

# Effect of Folic Acid Fortification of Food on Homocysteine-Related Mortality

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**BACKGROUND:** In 1998, the Food and Drug Administration mandated the fortification of food products with folic acid. The effect of this rule on mortality associated with homocysteine levels in patients with coronary artery disease is unknown.

**METHODS:** We studied 2481 consecutive patients with coronary artery disease who underwent coronary angiography between 1994 and 1999, and who had baseline homocysteine measurements and at least 2 years of follow-up. Patients were divided into prefortification (1994 to 1997, n = 1595) and post-fortification (1998 to 1999, n = 886) groups, as well as classified based on baseline homocysteine levels (normal to low, intermediate, and high). Homocysteine levels were measured by fluorescence polarization immunoassay. Mortality was determined by telephone survey or from a national Social Security database or hospital records.

**RESULTS:** After implementation of the fortification rule, median homocysteine levels declined from 13.8 to 12.3  $\mu\text{mol/L}$  ( $P < 0.001$ ), and the proportion of patients with high homocys-

teine levels ( $>15 \mu\text{mol/L}$ ) decreased from 41% (n = 650) to 28% (n = 249) ( $P < 0.001$ ). Overall, homocysteine was a modest risk factor for mortality (adjusted relative risk [RR] = 1.03 per  $\mu\text{mol/L}$ ; 95% confidence interval [CI]: 1.01 to 1.05;  $P = 0.006$ ). There was no significant interaction between fortification status and homocysteine category with mortality ( $P$  for interaction = 0.85). Two-year mortality was reduced minimally (7.8% [n = 124] to 7.2% [n = 64]; RR = 0.93; 95% CI: 0.68 to 1.27;  $P = 0.63$ ; adjusted RR = 0.97; 95% CI: 0.68 to 1.40), but was consistent with the expectation of a modest reduction in homocysteine levels.

**CONCLUSION:** Homocysteine is an independent, graded risk factor for mortality. Homocysteine levels decreased modestly after the fortification of food with folic acid, but the effects on mortality were minor and likely attributable to other factors, indicating the need for more aggressive measures to reduce homocysteine-associated cardiovascular risk. *Am J Med.* 2004; 116:158–164. ©2004 by Excerpta Medica Inc.

Homocysteine is an amino acid intermediary in the metabolic pathway of methionine (1,2). It has pro-oxidant properties and in high concentrations is a cause of endothelial dysfunction (2–4). Elevated levels have been associated with increased primary and secondary risk of ischemic heart disease, although the strength of these associations continues to be debated (2,5–9). Folic acid reduces circulating homocysteine levels (10), but whether this lowers coronary risk is not known and is being studied in large clinical trials (2,10).

Previously, we showed that homocysteine levels  $>16.5 \mu\text{mol/L}$  were associated with reduced survival in patients with established coronary artery disease (8), which is consistent with reports from studies of primary and secondary coronary risk (2,6,7). However, these observations predated the general implementation of folic acid fortification of food products in the United States (8).

On February 29, 1996, the Food and Drug Administration (FDA) announced changes in the fortification requirements for folic acid in grain-based foods (11). Implementation by manufacturers was required by January

1, 1998. The objective of the new rule was to prevent folic acid deficiency during pregnancy and to reduce the related risk of neural tube defects in newborns. Since fortification might also reduce homocysteine levels in those with or at risk of coronary artery disease (12), we conducted a study to assess whether the mandate on folic acid fortification had an effect on homocysteine levels and consequently on mortality in a large cohort of patients with coronary artery disease.

## METHODS

### Study Sample

The study sample consisted of 2481 patients selected from the cardiac catheterization laboratory registry of the Intermountain Heart Collaborative Study (13). Patients had to have undergone angiography, have one or more  $\geq 70\%$  stenotic lesions, and have provided written consent to a blood draw at the time of angiography for use in confidential research studies approved by the LDS Hospital Institutional Review Board. Each patient had a minimum of 2 years of clinical follow-up. (The study sample overlaps with that of a previous report [12], which was unselected for angiographic coronary artery disease and did not assess survival outcomes.)

Patients were divided into two groups based on enrollment date in relation to the 1998 mandate: a prefortification group, enrolled from 1994 to 1997 (n = 1595); and a

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postfortification group, enrolled from 1998 to 1999 (n = 886).

