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# Plasma Homocysteine as a Risk Factor for Vascular Disease

## The European Concerted Action Project

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**Context.**—Elevated plasma homocysteine is a known risk factor for atherosclerotic vascular disease, but the strength of the relationship and the interaction of plasma homocysteine with other risk factors are unclear.

**Objective.**—To establish the magnitude of the vascular disease risk associated with an increased plasma homocysteine level and to examine interaction effects between elevated plasma homocysteine level and conventional risk factors.

**Design.**—Case-control study.

**Setting.**—Nineteen centers in 9 European countries.

**Patients.**—A total of 750 cases of atherosclerotic vascular disease (cardiac, cerebral, and peripheral) and 800 controls of both sexes younger than 60 years.

**Measurements.**—Plasma total homocysteine was measured while subjects were fasting and after a standardized methionine-loading test, which involves the administration of 100 mg of methionine per kilogram and stresses the metabolic pathway responsible for the irreversible degradation of homocysteine. Plasma cobalamin, pyridoxal 5'-phosphate, red blood cell folate, serum cholesterol, smoking, and blood pressure were also measured.

**Results.**—The relative risk for vascular disease in the top fifth compared with the bottom four fifths of the control fasting total homocysteine distribution was 2.2 (95% confidence interval, 1.6-2.9). Methionine loading identified an additional 27% of at-risk cases. A dose-response effect was noted between total homocysteine level and risk. The risk was similar to and independent of that of other risk factors, but interaction effects were noted between homocysteine and these risk factors; for both sexes combined, an increased fasting homocysteine level showed a more than multiplicative effect on risk in smokers and in hypertensive subjects. Red blood cell folate, cobalamin, and pyridoxal phosphate, all of which modulate homocysteine metabolism, were inversely related to total homocysteine levels. Compared with nonusers of vitamin supplements, the small number of subjects taking such vitamins appeared to have a substantially lower risk of vascular disease, a proportion of which was attributable to lower plasma homocysteine levels.

**Conclusions.**—An increased plasma total homocysteine level confers an independent risk of vascular disease similar to that of smoking or hyperlipidemia. It powerfully increases the risk associated with smoking and hypertension. It is time to undertake randomized controlled trials of the effect of vitamins that reduce plasma homocysteine levels on vascular disease risk.

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ease.<sup>1,2</sup> The relationship has been reported for elevated plasma homocysteine levels measured with the subject fasting and after a methionine-loading test.<sup>2</sup> Despite the consistency of these reports, methodological problems make it difficult to be certain of the strength of the relationship and in particular of the independence of or interactions between elevated plasma homocysteine level and conventional risk factors.<sup>3</sup> Elucidation of these issues is an essential part of testing the hypothesis that the relationship between elevated plasma homocysteine level and vascular disease is causal.<sup>4,5</sup> The presence of interaction effects might modify current approaches to vascular disease prevention. In addition, few studies to date have examined the risk associated with elevated plasma homocysteine levels in women.<sup>6,8</sup>

Plasma homocysteine levels are determined by both genetic<sup>9-11</sup> and nutritional<sup>2</sup> factors. Of the latter, supplementation with folic acid in particular can reduce homocysteine levels.<sup>12-14</sup> This observation may have substantial public health implications.

With the use of the European Community Concerted Action structure,<sup>15</sup> a case-control study was designed with the following objectives: (1) to examine the relationships between plasma total homocysteine (tHcy) and vascular disease risk in male and female European subjects; (2) to study possible interaction effects between plasma tHcy level and conventional risk factors; and (3) to establish whether the blood levels of the nutrients that modulate plasma tHcy levels (red blood cell folate, cobalamin, and pyridoxal phosphate) relate to vascular disease. In this report, we concentrate on the first 2 objectives.

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CASE-CONTROL and prospective studies indicate that an elevated plasma homocysteine level is a powerful risk factor for atherosclerotic vascular dis-



