10% had never thawed. Previous evidence suggests tHcy is not altered with thawing and

refreezing (M.R. Malinow, MD, unpublished data, 1997). The plasma aliquots for vitamin measurements had never been thawed.

Laboratory Measurements

The Oregon Regional Primate Research Center staff measured tHcy, in duplicate, as the sum of free and bound homocysteine, homocysteine, and cysteine-homocysteine mixed disulfide using high-pressure liquid chromatography and electrochemical detection based on the method of Smolin and Schneider,²⁵ as previously described²⁶ with minor modifications.²⁷ The laboratory interspersed 3 quality-control samples with the study samples each day; these standards were 2 large, well-mixed pools obtained from American Red Cross plasma. The laboratory adjusted tHcy levels to the mean of the standard in each daily run to account for day-to-day method variability. The staff of the Oregon Regional Primate Research Center measured plasma folate and vitamin B₁₂ by the Quantiphase II Radioassay method supplied by Bio-Rad Diagnostics Group and PLP using a radioenzymatic assay supplied by Bühlmann Laboratories AG through American Laboratory Products Co. To assess laboratory reliability, ARIC included split-specimen, blinded duplicates prepared at the time of baseline blood drawing. This yielded the following Pearson coefficients: tHcy, $r=0.95$ ($n=77$); folate, $r=0.97$ ($n=29$); PLP, $r=0.90$ ($n=29$); and vitamin B₁₂, $r=0.91$ ($n=29$).

We determined the presence or absence of the T₈₃₃C and G₉₁₉A mutations and the 68-bp insertion of the CBS gene.^{28,29} To determine the C₆₇₇T mutation of the MTHFR gene, we used the method of Rozen and associates.³⁰

Data Analysis

We excluded participants with prevalent CHD, stroke, or transient ischemic attack but not the 5% of participants whose angina status was positive or unknown by the Rose questionnaire, because the questionnaire's validity, especially in women, has been questioned.³¹ We also excluded 6 participants with creatinine >2 mg/dL, 21 participants who had implausible values for energy intake, and 28 participants with missing tHcy values.

To determine the relation of tHcy with other variables, some of which may be confounders in this analysis, we categorized the cohort sample into fifths based on quintile cutpoints and used ANCOVA to compute age-, race-, and sex-adjusted mean levels or percentages of the other variables for each quintile. We also computed weighted correlations among pairs of vitamin variables or tHcy.

To test study hypotheses, we first used ANCOVA to compute age-, race-, and sex-adjusted geometric mean or percentage values of study variables for CHD cases versus the cohort after appropriate weighting for the stratified case-cohort sampling design. We used geometric means because tHcy and vitamin variables were right skewed.

We computed relative risks and 95% CIs of CHD in relation to categories of study variables using a weighted proportional hazards regression, accounting for the stratified random sampling and the case-cohort design by Barlow's method.³² We tested for trend in relative risks across quintiles (coded 1 to 5) using a χ^2 test. We performed supplemental analyses using continuous instead of categorical independent variables. We initially used 2 sex-specific models, 1 of which was adjusted for age, race (black, white), and ARIC field center. In the final multivariate model, we adjusted for sex, age, race, field center, and the other factors related to CHD in this sample: smoking status (never, former, current), total cholesterol, HDL cholesterol, hypertension, and diabetes. In addition, we adjusted dietary vitamin intake for energy intake by including it as a continuous variable in the models.

Results

Sample Characteristics

The sample included 232 incident CHD cases (146 definite or probable MI, 19 silent MI, 30 definite fatal CHD, and 37 revascularization procedure) and a reference cohort sample of

TABLE 1. Age-, Race-, and Sex-Adjusted Mean Levels (or Percentages) of Variables According to Quintiles of tHcy (ARIC, Cohort Sample Only)