### *Homocysteine Measurement and Classification*

Fasting plasma samples were separated from whole blood, refrigerated, and frozen within 4 hours at  $-70^{\circ}\text{C}$  until assayed with a fluorescence polarization immunoassay (Abbott Diagnostics, Chicago, Illinois). Patients were classified by baseline homocysteine levels:  $<10\ \mu\text{mol/L}$  (normal to low);  $10$  to  $15\ \mu\text{mol/L}$  (intermediate); and  $>15\ \mu\text{mol/L}$  (high), which were based on laboratory standards, previous literature reports, and our own experience (2,8).

### *Follow-up and Outcomes*

Patients were followed until death or April 2002, whichever was first. Deaths were determined from a telephone survey, by reviewing hospital and health department records, or by searching a national Social Security database. If death could not be determined from any of these sources, the patient was considered to be alive.

### *Covariables and Primary Endpoint Analysis*

The study adjusted for standard cardiac risk factors, including age, sex, hypertension, diabetes, smoking, hyperlipidemia, family history of early coronary artery disease, and C-reactive protein level. Diabetes was defined as a fasting blood glucose level  $\geq 126\ \text{mg/dL}$  or use of antidiabetic medications. Hypertension was defined as blood pressure  $\geq 140/90\ \text{mm Hg}$  or use of antihypertensive agents. Hyperlipidemia was defined as a total cholesterol level  $\geq 200\ \text{mg/dL}$ , a low-density lipoprotein (LDL) cholesterol level  $\geq 130\ \text{mg/dL}$ , or use of cholesterol-lowering medications. Family history of early coronary artery disease was defined as cardiovascular death, myocardial infarction, or coronary revascularization before the age of 65 years in a first-degree relative. Smoking included active or previous ( $>10$  pack-years) tobacco use. C-reactive protein levels  $\geq 1.2\ \text{mg/dL}$  were considered elevated.

The study also adjusted for admitting presentation (stable vs. unstable angina), coronary anatomy (one-, two-, or three-vessel disease), and type of initial treatment (medical only, percutaneous coronary intervention, or coronary artery bypass graft surgery). We also adjusted for comorbid diseases, such as a history of myocardial infarction, stroke, or heart failure, and renal insufficiency (renal failure or creatinine level  $>2\ \text{mg/dL}$ ). Key discharge medications (beta-blockers, angiotensin-converting enzyme [ACE] inhibitors, and statins) were also included in adjusted analyses (aspirin use was almost universal).

### *Statistical Analysis*

Comparisons were made using analysis of variance for continuous variables and chi-squared tests for discrete variables. Baseline demographic and laboratory data are

presented as means ( $\pm$  SD) for continuous variables and frequencies for discrete variables.

Survival statistics were used for risk determination using the Cox proportional hazards model. The primary outcome variable was all-cause death at 2 years after entry catheterization. (Specific cause of death was not uniformly available, but approximately 80% of deaths in the cohort were cardiovascular). Both univariable and multivariable (stepwise and forced entry) Cox regression analyses were performed using SPSS for Windows, version 11.5 (Chicago, Illinois), with homocysteine entered as a continuous variable (primary analysis). Mortality was assessed within the three homocysteine categories (secondary analysis). A two-sided  $P$  value  $\leq 0.05$  was considered significant.

## RESULTS

Patients in the pre- and postfortification groups generally had similar characteristics (Table 1). The mean ( $\pm$  SD) age was  $65 \pm 11$  years, and 77% were men. However, the postfortification cohort more often had a history of hypertension and hyperlipidemia, and a positive family history of coronary artery disease, but a slightly smaller proportion had three-vessel coronary disease. Patients enrolled after the 1998 mandate were also more likely to undergo percutaneous coronary intervention, but less likely to undergo coronary artery bypass graft surgery. Elevated C-reactive protein levels were less prevalent in this group, although beta-blockers, ACE inhibitors, and statins were used more frequently.