Table 1.—Inclusion and Exclusion Criteria for Cases and Controls

Inclusion Criteria	
<b>Cases</b>	
Age ≤60 y at diagnosis (made within previous 12 mo)	
Coronary heart disease	
Clinical evidence of angina or myocardial infarction	
Plus: 2-fold or more rise in cardiac enzymes with evolutionary ST-T changes or pathological Q waves alone or angiographic evidence of ≥70% stenosis of a major coronary artery	
Cerebrovascular disease	
Clinical evidence of stroke or transient ischemic attack	
Plus: carotid stenosis ≥50% on Doppler or angiography or unequivocal atherosclerotic plaque on angiography or computed tomographic evidence of cerebral infarction without demonstrable source of embolism	
Peripheral vascular disease	
Clinical evidence of intermittent claudication or clearly diminished foot pulses	
Plus: obstruction of 1 major peripheral artery on angiography or Doppler ankle-arm index <0.9	
<b>Controls</b>	
Age ≤60 y	
Free of overt disease, namely, clinical or investigative evidence of vascular, renal, hepatic, or metabolic disease	
Exclusion Criteria	
Age >60 y	
Recent (≤3 mo) major systemic illness, including myocardial infarction	
Evidence of nonatherothrombotic vascular disease, namely, vasculitis, fibromuscular dysplasia, dissection, or migraine	
Diabetes mellitus	
Renal disease	
Hepatic disease	
Thyroid disease	
Cardiomyopathy	
Pregnancy	
Anticonvulsant medication	
Overt psychiatric illness	
Chronic alcohol abuse	
Exposure to nitrous oxide (≤3 mo)	

## SUBJECTS AND METHODS

### Patients

Seven hundred fifty vascular disease cases and 800 control subjects younger than 60 years, of both sexes, were enrolled from 19 centers in 9 European countries. Inclusion and exclusion criteria are listed in Table 1. Cases were required to have defined clinical and objective investigative evidence of vascular disease. To reduce biases caused by risk factor treatment, centers were asked to recruit new or recently diagnosed cases wherever possible. Sixty-nine percent were recruited within 1 year of diagnosis. Exclusion criteria for both cases and controls included nonatherosclerotic vascular disease, cardiomyopathy, pregnancy, recent (within 3 months) systemic illness, diabetes mellitus, renal, thyroid, or psychiatric illness, anticonvulsant therapy, and recent (within 3 months) exposure to nitrous oxide.

### Controls

Controls were free of overt disease. Community-based controls drawn from random population samples, family prac-

tice registers, and occupational registers were considered optimal, and centers were asked to recruit such subjects from a geographical background similar to that of cases wherever possible. This necessarily pragmatic approach yielded slightly less than one half of controls from random community samples and one third from employee health insurance registers, while one sixth were hospital employees. Two percent of controls were hospital patients. Controls from the 3 main sources were similar in terms of the major variables studied and plasma tHcy levels.

### Variables Examined

Demographic, historical, risk factor, and diagnostic data were recorded in standardized format.<sup>16</sup> Drug and vitamin usage were noted. Fasting serological measurements were made at least 3 months after acute systemic illness, such as myocardial infarction, which may alter homocysteine levels.<sup>13,17</sup>

An elevated plasma tHcy level may be present in the fasting state or may be unmasked by means of a methionine-loading test. Blood samples for tHcy estimation were taken with the subjects fasting and 6 hours after administration of methionine, 100 mg/kg, by a standardized method.<sup>16,18</sup> Estimations of tHcy were performed centrally in Bergen, Norway, by means of a method involving reduction with sodium borohydride, derivatization with monobromobimane, high-performance liquid chromatography separation, and fluorescence detection.<sup>19</sup> Cobalamin and folate levels were estimated by radioimmunoassay and pyridoxal phosphate levels by enzymatic photometry with high-performance liquid chromatography separation at Mimelab-AB, Soraker, Sweden.

Smoking status was determined at the time of vascular diagnosis (cases) or at methionine loading (controls). Blood pressure readings were taken in duplicate both before and after the methionine-loading test.

### Definitions of Variables for Risk Analysis

Plasma tHcy level was analyzed as a categorical variable. An elevated tHcy level was defined as one in the top fifth of the control distribution (fasting, ≥12 μmol/L; postload, ≥38 μmol/L). The increase in tHcy level (postload minus the fasting value) also had a cutoff point defined by the top fifth of the control distribution (≥27 μmol/L). Relative risks were estimated with those in the bottom four fifths of the distribution used as reference.

Because of the large number of treated hypertensive patients and the effect of treatment on actual levels, we also ana-

lyzed blood pressure as a categorical variable. Hypertension was deemed to be present if the mean of the 4 readings was 160 mm Hg or more (systolic) or 95 mm Hg or more (diastolic), or the subject was taking antihypertensive medication.

Smoking was analyzed as a continuous variable in terms of the current total amount of tobacco smoked expressed in equivalent number of cigarettes per day. Relative risks for smoking were based on a comparison between the risk for a smoker of exactly 20 cigarettes per day compared with a nonsmoker.