Variable	tHcy Quintiles (in $\mu\text{mol/L}$)					P for Trend
	3.46–6.28	6.29–7.83	7.84–9.24	9.25–11.49	11.50–33.51	
Means (arithmetic, unless indicated)						
tHcy, $\mu\text{mol/L}\dagger$	5.3	7.0	8.5	10.0	14.2	...
Plasma folate, $\text{nmol/L}\dagger$	8.5	8.7	5.6	4.9	4.0	<0.01
Plasma PLP, $\text{nmol/L}\dagger$	54.6	37.0	34.5	28.0	14.8	<0.01
Plasma vitamin B ₁₂ , $\text{pmol/L}\dagger$	365	303	282	271	222	<0.01
Dietary folate, $\mu\text{g/d}\ddagger$	210	217	177	206	173	0.01
Dietary vitamin B ₆ , $\text{mg/d}\ddagger$	1.47	1.67	1.41	1.58	1.41	0.18
Dietary vitamin B ₁₂ , $\mu\text{g/d}\ddagger$	6.64	6.92	6.03	6.77	6.20	0.41
Systolic blood pressure, mm Hg	119	118	123	117	118	0.59
Total cholesterol, mg/dL	210	217	214	213	216	0.72
HDL cholesterol, mg/dL	56	56	54	53	52	0.15
Triglycerides, $\text{mg/dL}\dagger$	103	117	100	108	112	0.67
Fibrinogen, mg/dL	303	290	285	309	308	0.28
Body mass index, kg/m^2	25.6	26.5	28.5	26.8	27.3	0.12
Waist/hip ratio	0.89	0.90	0.92	0.92	0.92	0.03
Alcohol, g/wk	27	25	36	42	47	0.05
Sports index (low=1 to high=5)	2.44	2.48	2.59	2.50	2.25	0.30
Percentages						
Blacks	15	20	43	17	24	0.36
Current smoker	29	20	12	19	26	0.89
Hypertensive	11	29	35	29	35	0.02
Hypertension medication	10	20	23	22	24	0.09
Diabetes	8	4	2	8	3	0.57
Vitamin supplement (% yes)	44	33	41	22	10	<0.01
<i>CBS</i> insertion*	2.4	0.5	2.5	1.0	3.2	0.57
<i>MTHFR</i> C _{677T} *	2.3	4.7	7.4	8.2	16.6	0.01

*Homozygous for the mutation.

†Geometric mean.

‡Also adjusted for energy intake.

537 (of whom 10 were also CHD cases). Approximately 26% of the CHD case subjects were black, and 75% were men.

Correlates of tHcy

In the cohort sample, plasma folate, vitamin B₁₂, and PLP all showed graded inverse associations across tHcy quintiles (Table 1). The weighted correlations of tHcy with plasma folate, PLP, and vitamin B₁₂ were $r=-0.29$, $r=-0.20$, and $r=-0.28$, respectively. Correlations among the plasma vitamins were as follows: folate and vitamin B₁₂, $r=0.44$; folate and PLP, $r=0.48$; and PLP and vitamin B₁₂, $r=0.36$. The correlations of plasma vitamins with estimated dietary intake (without supplements) were as follows: folate, $r=0.15$; B₆ (with PLP), $r=0.24$; and B₁₂, $r=0.04$. However, dietary intake of the 3 vitamins from foods was not consistently associated with plasma tHcy in a graded fashion (Table 1). The prevalence of vitamin supplement use decreased across tHcy quintiles and was especially low (10%) in the highest quintile of tHcy. Vitamin users had higher age-, sex-, race-, and field center-adjusted mean plasma levels of vitamins

than did nonusers for folate (15 versus 8 nmol/L), PLP (98 versus 34 nmol/L), and vitamin B₁₂ (359 versus 295 pmol/L). Waist/hip ratio, alcohol intake, and hypertension were weakly but positively associated with plasma tHcy concentration, but other CHD risk factors were not.

We detected no homozygotes or heterozygotes for the G_{919A} mutation of the *CBS* gene and only 3 heterozygotes for the T_{833C} mutation (1 case, 2 noncases). There was no association between prevalence of the *CBS* insertion and tHcy (Table 1). The prevalences of heterozygosity and homozygosity of the thermolabile *MTHFR*_{C677T} mutation were 37% and 9%. *MTHFR*_{C677T} homozygosity increased across each quintile of tHcy (Table 1), and the association carried a P value for trend of 0.01.

Mean Differences Between CHD Cases and Noncases

Compared with the noncases, participants who subsequently developed CHD tended to have a higher baseline mean tHcy concentration; lower mean plasma concentrations of folate,

TABLE 2. Age-, Race-, and Sex-Adjusted Geometric Mean Levels (or Percentages) of Study Variables in Incident CHD Cases Versus Noncases (ARIC)

Variable	Cases (n=232)	Noncases (n=527)	P*
tHcy, $\mu\text{mol/L}$	8.86	8.53	0.24
Plasma folate, nmol/L	5.38	6.12	0.21
Plasma PLP, nmol/L	19.0	31.5	<0.01
Plasma vitamin B ₁₂ , pmol/L	271	286	0.18
Dietary folate, $\mu\text{g/d}\dagger$	195	197	0.78
Dietary PLP, mg/d†	1.45	1.51	0.13
Dietary vitamin B ₁₂ , $\mu\text{g/d}\dagger$	6.27	6.50	0.45
Vitamin supplement use, %	22.8	29.1	0.17

*Test of difference between cases and the cohort.

†Also adjusted for energy intake.