### *Changes in the Distribution of Homocysteine Levels*

Median homocysteine levels dropped modestly after the fortification rule was implemented, from  $13.8$  to  $12.3\ \mu\text{mol/L}$  ( $P < 0.001$ ; Figure 1). However, homocysteine levels were broad ranging and overlapped for the two cohorts (Figure 1). The fortification rule also affected the distribution of patients in the three homocysteine categories ( $P < 0.0001$ ; Figure 2). There was a significant reduction in the proportion of subjects with high homocysteine levels ( $>15\ \mu\text{mol/L}$ ), from 41% ( $650/1595$ ) to 28% ( $249/886$ ) ( $P < 0.001$ ). In contrast, there was an increase in the proportion of patients with normal-to-low homocysteine levels ( $<10\ \mu\text{mol/L}$ ), from 18% ( $n = 281$ ) to 26% ( $n = 234$ ) ( $P < 0.001$ ). The proportion of patients with intermediate homocysteine levels was relatively unchanged (42% [ $n = 664$ ] to 45% [ $n = 403$ ]).

### *Association of Homocysteine and Folic Acid Supplementation with Mortality*

Overall 2-year mortality was 7.8% ( $124/1595$ ) before and 7.2% ( $64/886$ ) after the rule change, an insignificant reduction (relative risk [RR] = 0.93; 95% confidence inter-

**Table 1.** Characteristics of the Study Groups (n = 2481)

Characteristic	Prefortification (1994 to 1997) (n = 1595)	Postfortification (1998 to 1999) (n = 886)
	Number (%) or Mean $\pm$ SD	
Age (years)	65 $\pm$ 11	65 $\pm$ 12
Male sex	1239 (78)	665 (75)
Systemic hypertension	878 (55)	551 (62)*
Hyperlipidemia	833 (52)	538 (61)*
Diabetes mellitus	296 (19)	188 (21)
Family history of coronary artery disease	538 (34)	346 (39) <sup>†</sup>
Cigarette smoking	418 (26)	228 (26)
Renal insufficiency	92 (6.1)	54 (5.9)
History of heart failure	210 (13)	133 (15)
History of myocardial infarction	380 (24)	236 (27)
History of stroke	22 (1)	28 (3) <sup>†</sup>
Admitting presentation		
Stable angina	706 (44)	387 (44)
Unstable angina	515 (32)	281 (32)
Myocardial infarction	374 (23)	218 (25)
Diseased vessels		
1	585 (37)	379 (43)
2	442 (28)	227 (26)
3	568 (36)	280 (32)
Elevated C-reactive protein level <sup>‡</sup>	1021 (64)	478 (54)*
Medications at discharge		
Statin	315 (20)	346 (39)*
Beta-blocker	931 (58)	596 (67)*
Angiotensin-converting enzyme inhibitor	367 (23)	276 (31)*
Treatment at initial hospitalization		
Medical therapy only	699 (44)	414 (47)
Percutaneous coronary intervention	396 (25)	309 (35)
Coronary artery bypass graft surgery	500 (31)	163 (18)*

\*  $P \leq 0.001$ .<sup>†</sup>  $P \leq 0.05$ .<sup>‡</sup> Defined as  $\geq 1.2$  mg/dL.

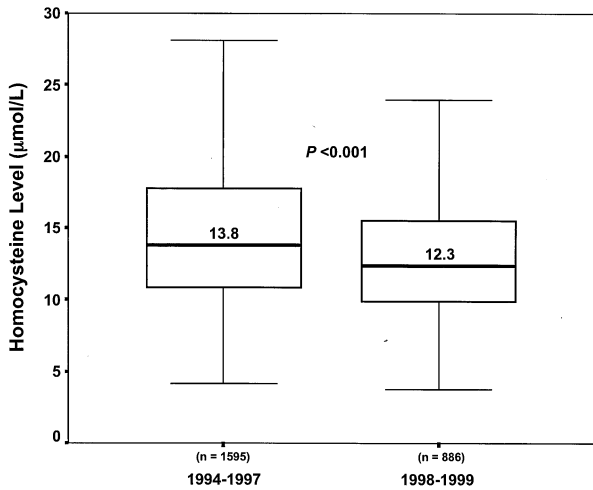
val [CI]: 0.68 to 1.27;  $P = 0.63$ ). Still, homocysteine was predictive of 2-year mortality in both unadjusted (RR = 1.033 per  $\mu\text{mol/L}$ ; 95% CI: 1.015 to 1.051;  $P < 0.001$ ) and adjusted (RR = 1.027 per  $\mu\text{mol/L}$ ; 95% CI: 1.008 to 1.046;  $P = 0.006$ ) analyses (Table 2). Homocysteine category was less predictive of mortality (unadjusted RR = 1.36 per category, 95% CI: 1.10 to 1.68,  $P = 0.004$ ; adjusted RR = 1.23 per category, 95% CI: 0.97 to 1.55,  $P = 0.09$ ). The adjusted relative risk for high versus normal-to-low homocysteine levels was 1.43 (95% CI: 0.88 to 2.32;  $P = 0.15$ ).