Serum cholesterol level was also analyzed as a continuous variable, and relative risks were based on a comparison of risks in subjects at the top and bottom quintiles of the cholesterol distribution in controls, 7.1 mmol/L (275 mg/dL) and 4.9 mmol/L (189 mg/dL), respectively.

Each case was categorized as one of coronary heart, cerebrovascular, or peripheral vascular disease. If a case had several diagnoses, the major manifestation was used.

### Statistical Methods

Logarithmic transformations and geometric means were used for variables showing a marked positive skew. Analysis of variance, least-squares regression, and *t* test and  $\chi^2$  test were used for initial data analysis with 2-tailed levels of significance.

Risk analyses were performed by means of conditional logistic regression for all vascular disease and for the 3 subcategories separately. All of the controls were used for each category, and all risk analyses were stratified by age group (<40, 40-49, and ≥50 years), sex where relevant, and the 19 centers. Odds ratios, which are described in this article as relative risks, together with their 95% confidence intervals (CIs), were derived from the regression coefficient with dummy variables used for the dose-response analysis.

Two risk factors interact with each other if the joint effect of the 2 factors in combination is different from that expected on the basis of their separate effects. Two models may be used to determine interaction—the additive and the multiplicative<sup>20</sup>—but without a great deal of knowledge about the processes that lead to disease, it is impossible to know which model is more appropriate. The multiplicative model tends to be the more commonly used because it is the model underlying logistic regression,<sup>21</sup> but there have been recent suggestions that cardiovascular risk factors may not act multiplicatively.<sup>22</sup>

For the calculation of the observed relative risk in those exposed to 2 factors, a multiplicative interaction term



Table 2.—Distribution of Major Variables in Cases and Controls\*

	Controls	Cases				P†
		All	CHD	CVA	PVD	
No.	800	750	383	211	156	...
Mean age, y	43.9	47.2	48.8	43.7	47.8	<.001
Sex, No. (%) male	570 (71.3)	544 (72.5)	322 (84.1)	112 (53.1)	110 (70.5)	.57
GMT fasting total homocysteine, $\mu\text{mol/L}$	9.73	11.25	11.16	11.11	11.67	<.001
No. (%) in top fifth	160 (20.0)	274 (36.5)	138 (36.0)	73 (34.6)	63 (40.4)	<.001
GMT postload total homocysteine, $\mu\text{mol/L}$	30.32	35.54	34.44	36.59	36.93	<.001
No. (%) in top fifth	160 (20.0)	285 (38.0)	135 (35.3)	87 (41.2)	63 (40.4)	<.001
GMT total homocysteine increase, $\mu\text{mol/L}‡$	20.3	23.6	22.9	24.8	24.3	<.001
No. (%) in top fifth	160 (20.0)	241 (32.1)	107 (27.9)	77 (36.5)	57 (36.5)	<.001
Mean serum cholesterol, mmol/L (mg/dL)	5.98 (231)	6.39 (247)	6.44 (249)	6.20 (240)	6.51 (252)	<.001
Mean LDL cholesterol, mmol/L (mg/dL)	4.05 (157)	4.42 (171)	4.50 (174)	4.25 (164)	4.45 (172)	<.001
Mean HDL cholesterol, mmol/L (mg/dL)	1.32 (51)	1.14 (44)	1.10 (43)	1.22 (47)	1.14 (44)	<.001
Mean HDL/total cholesterol	0.23	0.19	0.18	0.21	0.18	<.001
Mean serum triglycerides, mmol/L (mg/dL)	1.34 (119)	1.85 (164)	1.9 (168)	1.62 (143)	2.0 (177)	<.001
Mean systolic blood pressure, mm Hg	127.0	130.7	127.5	131.4	137.8	<.001
Mean diastolic blood pressure, mm Hg	81.5	82.2	81.4	82.7	83.7	.15
No. (%) with hypertension§	96 (12.0)	289 (38.5)	152 (39.7)	74 (35.1)	63 (40.4)	<.001
No. (%) with any current tobacco use§	267 (33.4)	408 (54.4)	179 (46.7)	109 (51.7)	120 (76.9)	<.001
Mean equivalent cigarettes/d in smokers	16.8	22.4	24.7	19.3	21.7	<.001
GMT red blood cell folate, nmol/L	840.46	811.68	845.67	767.95	778.82	.1
GMT cyanocobalamin, pmol/L	234.62	236.1	232.41	243.47	235.36	.80
GMT pyridoxal phosphate, nmol/L	31.11	26.32	26.92	27.52	22.73	<.001
No. (%) taking vitamins (pyridoxal/cyanocobalamin/folate)	44 (5.5)	14 (1.9)	5 (1.3)	5 (2.4)	4 (2.6)	<.001
Mean serum creatinine, $\mu\text{mol/L}$ (mg/dL)	68.9 (0.78)	70.7 (0.80)	73.4 (0.83)	68.9 (0.78)	68.1 (0.77)	.06

