

## ORIGINAL CONTRIBUTION

# Homocysteine and Risk of Cardiovascular Disease Among Postmenopausal Women

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SEVERAL MECHANISMS HAVE BEEN proposed linking hyperhomocystinemia to vascular damage, and it has been hypothesized that elevated levels of total plasma homocysteine represent an important modifiable risk factor for atherothrombotic disease.<sup>1,2</sup> A number of retrospective and cross-sectional studies provide support for this hypothesis.<sup>3</sup> However, because homocysteine levels may increase following acute myocardial infarction (MI)<sup>4</sup> or stroke,<sup>5</sup> any observed association could, at least in theory, be a result rather than a cause of acute vascular occlusion.

In contrast, in prospective studies, homocysteine levels are ascertained prior to the onset of thrombosis and, thus, reduce the potential for this type of bias. The results of such studies, however, have been inconsistent. For example, prospective studies from the United States<sup>6</sup> and Finland<sup>7</sup> report no evidence of association between baseline homocysteine level and subsequent coronary heart disease risk, whereas another study of middle-aged US men reported a positive association in the subgroup in the top 5% of the homocysteine level distribution<sup>8</sup> that was no longer present with long-term follow-up.<sup>9</sup> In this latter study, there were no associations between homocysteine and risk of thromboembolic stroke<sup>10</sup> or incident angina pec-

**Context** Individuals with elevated levels of homocysteine tend to have higher prevalence of cardiovascular disease. However, prospective studies of homocysteine are inconsistent and data among women are limited.

**Objective** To determine whether elevated homocysteine levels in healthy postmenopausal women predict risk of developing cardiovascular disease.

**Design** Prospective, nested case-control study with a mean 3-year follow-up.

**Setting** The Women's Health Study, an ongoing US primary prevention trial initiated in 1993.

**Participants** From a total cohort of 28 263 postmenopausal women with no history of cardiovascular disease or cancer at baseline, 122 women who subsequently experienced cardiovascular events were defined as cases, and 244 age- and smoking status-matched women who remained free of disease during follow-up were defined as controls.

**Main Outcome Measures** Incidence of death due to cardiovascular disease, non-fatal myocardial infarction (MI), stroke, percutaneous transluminal coronary angioplasty, or coronary artery bypass graft by baseline homocysteine level.

**Results** Of the 122 cases, there were 85 events of MI or stroke and 37 coronary revascularizations. Case subjects had significantly higher baseline homocysteine levels than controls (14.1 vs 12.4  $\mu\text{mol/L}$ ,  $P = .02$ ). Subjects with homocysteine levels in the highest quartile had a 2-fold increase in risk of any cardiovascular event (relative risk [RR], 2.0; 95% confidence interval [CI], 1.1-3.8). This effect was largely due to an excess of cases with high levels of homocysteine; the RR for those with homocysteine levels at or higher than the 95th percentile (20.7  $\mu\text{mol/L}$ ) was 2.6 (95% CI, 1.1-5.7). Risk estimates were independent of traditional risk factors and were greatest for the end points of MI and stroke (RR for those with baseline homocysteine levels in the top quartile, 2.2, 95% CI, 1.1-4.6). Self-reported multivitamin supplement use at study entry was associated with significantly reduced levels of homocysteine ( $P < .001$ ). However, the association between increasing quartile of homocysteine level and risk of MI or stroke remained significant in analyses controlling for baseline multivitamin supplement use ( $P = .003$  for trend), and subgroup analyses limited to women who were ( $P = .02$  for trend) or were not ( $P = .04$  for trend) taking multivitamin supplements.

**Conclusions** Among healthy postmenopausal US women, elevated levels of homocysteine moderately increased the risk of future cardiovascular disease. Whether lowering the homocysteine level reduces risk of cardiovascular events requires testing in randomized controlled trials.

JAMA 1999;281:1817-1821

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toris.<sup>11</sup> Similarly, among men enrolled in the prospective Atherosclerosis Risk in Communities (ARIC) study, no association was found between homocysteine levels and subsequent coronary events.<sup>12</sup>

On the other hand, prospective studies from Norway<sup>13,14</sup> and Great Brit-

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ain<sup>15</sup> demonstrate positive associations with coronary heart disease, while other studies suggest positive associations for peripheral arterial disease<sup>16</sup> and stroke.<sup>17</sup> Furthermore, among women enrolled in the ARIC study, a positive univariate association was reported, however, after adjustment for other coronary risk factors, this effect was no longer statistically significant.<sup>12</sup> Thus, recent prospective data are less consistent than prior retrospective and cross-sectional data.