When 2-year mortality was evaluated for the three homocysteine categories divided by pre- and postfortification status (Table 3), no significant interaction between time (pre- vs. postfortification) and homocysteine category with mortality was found ( $P$  for interaction = 0.85). An exploratory analysis within the normal-to-low homo-

cysteine category suggested a decreasing mortality trend (7.1% [20/281] to 3.4% [8/234],  $P = 0.06$ ).

After accounting for differences in baseline characteristics and other covariables except homocysteine, the relative mortality risk of postfortification compared with prefortification status was 0.97 (95% CI: 0.68 to 1.40). After further adjustment for differences in homocysteine category, the relative mortality risk of postfortification compared with prefortification status was 1.03 (95% CI: 0.71 to 1.48).

**Prefortification group.** When analysis was restricted to the prefortification group, homocysteine was found to be a weak predictor of mortality (unadjusted RR = 1.033 per  $\mu\text{mol/L}$ , 95% CI: 1.012 to 1.055,  $P = 0.002$ ; adjusted RR = 1.025, 95% CI: 1.003 to 1.047,  $P = 0.02$ ), and homocysteine category as a predictor did not achieve significance (unadjusted RR = 1.28 per category, 95% CI: 0.99



**Figure 1.** Distribution of homocysteine levels in the prefortification (1994 to 1997) and postfortification (1998 to 1999) cohorts. The boxes represent the median homocysteine values with 25th to 75th percentiles; the whiskers represent 95th percentiles.

to 1.66,  $P = 0.06$ ; adjusted RR = 1.17, 95% CI: 0.87 to 1.57,  $P = 0.30$ ).

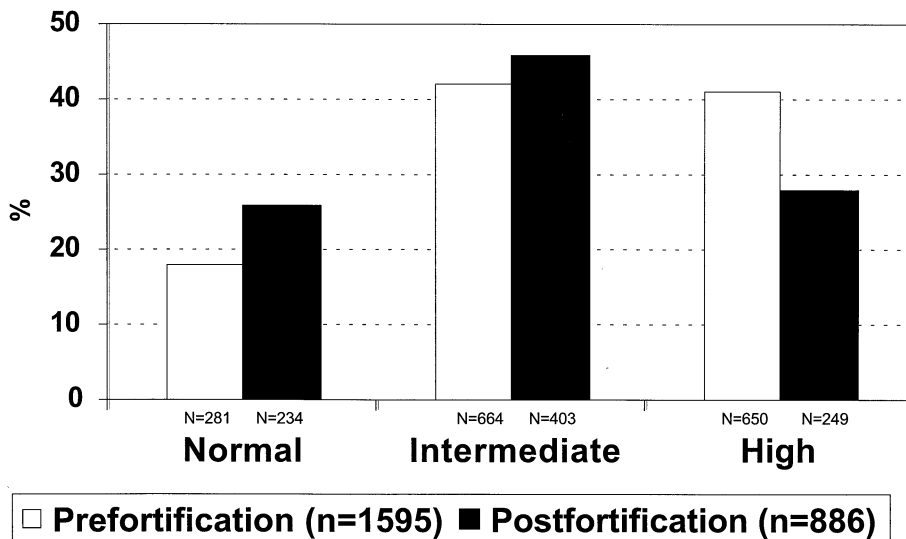
**Postfortification group.** For the postfortification group, estimates of relative risk were similar to or greater than for the overall cohort. For homocysteine, the unadjusted relative risk was 1.04 per  $\mu\text{mol/L}$  (95% CI: 1.00 to 1.07;  $P = 0.04$ ), and the adjusted relative risk was 1.03 (95% CI: 0.99 to 1.08,  $P = 0.12$ ). For homocysteine category, the unadjusted relative risk was 1.51 per category (95% CI:

1.06 to 2.15,  $P = 0.02$ ), and the adjusted relative risk was 1.41 (95% CI: 0.93 to 2.14,  $P = 0.11$ ). Both the intermediate (unadjusted RR = 2.61, 95% CI: 1.19 to 5.74,  $P = 0.02$ ; adjusted RR = 2.26, 95% CI: 0.95 to 5.37,  $P = 0.06$ ) and high (unadjusted RR = 2.76, 95% CI: 1.20 to 6.33,  $P = 0.02$ ; adjusted RR = 2.33, 95% CI: 0.95 to 5.37,  $P = 0.06$ ) homocysteine groups were associated with greater mortality than the normal-to-low referent group.