\*CHD indicates coronary heart disease; CVA, cerebrovascular disease; PVD, peripheral vascular disease; GMT, geometric mean titer; LDL, low-density lipoprotein; and HDL, high-density lipoprotein.

†Comparison of all vascular cases and controls.

‡Postload minus fasting total homocysteine.

§See text.

||One case missing.

was included in the logistic model even if it was statistically nonsignificant, thus avoiding the otherwise automatic acceptance of a multiplicative model. The expected joint relative risks, under both the multiplicative and additive models, were based on the individual relative risks for each factor on its own. Significance of departure from a particular model was based on hypothesis tests on the regression coefficients.<sup>23</sup> The interaction analysis employed a model that included either fasting or postload tHcy level, the 3 conventional risk factors (cholesterol, smoking, and blood pressure), and the 3 interactions between the risk factors and the particular tHcy level, with age, sex, and center as stratification variables.

The need to examine interaction effects between tHcy and the 3 conventional risk factors necessitated a larger sample than that of earlier studies. The chosen sample size was based on a particular quantification of an interaction between 2 continuous variables and is described elsewhere.<sup>16</sup>

## RESULTS

### Case-Control Differences

Cases were slightly older, had higher plasma tHcy, cholesterol, triglyceride, and systolic blood pressure levels, and

used more tobacco than controls ( $P < .001$ ). Serum cobalamin levels were similar in cases and controls. Red blood cell folate levels were nonsignificantly lower and serum pyridoxal phosphate levels significantly lower in cases than in controls (Table 2). Cases were significantly less likely than controls to be taking supplements containing these vitamins. Users of supplements containing folic acid, cobalamin, or pyridoxine had a relative risk of 0.38 (95% CI, 0.2-0.72) compared with nonusers (adjusted for conventional risk factors).

Geometric mean fasting tHcy levels were 16% (95% CI, 12%-20%) and postload tHcy levels 17% (95% CI, 13%-21%) higher in cases than in controls. Similar differences were noted in each vascular disease category. Arithmetic mean tHcy levels were 20% higher in cases for both fasting and postload values. The case-control differences were explained by a shift in the case distribution to the right with a more pronounced positive skew in cases (Figure 1). In controls, fasting tHcy levels were significantly and independently higher in older subjects and in men. Postload levels showed a similar association with age but not sex. Despite variation in tHcy levels between centers, no clear geographical trend was apparent, perhaps because of small num-

bers in some southern European centers. The increase in plasma tHcy level (postload minus fasting) was also significantly higher in cases than in controls (Table 2).

Relationships between fasting and postload total tHcy level were also examined. Although the measures were highly correlated (Figure 2), different persons with elevated tHcy levels were identified by each measure. A total of 30.1% (241/800) of controls had elevated tHcy levels, either fasting, postload, or both, compared with 50% (375/750) of cases; 13.5% (101/750) of all cases (26.9% [101/375] of cases with elevated tHcy levels) would not have been so classified if the postload estimation had not been available (Figure 3). An elevated increase in plasma tHcy level was defined as an increase (postload minus fasting) above the top quintile in controls. A further 18 controls (2.3%) and 13 cases (1.7%) with elevated increases who did not have elevated fasting or postload levels were identified by this additional measure.