To provide further prospective data in women, we evaluated baseline levels of homocysteine among apparently healthy participants in the Women's Health Study (WHS), an ongoing primary prevention trial among 39 876 postmenopausal women with no history of cardiovascular disease or cancer,<sup>18</sup> and related these levels to the future risk of experiencing cardiovascular events.

## METHODS

We used a prospective, nested case-control study design among participants in the WHS, an ongoing, ran-

domized, double-blind, placebo-controlled trial of aspirin (100 mg on alternate days) and vitamin E (600 IU on alternate days) in the primary prevention of cardiovascular disease and cancer Cohort assembly for the WHS occurred between 1993 and 1995. Baseline blood samples were obtained in EDTA from 28 263 (71%) of WHS study participants and stored in liquid nitrogen until analysis.

In this study, cases were defined as WHS participants who provided an adequate baseline blood sample and subsequently experienced a cardiovascular event (defined as death due to coronary heart disease, nonfatal MI or stroke, percutaneous transluminal coronary angioplasty, or coronary artery bypass graft) during a 3-year follow-up period. The diagnosis of MI was made if symptoms met World Health Organization criteria and the event was associated with cardiac enzyme abnormalities or diagnostic electrocardiogram changes. The diagnosis of stroke was made if the patient had a new neurologic deficit lasting more than 24 hours; computed tomography or magnetic reso-

nance imaging scans were available in the majority of cases. Reported percutaneous transluminal coronary angioplasty and coronary artery bypass graft procedures were confirmed by hospital records. Coronary heart disease deaths were confirmed by autopsy reports, death certificates, and circumstances of death. All end points were adjudicated by a committee of cardiologists and neurologists.

For each case subject who had a confirmed cardiovascular event during follow-up, 2 control subjects of the same age ( $\pm 1$  year) and smoking status (past, current, or never) were selected from the remaining WHS participants who also provided baseline blood samples and who reportedly remained free of cardiovascular disease during the 3-year follow-up. Using these matching criteria, 122 cases and 244 controls were selected and form the basis for these analyses.

Baseline plasma samples from each case and control subject were thawed and assayed for total plasma homocysteine level using the IMx homocysteine assay (Abbott Laboratories, Abbott Park, Ill).<sup>19,20</sup> The assay coefficient of variation ranges from 3.7% to 5.3% when measuring samples between 5 and 25  $\mu\text{mol/L}$ . All investigators and laboratory personnel were blinded to the subjects' clinical details. Case and control specimens were handled identically and in blinded fashion throughout the blood collection, storage, retrieval, and analysis process. Samples were analyzed in triplets, with the position of the cases varied at random within triplets to avoid systematic bias and interassay variability.

Means and proportions for baseline cardiovascular risk factors were calculated for cases and controls. The significance of differences in means between the case and control groups was tested using the *t* test, while the significance of differences in proportions was tested using the  $\chi^2$  statistic. Tests for trends were used to assess for evidence of a linear relationship between increasing level of homocysteine and the risk of future coronary events af-

**Table 1.** Baseline Clinical Characteristics of Cases and Controls

	Cases	Controls	P Value*
No of subjects	122	244	
Age, mean, y	59.3	59.3	
Smoking status, %			
Past	29.5	29.5	
Current	27.9	27.9	
Never	42.6	42.6	
Hyperlipidemia, %	45.9	28.3	.001
Hypertension, %	55.5	31.3	.001
Family history of premature myocardial infarction, %	21.3	12.7	.04
Diabetes, %	9.8	2.1	.001
Body mass index, mean, kg/m <sup>2</sup>	27.1	26.0	.05
Alcohol use, %			
Rarely/never	45.1	46.7	.60
Monthly	14.8	13.9	
Weekly	27.9	31.2	
Daily	12.3	8.2	
Exercise frequency, %			
Rarely/never	44.3	44.7	.90
<Once per wk	21.3	20.1	
1-3 times per wk	27.9	27.1	
$\geq 4$ times per wk	6.6	8.2	
Current use of hormone replacement therapy, %	44.3	41.0	.50
Current multivitamin supplement use, %	40.0	31.3	.10

\*Ellipses indicate matching criteria.