**Interim group.** An exploratory analysis was performed in the subgroup of patients enrolled during the transition period after the announcement of the fortification rule in February 1996 and before its required implementation in January 1998. There was no evidence of improvement in median homocysteine concentrations (13.9 vs. 13.6  $\mu\text{mol/L}$ ,  $P = 0.73$ ) or mortality (7.8% [54/695] vs. 7.8% [70/900]; Table 3) when compared with the preannouncement subgroup.

## DISCUSSION

We found that after implementation of an FDA-mandated rule requiring fortification of food products with folic acid, there was a redistribution within homocysteine risk categories in a cohort of nearly 2500 patients with coronary artery disease. This was associated with a modest overall reduction in median homocysteine levels in the postfortification cohort. Generally, the relation of homocysteine to mortality risk was unchanged by fortification, although an improving survival trend was suggested in the normal-to-low homocysteine group, which might be due to chance or to a modest beneficial effect of



**Figure 2.** Effect of the folic acid fortification rule on the distribution of patients with coronary artery disease in the three homocysteine categories. Homocysteine levels were classified as normal or low ( $< 10 \mu\text{mol/L}$ ), intermediate (10 to 15  $\mu\text{mol/L}$ ), and high ( $> 15 \mu\text{mol/L}$ ).

**Table 2.** Regression Model for the Prediction of 2-Year Mortality\*†

Variable	Hazard Ratio (95% Confidence Interval)	P Value
Homocysteine (per $\mu\text{mol/L}$ )	1.03 (1.01–1.05)	0.006
Postfortification time	1.2 (0.84–1.6)	0.36
Age (per decade)	1.4 (1.2–1.6)	<0.001
Elevated C-reactive protein level	2.2 (1.5–3.2)	<0.001
Diabetes	1.8 (1.3–2.5)	<0.001
History of heart failure	2.5 (1.8–3.5)	<0.001
Beta-blocker use	1.8 (1.3–2.6)	0.001
Diseased vessels		
1	1.0	
2	1.4 (0.92–2.1)	0.12
3	1.7 (1.2–2.5)	0.004
Hyperlipidemia	0.61 (0.45–0.84)	0.002
Statins at discharge	0.57 (0.38–0.86)	0.007
Treatment at initial hospitalization		
Medical therapy only	1.0	
Percutaneous coronary intervention	0.87 (0.59–1.3)	0.48
Coronary artery bypass graft surgery	0.52 (0.35–0.77)	0.001

\* No difference in slope of risk function was found for homocysteine between the different time periods when an interaction term (homocysteine \* time period) was entered into the model ( $P$  for interaction = 0.85).

† Other variables considered for entry but excluded from this model were sex, systemic hypertension, family history of coronary artery disease, cigarette smoking, renal insufficiency, history of myocardial infarction, history of stroke, baseline clinical presentation, and prescription of an angiotensin-converting enzyme inhibitor at discharge.

folic acid, perhaps acting independent of homocysteine (7). Overall, however, the effect of fortification on survival was minor at best and likely attributable to other factors, indicating the need for more aggressive measures to reduce homocysteine-associated cardiovascular risk (2,10).

Our findings are consistent with and extend other reports demonstrating the ability of folic acid fortification of food and vitamin supplementation, with or without vitamin B<sub>6</sub> (pyridoxine) or B<sub>12</sub>, to reduce plasma homocysteine levels (10,11,14–21). In one primary risk cohort of 350 subjects (21), fortification reduced mean homocysteine levels by 0.7  $\mu\text{mol/L}$ , as well as the prevalence of high homocysteine levels (>13  $\mu\text{mol/L}$ ) by 9%.