### Vascular Disease Risk and Elevated Plasma tHcy Levels

In the univariate analysis using present definitions, both elevated fasting and postload tHcy levels were as strongly related to vascular disease and

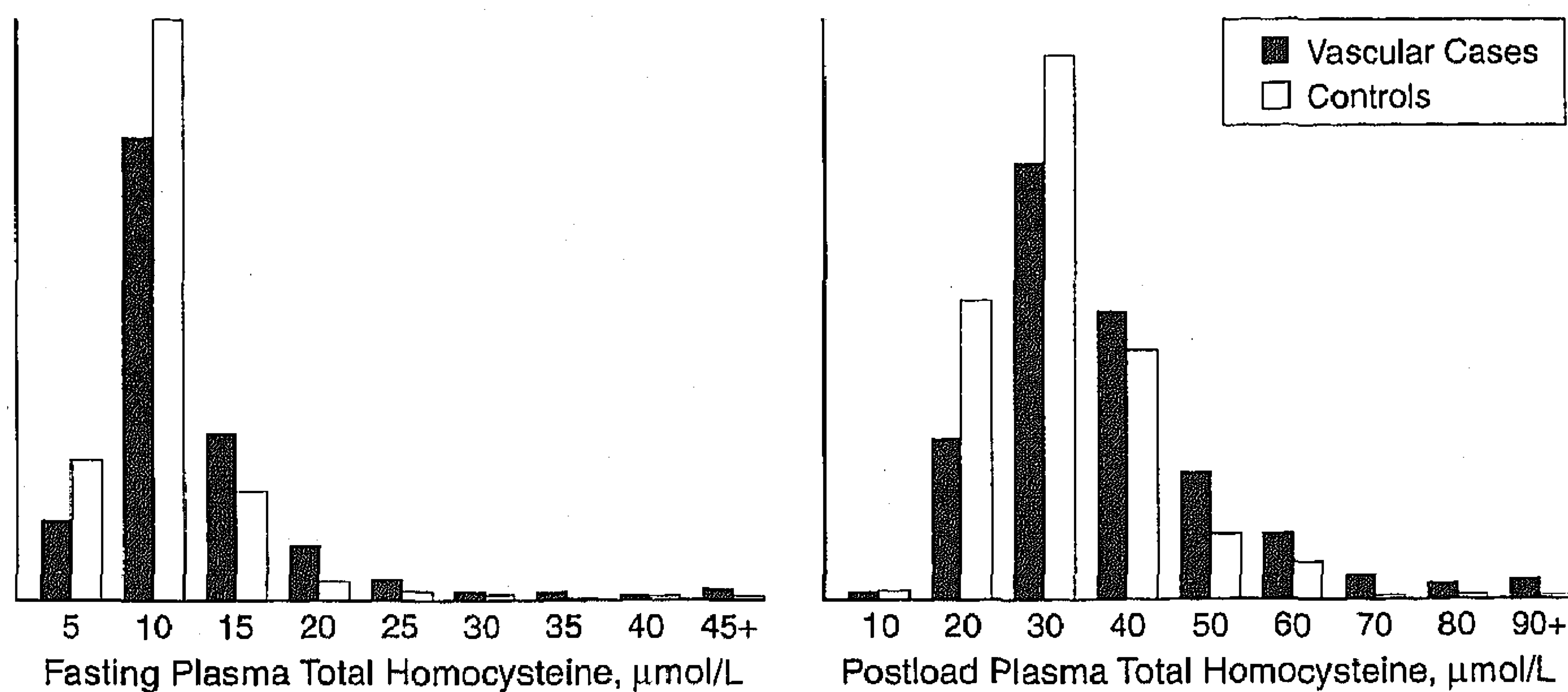


Figure 1.—Distribution of fasting and postload plasma total homocysteine levels in vascular disease cases (n=750) and controls (n=800).