ter dividing the sample into quartiles, defined by the distribution of the control values. Adjusted estimates of risk were obtained using logistic regression models that, in addition to accounting for the matching variables of age and smoking status, controlled for randomized treatment assignment, history of hyperlipidemia, history of hypertension, body mass index (weight in kilograms divided by the square of height in meters), exercise frequency (per week), history of diabetes, and parental history of MI before age 60 years. To compare our results with those of previous investigations, we further computed from these linear models the relative risk (RR) associated with each 5- $\mu\text{mol/L}$  increase in total plasma homocysteine. In addition, because some prior studies have suggested that a threshold effect exists for homocysteine, the data were also analyzed using prespecified cutoffs at the 50th, 75th, 90th, and 95th percentiles of homocysteine level among the control values. Additional analyses were performed after adjustment for baseline multivitamin supplement intake and in subgroups limited to women who did and did not report multivitamin supplement use at study entry. All *P* values are 2-tailed and all confidence intervals (CIs) were calculated at the 95% level.

## RESULTS

Due to matching, the distributions of age and smoking status were virtually identical in the case and control groups. As expected in a study of cardiovascular disease, cases were more likely to have a history of hyperlipidemia, obesity, hypertension, or diabetes or a family history of premature MI. There were no significant differences for self-reported levels of alcohol consumption, exercise frequency, hormone replacement therapy, or multivitamin supplement use at baseline (TABLE 1).

Of the 122 cases, there were 85 events of MI or stroke and 37 coronary revascularizations. Overall, study participants who subsequently experienced cardiovascular events (cases) had significantly higher baseline homocys-

teine levels than those who remained free of reported cardiovascular disease during follow-up (controls) (14.1 vs 12.4  $\mu\text{mol/L}$ ; *P* = .02). Similar effects were seen in subgroup analyses of those who did and did not report baseline use of multivitamin supplements (TABLE 2).

In age- and smoking status-matched analyses, the risk of any future cardiovascular event appeared to increase with increasing level of homocysteine such that the RRs for women in the lowest (referent) to highest quartiles of homocysteine level at baseline were 1.0, 1.1, 1.1, and 2.0 (*P* = .02 for trend). For the end points of MI or stroke, age- and smoking status-matched risks for individuals in the lowest (referent) to highest quartiles of homocysteine level were 1.0, 0.7, 1.6, and 2.2 (*P* = .004 for trend) (TABLE 3).

In continuous logistic regression analyses, which evaluated for evidence of a linear relationship, each increase in homocysteine concentration of 5  $\mu\text{mol/L}$  was associated with a 20% increase in risk of any cardiovascular event (*P* = .03). After adjustment for baseline differences in other coronary risk factors, each 5- $\mu\text{mol/L}$  increase in homocysteine was associated with a 24% increase in risk (*P* = .05).

To evaluate for evidence of nonlinear effects, we computed RRs across a series of prespecified cut points defined by the control distribution. As shown in TABLE 4, the age- and smoking status-matched risks of future cardiovascular events for those with baseline homocysteine levels at or higher than the 50th, 75th, 90th, and 95th percentiles (as defined by the control distribution) were 1.5 (*P* = .09), 1.9 (*P* = .007), 2.0 (*P* = .03), and 2.6 (*P* = .02), respectively. Risk estimates were minimally altered after controlling for baseline differences in body mass index, exercise frequency, hypertension, hyperlipidemia, diabetes, and family history of premature MI (Table 4). Controlling for hormone replacement therapy had no effect on these findings.

Thirty-four percent of the study participants reported use of multivitamin supplements at study entry, when blood samples were obtained. Overall, control women who reported taking multivitamin supplements had significantly lower mean levels of homocysteine compared with control women not taking multivitamin supplements (10.9 vs 13.1  $\mu\text{mol/L}$ , *P* < .001). Nonetheless, additional control for multivitamin supplement use had little effect on the association of baseline homocysteine levels with

**Table 2.** Baseline Levels of Total Plasma Homocysteine Among Cases and Controls

	Homocysteine Concentration, Baseline Mean (SD), $\mu\text{mol/L}$		<i>P</i> Value
	Cases	Controls	
All study participants	14.1 (8.0)	12.4 (5.8)	.02
Multivitamin supplement users	12.6 (4.2)	10.9 (3.9)	.01
Non-multivitamin supplement users	15.1 (9.7)	13.1 (6.4)	.08

**Table 3.** Relative Risk of Future Cardiovascular Events According to Baseline Concentration of Total Plasma Homocysteine\*