PLP, and vitamin B₁₂; and lower supplemental vitamin use (Table 2). However, only the difference in mean plasma PLP (19.0 nmol/L in CHD cases versus 31.5 nmol/L in noncases) was statistically significant. Mean tHcy (in $\mu\text{mol/L}$) was 10.5 for participants with definite or probable MI, 9.8 for silent MI, 11.1 for definite fatal CHD, and 11.2 for revascularization ($P=0.63$ for difference).

Relative Risks of CHD

Initial modeling of CHD incidence suggested interactions ($P\leq 0.10$) of sex with tHcy, plasma PLP, plasma folate, and vitamin supplement intake, so we used sex-specific models initially. As Table 3 illustrates, there was a positive association of tHcy with CHD incidence in women (P for trend=0.04), with an age-, race-, and center-adjusted relative risk of 2.53 for the highest quintile. There was no association in men. The relative risk of CHD for those in the upper 10th percentile of tHcy versus the lowest quintile was 3.48 (95% CI=0.96 to 12.6) in women but was 1.07 (95% CI=0.3 to 3.4) in men. There also were statistically significant age-, race-, and center-adjusted inverse associations of CHD with plasma PLP in both sexes and with plasma folate in women. Relative risks ranged from 0.36 to 0.48 for the fifth versus first quintile (Table 3). There was no significant association of CHD with dietary measures of B-vitamin intake (not shown). However, the age-, race-, and center-adjusted relative risk of CHD for vitamin supplement users versus nonusers was 0.47 (95% CI=0.22 to 0.97) in women but 1.02 (95% CI=0.6 to 1.9) in men.

The C₆₇₇T *MTHFR* mutation and the *CBS* insertion were not associated with CHD incidence. For example, adjusted for age, race, and center, the relative risks for heterozygosity and homozygosity for the C₆₇₇T mutation of the *MTHFR* gene

TABLE 3. Sex-Specific Age-, Race-, and Center-Adjusted Relative Risks (95% CIs) of CHD in Relation to Quintiles of Plasma tHcy or Vitamin Concentrations (ARIC)

Variable	Sex	Quintiles					P for Trend
		1	2	3	4	5	
tHcy	Women						
	RR	Ref	0.76	0.89	1.71	2.53	0.04
	95% CI	...	0.3–2.2	0.3–2.7	0.6–4.8	0.9–7.5	
	Men						
	RR	Ref	0.71	0.98	0.88	0.87	0.92
	95% CI	...	0.2–2.3	0.3–3.0	0.3–2.6	0.3–2.6	
Plasma folate	Women						
	RR	Ref	0.81	0.88	0.34	0.39	0.003
	95% CI	...	0.3–2.0	0.3–2.2	0.1–0.97	0.1–1.06	
	Men						
	RR	Ref	0.61	0.68	0.87	1.01	0.65
	95% CI	...	0.3–1.3	0.3–1.4	0.4–1.8	0.5–2.2	
Plasma PLP	Women						
	RR	Ref	0.49	0.36	0.17	0.36	0.002
	95% CI	...	0.2–1.1	0.1–1.01	0.05–0.6	0.1–0.98	
	Men						
	RR	Ref	1.29	0.77	0.54	0.48	0.02
	95% CI	...	0.6–2.7	0.4–1.6	0.3–1.1	0.2–1.04	
Plasma vitamin B ₁₂	Women						
	RR	Ref	0.74	1.00	0.33	0.62	0.06
	95% CI	...	0.2–2.4	0.4–2.5	0.1–1.04	0.2–1.7	
	Men						
	RR	Ref	0.70	1.01	0.67	1.22	0.85
	95% CI	...	0.4–1.4	0.5–2.0	0.3–1.3	0.6–2.6	

Ref indicates reference. Quintile cutpoints were: folate (3.1, 6.4, 10.6, and 17.9 nmol/L); PLP (21.0, 31.9, 45.8, and 78.9 nmol/L); and vitamin B₁₂ (219, 282, 339, and 437 pmol/L).

TABLE 4. Multivariately Adjusted* Relative Risks (95% CIs) of CHD in Relation to Quintiles of Plasma tHcy or Vitamin Concentrations (ARIC)

Variable	Quintiles					P for Trend
	1	2	3	4	5	
tHcy						
RR	Ref	0.79	1.43	1.28	1.28	0.29
95% CI	...	0.3–2.2	0.6–3.7	0.5–3.3	0.5–3.2	
Plasma folate						
RR	Ref	0.73	0.82	0.82	0.66	0.14
95% CI	...	0.4–1.5	0.4–1.7	0.4–1.7	0.3–1.5	
Plasma PLP						
RR	Ref	0.81	0.63	0.53	0.28	0.001
95% CI	...	0.4–1.6	0.3–1.2	0.2–1.2	0.1–0.7	
Plasma vitamin B₁₂						
RR	Ref	0.71	0.95	0.53	0.81	0.13
95% CI	...	0.4–1.4	0.5–1.8	0.3–1.1	0.4–1.8	

Ref indicates reference.