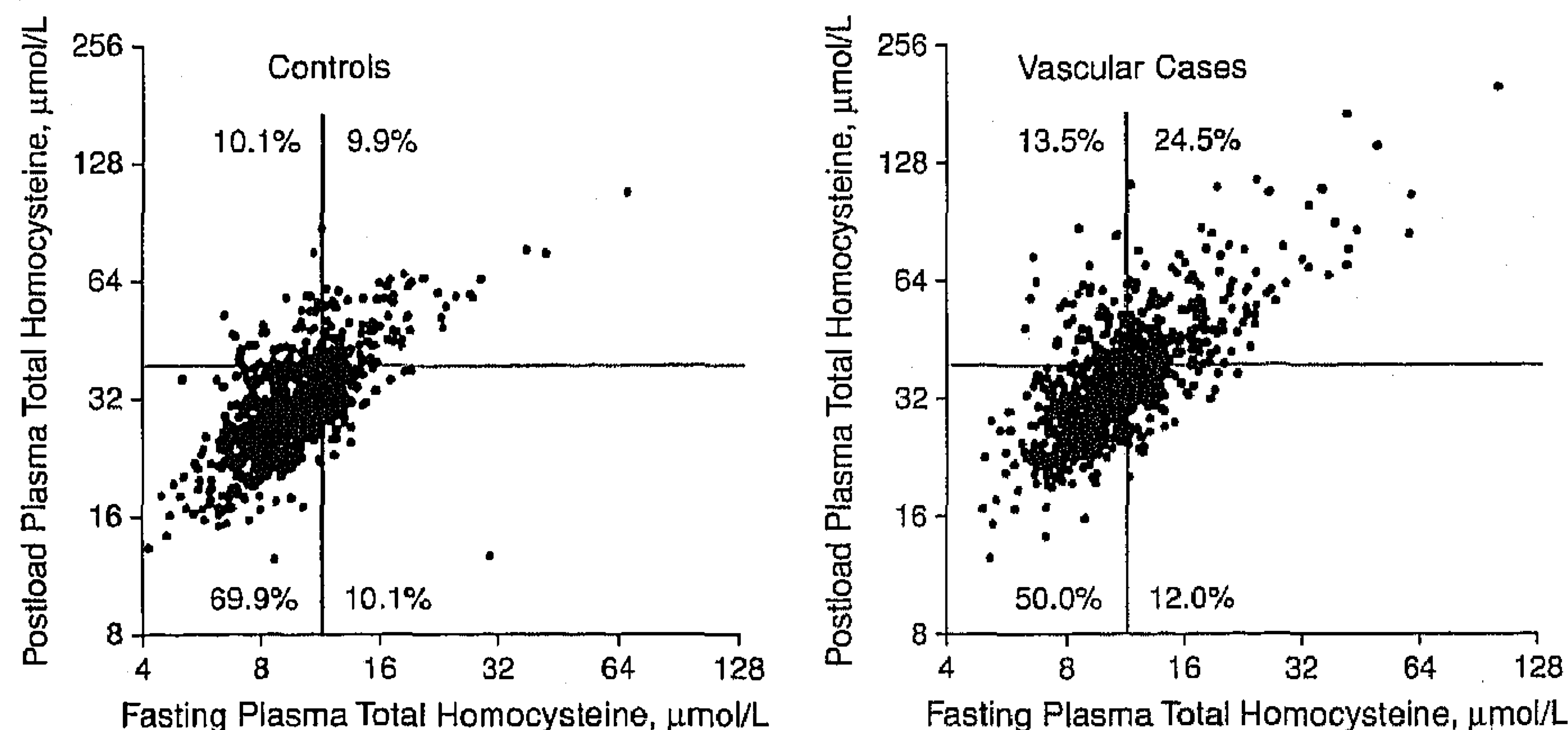


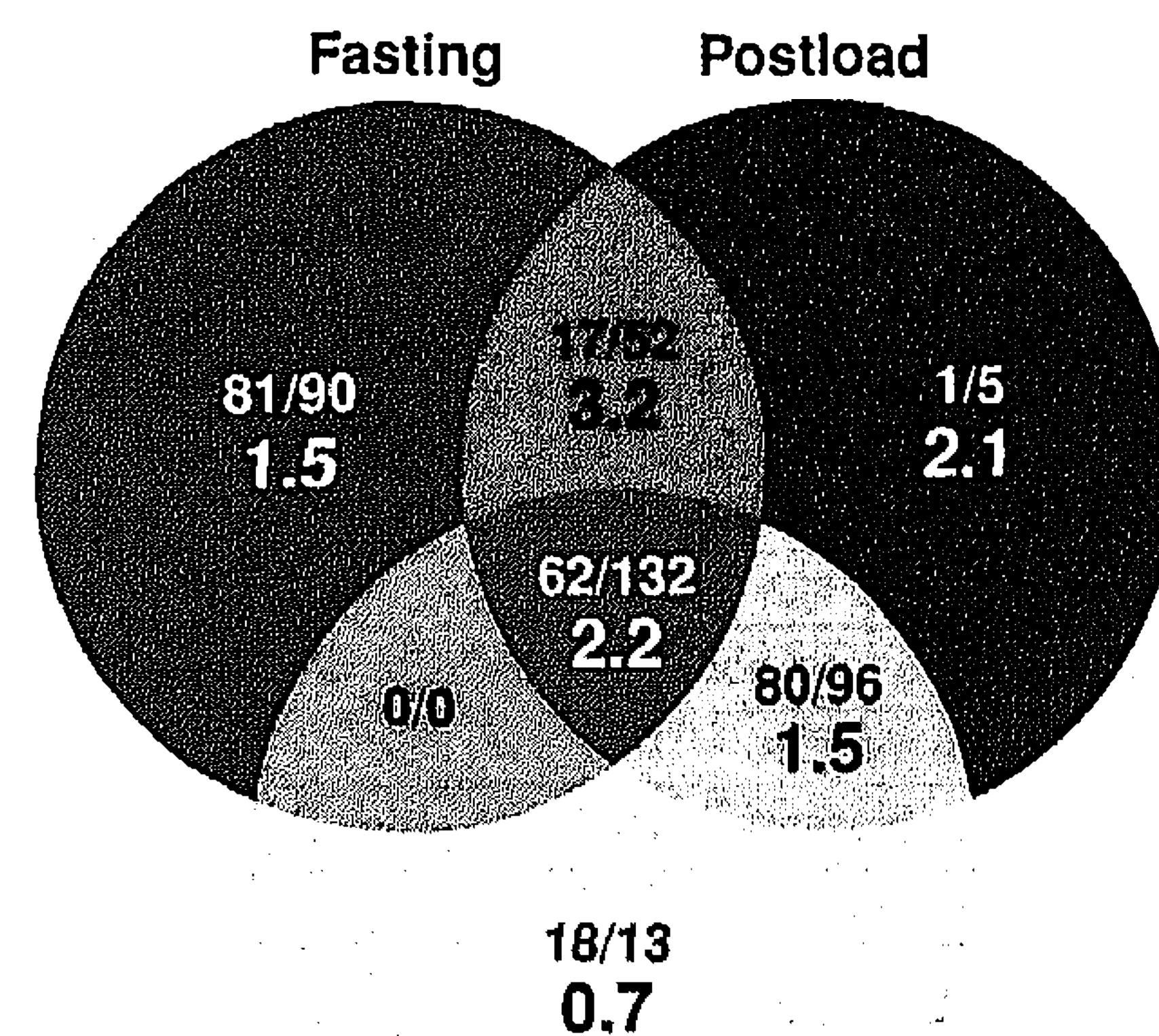
Figure 2.—The association between fasting and postload plasma total homocysteine levels in all vascular disease cases and controls. The reference lines are at the control quintile cutoff points used to define elevated values. The percentages in circles are the proportion of controls (or cases) with combinations of elevated fasting and postload homocysteine levels. For controls the regression equation was  $\log(\text{postload total homocysteine level}) = 1.82 + 0.70 \log(\text{fasting total homocysteine level})$ ;  $r = 0.69$ ;  $P < .001$ . For cases the equation was  $\log(\text{postload total homocysteine level}) = 1.98 + 0.66 \log(\text{fasting total homocysteine level})$ ;  $r = 0.70$ ;  $P < .001$ . Note: log refers to natural log.

its 3 categories as cholesterol level and smoking (Table 3). Postload risk was greater in women (especially those with coronary heart disease) than in men, but this was not statistically significant. Hypertension was insignificantly more strongly related to risk than elevated tHcy was. Examination of the increase (postload minus fasting level) in tHcy level tended to result in somewhat lower risk estimates (Table 3).

When adjusted for the presence of the conventional risk factors as well as age and center, the relative risks for both elevated fasting and postload tHcy levels were reduced only marginally and remained independent and strong predictors of vascular disease despite the presence of interaction effects (see below).