Homocysteine Level Quartile	Homocysteine Level, Range, $\mu\text{mol/L}$	Any Cardiovascular Event		Myocardial Infarction or Stroke	
		Crude RR (95% CI)	Adjusted RR (95% CI)	Crude RR (95% CI)	Adjusted RR (95% CI)
1	<9.54	1.0 (Referent)	1.0 (Referent)	1.0 (Referent)	1.0 (Referent)
2	9.54-11.19	1.1 (0.6-2.2)	1.1 (0.6-2.2)	0.7 (0.3-1.6)	0.7 (0.3-1.7)
3	11.20-13.26	1.1 (0.5-2.1)	1.2 (0.6-2.3)	1.6 (0.7-3.3)	1.7 (0.8-3.6)
4	>13.26	2.0 (1.1-3.8)	2.3 (1.2-4.3)	2.2 (1.1-4.6)	2.4 (1.2-5.0)

\*Data are shown for any cardiovascular event and for the subgroups of myocardial infarction or stroke. Relative risks (RRs) are shown in crude analyses and after adjustment for self-reported multivitamin supplement intake at study entry. CI indicates confidence interval.

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subsequent risk of cardiovascular disease (Table 3).

To further evaluate this issue, we performed a subgroup analysis limited to women taking multivitamin supplements at baseline (TABLE 5). In this subgroup, the overall distribution of homocysteine level was lower than in the cohort as a whole, and those with baseline levels of homocysteine at or higher than the 75th percentile of the reduced control distribution had a 2- to 3-fold increase in risk of any cardiovascular event compared with those with lower levels (RR, 2.6; 95% CI, 1.2-5.7,  $P = .02$ ). Furthermore, the RRs in this subgroup for any cardiovascular event increased from lowest (referent) to highest quartiles of baseline homocysteine concentration (RR, 1.0, 0.9, 1.6, and 3.1, respectively,  $P = .01$  for trend). Similar increases in risk were observed across quartiles of baseline homocysteine level in the subgroup of women who were not taking multivitamin supplements at study initiation ( $P = .04$  for trend) (Table 5).

**COMMENT**

In this prospective study of apparently healthy postmenopausal women, those with elevated baseline plasma concentrations of homocysteine had increased risk of future cardiovascular events, particularly at the highest levels of homocysteine. This association appeared to be independent of several cardiovascular risk factors. Furthermore, baseline multivitamin supplement use in this cohort was associated with significantly reduced homocysteine levels. However, in these data, the association between homocysteine level and cardiovascular risk remained significant in analyses controlling for baseline multivitamin supplement use, as well as in subgroup analyses limited to those women who were and were not taking multivitamin supplements at study entry.

Previous prospective studies of homocysteine level as a risk factor for coronary heart disease have been inconsistent, with both positive<sup>8,12,17</sup> and null<sup>6,7,9,12</sup> results reported. Furthermore, prior studies of homocyste-

ine level in healthy populations have predominantly been limited to men. Thus, the current data from postmenopausal women add to the accumulating evidence regarding homocysteine level as a marker for cardiovascular risk. Our finding that each 5- $\mu\text{mol/L}$  increase in homocysteine level was associated with a 20% increase in risk is consistent with estimates from prior retrospective<sup>3</sup> studies and some, but not all, prospective studies.<sup>15</sup> In addition, our data among apparently healthy women are consistent with subgroup analyses from the recent ARIC study, in which a positive association was found in univariate analyses for women but not for men.<sup>12</sup> Whether these observations indicate a chance effect or provide evidence of effect modification by sex will require future studies.

Our observation that multivitamin supplement use was associated with reduced homocysteine level is also consistent with prior studies demonstrating that folic acid intake decreases total plasma homocysteine level.<sup>21,22</sup> In these data, however, a statistically significant association between homocysteine concentration and cardiovascular risk was observed in the total cohort after adjustment for multivitamin supplement use, as well as in subgroup analyses stratified by multivitamin supplement use.

Potential limitations of these data merit consideration. Our study measured homocysteine levels only once, at study entry, and thus are susceptible to regression-dilution bias. Any such bias, however, would tend to lead to an underestimation of true effects and would

**Table 4.** Crude and Adjusted Relative Risk of Future Cardiovascular Events Among Subjects With Baseline Homocysteine Levels at or Higher Than the 50th, 75th, 90th, and 95th Percentiles of the Control Distribution\*