*Adjusted for sex, age, race, field center, total and HDL cholesterol, hypertension, diabetes, and smoking status.

were 1.48 and 0.59, respectively, in women and 1.33 and 0.84 in men (all $P > 0.10$). The relative risks for heterozygosity and homozygosity for the *CBS* insertion mutation were 1.85 and 1.83, respectively, in women and 1.01 and 0.26 in men (all $P > 0.05$).

Although the simpler regression models suggested potential differences in associations by sex (Table 3), interactions by sex were not significant (all $P > 0.09$) after other risk factors were considered. This fact, plus the small number of events in women, led us to pool men and women for final modeling. As Table 4 shows, the multivariately adjusted association of CHD with plasma PLP was negative and statistically significant, with a relative risk for the highest versus lowest quintile of 0.28 (95% CI=0.1 to 0.7). Associations with CHD were positive for tHcy and negative for plasma folate and B₁₂, although none of these was statistically significant. The multivariately adjusted relative risk of CHD for vitamin supplement use was 0.79 (95% CI=0.5 to 1.4). Reanalysis of Table 4 excluding the blacks did not change the conclusions.

Discussion

In this prospective study, we found fasting tHcy to be associated positively and relatively strongly at high levels with age-, race-, and center-adjusted incidence of CHD in women, although not in men. Yet, adjustment for other CHD risk factors abolished the association, suggesting that tHcy was not independently associated with CHD. In this same population, we previously observed a nonsignificant, positive cross-sectional association in both men and women between tHcy and carotid intima-media thickness.³³ Most other cross-sectional case-control studies have shown an association between tHcy and CHD,^{1,34,35} but they have not uniformly controlled for all confounding variables and are prone to survival bias. Furthermore, tHcy is often elevated after an acute coronary event.³⁶ Although cross-sectional studies have

typically measured tHcy in cases at least 3 months after any acute CHD events, tHcy may have remained as a consequence of CHD.

In contrast with the cross-sectional studies, a majority of prospective epidemiological studies of subjects initially free of CHD, published either in full^{2–8} or in abstract,^{37,38} do not show an association of tHcy and CHD incidence. This suggests that elevated tHcy may be a consequence, not a cause, of CHD.⁶ Recent evidence suggests that endothelial dysfunction may raise plasma tHcy.³⁹ In patients with CHD, elevated tHcy strongly predicts a poor outcome,⁴⁰ further suggesting that it reflects the severity of CHD and possibly the risk of thrombosis.

Plasma PLP, B₁₂, and folate, which are cofactors in homocysteine metabolism, were moderately strong correlates of tHcy, as has been documented previously.^{1,34,35,41–43} We found moderately strong inverse associations of CHD with plasma PLP, folate, and vitamin B₁₂ in women and with PLP in men. However, in multivariate analysis pooling men and women, only PLP remained independently associated with CHD. Previous evidence is not entirely consistent but suggests folate and PLP but not vitamin B₁₂ concentrations in the blood are associated negatively with CHD occurrence.^{7,9,34,35,41–43} Only 2 of these previous studies^{7,9} were prospective. Vitamin B₆ deficiency can cause atherosclerosis in animal models, and other possible mechanisms of how vitamin B₆ might protect against CHD have been hypothesized.⁴⁴

In contrast with a recent large study,¹¹ we found no significant association between questionnaire assessments of B vitamins from food and CHD incidence. On the other hand, we found that vitamin supplement use was associated with reduced risk of CHD in the age-, race-, and field center-adjusted model for women. Users of vitamin supplements had higher plasma B-vitamin concentrations than nonusers, sug-

gesting that vitamin supplementation contributed to the inverse association between plasma PLP and CHD.

Despite the fact that *MTHFR*_{C677T} homozygosity was associated with higher tHcy levels, we found no association of *MTHFR*_{C677T} with CHD incidence. Most recent reports,^{10,45-47} but not all,⁴⁸⁻⁵⁰ have also found no association of *MTHFR*_{C677T} with CHD. The T_{833C} and G_{919A} mutations of the *CBS* gene together account for $\geq 50\%$ of the mutant alleles in patients with homocystinuria.^{28,51} Yet, heterozygosity for these 2 mutations in this population-based sample were too rare to be important determinants of elevated tHcy or of CHD risk. We also found no association of CHD with the *CBS* 68-bp insertion mutation, consistent with a previous report.²⁹

Our prospective findings add uncertainty to conclusions derived mostly from cross-sectional studies that tHcy is a major, independent, causative risk factor for CHD. Our findings point more strongly to the possibility that vitamin B₆ offers independent protection. Randomized trials, some of which are under way, are needed to better clarify the interrelationships of tHcy, B vitamins, and cardiovascular disease.

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