The simultaneous effect on vascular disease of an elevated fasting, an elevated postload, and an elevated increase

in tHcy levels was examined in a single logistic regression model (adjusting for the 3 classic risk factors). The results are presented in Figure 3. Although an elevated increase in tHcy level identified an extra 18 controls and 13 cases who did not have elevated fasting or postload levels, there was no independent effect on risk associated with an elevated increase in tHcy. On the other hand, an elevated postload level contributed independently to risk. For risk analyses, therefore, the effects of an elevated increase in tHcy level are not considered further. Fasting and postload elevations in tHcy had independent effects on risk and had a multiplicative effect when present together. The relative risk for a subject with an elevated fasting tHcy level only was 1.6 (95% CI, 1.0-2.2) and that for an elevated postload level only was 1.5 (95% CI, 1.0-2.2),



Increase (Postload - Fasting)

All Fasting, 1.9 All Postload, 1.8 All Increase, 1.4

Figure 3.—Numbers of subjects (controls/cases) with elevated fasting, elevated postload, and elevated increase of plasma total homocysteine level with associated relative risks (shown in large bold type) of vascular disease (adjusted for age, sex, center, and other risk factors). Relative risks for all subjects with elevated fasting, elevated postload, or elevated increase in total homocysteine level are shown separately (last line of type).

while for a subject with both elevated fasting and postload levels, the relative risk was 2.5 (95% CI, 1.7-3.5).