Percentile	Homocysteine Level, $\mu\text{mol/L}$	Any Event		Myocardial Infarction or Stroke	
		Crude RR (95% CI)	Adjusted RR (95% CI)	Crude RR (95% CI)	Adjusted RR (95% CI)
$\geq 50\text{th}$	11.19	1.5 (0.9-2.3)	1.2 (0.7-2.0)	2.3 (1.3-3.9)	1.9 (1.0-3.4)
$\geq 75\text{th}$	13.26	1.9 (1.2-3.1)	2.0 (1.1-3.4)	2.1 (1.2-3.5)	2.2 (1.2-4.0)
$\geq 90\text{th}$	17.29	2.0 (1.1-3.7)	1.7 (0.8-3.7)	2.3 (1.1-4.5)	1.9 (0.9-4.3)
$\geq 95\text{th}$	20.70	2.6 (1.1-5.7)	3.4 (1.3-8.8)	3.3 (1.4-7.7)	4.6 (1.7-12.3)

\*Crude analysis was matched on age and smoking status and controlled for randomized treatment assignment. Adjusted analysis was matched on age and smoking status and controlled for randomized treatment assignment, body mass index (weight in kilograms divided by the square of height in meters), hyperlipidemia, exercise frequency, hypertension, diabetes, and family history of premature myocardial infarction. RR indicates relative risk; CI, confidence interval.

**Table 5.** Relative Risk of Future Cardiovascular Events According to Baseline Concentration of Total Plasma Homocysteine\*

Homocysteine Level Quartile	Women Taking Multivitamin Supplements			Women Not Taking Multivitamin Supplements		
	Homocysteine Level, Range, $\mu\text{mol/L}$	Any Event, RR (95% CI)	MI or Stroke, RR (95% CI)	Homocysteine Level, Range, $\mu\text{mol/L}$	Any Event, RR (95% CI)	MI or Stroke, RR (95% CI)
1	<8.17	1.0 (Referent)	1.0 (Referent)	<9.94	1.0 (Referent)	1.0 (Referent)
2	8.17-10.30	0.9 (0.3-3.2)	0.9 (0.2-4.3)	9.94-11.67	1.2 (0.5-2.9)	1.2 (0.4-3.3)
3	10.31-11.64	1.6 (0.5-5.2)	1.3 (0.3-5.7)	11.68-13.95	1.0 (0.4-2.4)	1.7 (0.6-4.4)
4	>11.64	3.1 (1.1-9.1)	3.7 (1.1-13.4)	>13.95	1.9 (0.9-4.2)	2.4 (1.0-5.9)

\*Data are shown for the subgroups of women taking and not taking multivitamin supplements at study entry. MI indicates myocardial infarction; RR, relative risk; and CI, confidence interval.

not yield a false-positive result. Also, because the follow-up period for our study was relatively short (3 years), we cannot exclude the possibility that homocysteine levels are elevated because of the presence of subclinical atherosclerosis. This possibility has been raised in at least 1 prior prospective study of men in which homocysteine level was found to be a better predictor of short-term risk than long-term risk.<sup>8,9</sup> On the other hand, because the blood samples in our study were obtained prior to the onset of first cardiovascular events, we can exclude the possibility that the elevations observed in our data are a result rather than a cause of acute vascular occlusion. In addition, we did not ascertain allele status for the methylenetetrahydrofolate reductase gene in this population. However, a series of studies has reported that methylenetetrahydrofo-

late reductase status alone is a poor indicator of risk and that assessment of this polymorphism does not substantially add to the predictive value of homocysteine levels.<sup>12,23,25</sup> Moreover, as in any prospective epidemiological study, the potential for residual confounding cannot be excluded. In our analysis, however, we matched on smoking status and age and additionally controlled for body mass index, hyperlipidemia, exercise frequency, hypertension, diabetes, hormone replacement therapy, and family history of premature coronary artery disease. Thus, we believe the magnitude of any residual confounding is likely to be small.

In conclusion, in this prospective study, women with higher levels of homocysteine at baseline had higher risks of future cardiovascular disease. These risks, however, were modest in abso-

lute size and smaller than those observed in this cohort for other emerging cardiovascular risk factors, such as high-sensitivity C-reactive protein.<sup>26</sup> It is clear that folate supplementation reduces homocysteine level,<sup>21,22</sup> but whether folic acid reduces the risk of cardiovascular disease and, if so, whether such an effect is mediated by lowering homocysteine level remains a research question that must be tested in randomized controlled trials. We believe the current totality of evidence is insufficient on which to base rational clinical decisions for individual patients or for the general public in terms of any recommendation to screen for homocysteine level.

**Funding/Support:** This project was supported by grants from the National Heart, Lung, and Blood Institute, Bethesda, Md, and by an Established Investigator Award to Dr Ridker from the American Heart Association, Dallas, Tex.