In several observational studies, elevated plasma homocysteine levels have been associated with increased primary and secondary risk of cardiovascular disease

(2,5–8). Homocysteine-related risk also has been reported after acute coronary syndromes (22) and coronary angioplasty (23) and for type 2 diabetic patients (24). However, the strength of these associations has varied (2,5–8,25). A recent meta-analysis of 30 studies found that a 25% lower homocysteine level was associated with an 11% lower risk of coronary artery disease and a 19% lower risk of stroke (26). Another meta-analysis also supported homocysteine as an independent, modest risk predictor (9). Similarly, a meta-analysis of studies that assessed the risk of the methylenetetrahydrofolate reductase gene 677C→T polymorphism, which impairs homocysteine metabolism and increases homocysteine levels, reported a small increase in coronary risk for the TT genotype (+16%) compared with the CC genotype in the setting of low-folate dietary status (27).

If the estimated effect of homocysteine on mortality is

**Table 3.** Two-Year Mortality for Each Time Period According to Homocysteine Category

Time Period	Homocysteine Category ( $\mu\text{mol/L}$ )		
	<10	10 to 15	>15
	Number (%)		
Prefortification (8/94 to 12/97)	20/281 (7.1)	41/664 (6.2)	63/650 (9.7)
Preannouncement (8/94 to 2/96)	10/151 (6.6)	16/253 (6.3)	28/291 (9.6)
Interim (3/96 to 12/97)	10/130 (7.7)	25/411 (6.1)	35/359 (9.7)
Postfortification (1/98 to 11/99)	8/234 (3.4)	34/403 (8.4)	22/249 (8.8)

taken as accurate (RR = 1.027 per  $\mu\text{mol/L}$  homocysteine change), a median reduction of 1.5  $\mu\text{mol/L}$  yields a relative risk of 0.96, close to the observed result (adjusted RR = 0.97). A slightly larger benefit (RR = 0.95) is predicted using results of a meta-analysis (26). Thus, our data are compatible with an effect of the fortification policy on mortality, but a sample of 10,500 to 21,500 patients would be required to adequately power a study (80% power,  $\alpha$  of 0.05) to detect such modest reductions.

A causal role for homocysteine in vascular disease is supported by observations on rare genetic diseases, experimental models, and homocysteine-induced endothelial dysfunction in humans (2–4). Endothelial toxic damage can be caused by generation of reactive oxygen species (2,4), impaired production of endothelial-derived nitric oxide, stimulation of smooth muscle cell proliferation, and oxidation of LDL (2). Viridis et al (28) found that in both normotensive and hypertensive subjects with high homocysteine levels ( $>14.6 \mu\text{mol/L}$ ), vitamin C reversed the blunted response to acetylcholine, suggesting that hyperhomocysteinemia might impair endothelium-dependent vasodilation through an oxidant stress-related mechanism.

Folic acid might reduce the risk of coronary events by lowering homocysteine levels as well as by homocysteine-independent mechanisms (29). Doshi et al (29) studied the effects of folic acid therapy on endothelial dysfunction, an early predictor of vascular pathology, before and after folate-related changes in homocysteine levels. Folic acid (5 mg) improved endothelial function, as assessed by forearm flow-mediated dilatation, within 2 to 4 hours, whereas plasma homocysteine levels did not differ. The authors argued that the acute benefits of high-dose folic acid on endothelial function, and possibly its chronic benefits, might be independent of homocysteine. Our study is consistent with, but not conclusive for, a homocysteine-independent mechanism in patients with normal-to-low levels. Nevertheless, overall survival and survival in those with higher homocysteine levels did not improve, providing no evidence of a benefit of folic acid fortification that is independent of homocysteine.

This study shares the limitations of all nonrandomized, observational studies, including the possibility of selection bias and unadjusted confounding. However, it has the advantage of a large sample size and prospective patient and data entry. Another limitation is the lack of information on vitamin intake; thus, the possible effects of increasing vitamin use over time cannot be excluded. Other changes in medical therapies also might have influenced mortality trends. Any unadjusted confounding would tend to further diminish the insignificant mortality trend.

In conclusion, we found that homocysteine levels decreased modestly as a result of the fortification of food

with folic acid in a large cohort of patients with angiographically documented coronary artery disease. Still, high homocysteine levels remained prevalent. The effects on mortality were minor, but consistent with the modest predicted effects of fortification. Taken together, these results support ongoing clinical trials of the benefits of higher-dose, pharmacologic supplementation with folic acid on homocysteine-related cardiovascular risk (30).

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