#### REFERENCES

- McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of atherosclerosis. *Am J Pathol* 1969;56:111-128.
- Welch GN, Loscalzo J. Mechanisms of disease: homocysteine and atherothrombosis. *N Engl J Med* 1998;338:1042-1050.
- Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. *JAMA* 1995;274:1049-1057.
- Egerton W, Silberberg J, Crooks R, Ray C, Dudman N. Serial measures of plasma homocysteine after acute myocardial infarction. *Am J Cardiol* 1996;77:759-761.
- Lindgren A, Brattstrom L, Norrving B, Hultberg B, Anderson A, Johansson BB. Plasma homocysteine in the acute and convalescent phases after stroke. *Stroke* 1995;26:795-800.
- Evans RW, Shaten J, Hempel JD, Cutler JA, Kuller LH. Homocysteine and risk of cardiovascular disease in the Multiple Risk Factor Intervention Trial. *Arterioscler Thromb Vasc Biol* 1997;17:1947-1953.
- Alfthan G, Pekkanen J, Juhanen M, et al. Relation of serum homocysteine and lipoprotein(a) concentrations to atherosclerotic disease in a prospective Finnish population-based study. *Atherosclerosis* 1994;106:9-19.
- Stampfer MJ, Malinow MR, Willett WC, et al. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. *JAMA* 1992;268:877-881.
- Chasan-Taber L, Selhub J, Rosenberg IH, et al. A prospective study of folate and vitamin B6 and risk of myocardial infarction in US physicians. *J Am Coll Nutr* 1996;15:136-143.
- Verhoef P, Hennekens CH, Malinow MR, Kok FJ, Willett WC, Stampfer MJ. A prospective study of plasma homocyst(e)ine and risk of ischemic stroke. *Stroke* 1994;25:1924-1930.
- Verhoef P, Hennekens CH, Allen RH, Stabler SP, Willett WC, Stampfer MJ. Plasma total homocysteine and risk of angina pectoris with subsequent coronary artery bypass surgery. *Am J Cardiol* 1997;79:799-801.
- Folsom AR, Nieto FJ, McGovern PG, et al. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins. *Circulation* 1998;98:204-210.
- Arnesen E, Refsum H, Bonna KH, Ueland PM, Forde OH, Nordrehaug JE. Serum total homocysteine and coronary heart disease. *Int J Epidemiol* 1995;24:704-709.
- Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SM. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 1997;337:230-236.
- Wald NJ, Watt HC, Law MR, Weir DG, McPartlin J, Scott JM. Homocysteine and ischemic heart disease: results of a prospective study with implications regarding prevention. *Arch Intern Med* 1998;158:862-867.
- Taylor LM, LeFrang RD, Harris EJ, Porter JM. The association of elevated plasma homocysteine with progression of symptomatic peripheral arterial disease. *J Vasc Med Biol* 1991;3:128-136.
- Perry IJ, Refsum H, Normse RW, Ebrahim SB, Ueland PM, Shaper AG. Prospective study of serum total homocysteine concentrations and risk of stroke in middle aged British men. *Lancet* 1995;346:1395-1398.
- Buring JE, Hennekens CH, for the Women's Health Study Research Group. The Women's Health Study summary of the study design. *J Myocardial Ischemia* 1992;4:27-29.
- Shipchandler MT, Moore EG. Rapid, fully automated measurement of plasma homocyst(e)ine with the Abbott IMx analyzer. *Clin Chem* 1995;41:991-995.
- Alfheim A, Auerbekk A, Holets-McCormack S, Shih J. Development of an assay for homocysteine on an automated immunoassay analyzer [abstract]. *Clin Chem* 1998;44(suppl):A170.
- Malinow MR, Duell PB, Hess DL, et al. Reduction of plasma homocysteine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease. *N Engl J Med* 1998;338:1009-1015.
- Selhub J, Jacques PE, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* 1993;270:2693-2698.
- Verhoef P, Rimm EB, Hunter DJ, et al. A common mutation in the methylenetetrahydrofolate reductase gene and risk of coronary heart disease: results among US men. *J Am Coll Cardiol* 1998;32:353-359.
- Ma J, Stampfer MJ, Hennekens CH, et al. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. *Circulation* 1996;94:2410-2416.
- Brattstrom L, Wilcken DEL, Ohrvik J, Brudin L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. *Circulation* 1998;98:2520-2526.
- Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation* 1998;98:731-733.