Figure 4 demonstrates the possibility of a dose-reponse effect between tHcy level and vascular disease risk. Risk begins to rise from the middle of the distribution, with the increase most apparent beyond the eighth decile (top quintile). For this reason, the top fifth of the control distribution was compared with the bottom four fifths in deriving relative risks. Comparing the top 10th with the bottom 10th, subjects had a relative risk of 3.1 (95% CI, 1.9-5.2) for fasting tHcy and 3.7 (95% CI, 2.2-6.1) for postload tHcy. When tHcy was considered as a continuous variable, the relative risk per 5- $\mu\text{mol/L}$  increment in fasting tHcy level was 1.35 (95% CI, 1.1-1.6) for men and 1.42 (95% CI, 0.99-2.05) for women.

### Interactions Between Plasma tHcy Level and Other Risk Factors

Figure 5 and Table 4 show the relative risks in various combinations of tHcy levels and the conventional risk factors. In men, elevated fasting tHcy level showed an additive effect with cholesterol level and greater than multiplicative interaction with smoking and blood pressure. Elevated postload tHcy level produced interaction effects that were greater than multiplicative with cholesterol level and greater than additive with smoking and blood pressure. Similar interaction effects were apparent in wom-



Table 3.—Relative Risks (95% Confidence Intervals) for Elevated Fasting, Elevated Postload, and Elevated Increase in Plasma Total Homocysteine and Conventional Risk Factors (Adjusted for Age and Center)\*

	Relative Risk (95% Confidence Interval)			
	All Vascular	CHD	CVD	PVD
<b>Men (univariate)</b>				
Elevated total homocysteine†				
Fasting	2.2 (1.6-2.9)	2.0 (1.4-2.8)	2.1 (1.3-3.5)	2.6 (1.5-4.5)
Postload	1.9 (1.4-2.5)	2.0 (1.4-2.9)	2.1 (1.3-3.6)	1.3 (0.8-2.3)
Increase	1.5 (1.1-2.0)	1.5 (1.0-2.1)	1.7 (1.0-2.9)	1.2 (0.7-2.1)
Hypercholesterolemia‡	1.4 (1.1-1.7)	1.4 (1.1-1.8)	1.5 (1.0-2.2)	1.5 (1.0-2.3)
Tobacco smoking§	2.2 (1.8-2.7)	1.8 (1.5-2.3)	1.9 (1.3-2.8)	4.4 (2.9-6.7)
Hypertension	3.9 (2.8-5.3)	3.6 (2.5-5.2)	4.8 (2.7-8.4)	3.8 (2.1-6.8)
<b>Female (univariate)</b>				
Elevated total homocysteine†				
Fasting	1.9 (1.1-3.2)	2.3 (1.1-4.8)	1.9 (1.0-3.8)	0.5 (0.2-1.9)
Postload	2.8 (1.7-4.5)	4.2 (1.9-8.7)	1.9 (1.0-3.7)	2.1 (0.9-5.1)
Increase	1.9 (1.2-3.1)	2.6 (1.3-5.5)	1.5 (0.8-2.8)	2.3 (1.0-5.5)
Hypercholesterolemia‡	1.6 (1.1-2.3)	1.4 (0.8-2.3)	1.5 (0.9-2.6)	3.3 (1.5-6.9)
Tobacco smoking§	4.0 (2.4-6.5)	2.6 (1.3-5.3)	3.5 (2.0-6.2)	5.6 (2.5-12.3)
Hypertension	8.5 (4.3-17.0)	10.4 (4.4-24.4)	6.9 (2.7-17.8)	14.1 (4.1-48.0)
<b>Both sexes (univariate)</b>				
Elevated total homocysteine†				
Fasting	2.1 (1.6-2.7)	2.1 (1.5-2.8)	2.0 (1.4-3.1)	2.0 (1.2-3.1)
Postload	2.1 (1.6-2.7)	2.3 (1.7-3.2)	2.1 (1.4-3.1)	1.5 (1.0-2.4)
Increase	1.6 (1.2-2.0)	1.7 (1.2-2.3)	1.6 (1.1-2.4)	1.4 (0.9-2.3)
<b>Both sexes (adjusted for conventional risk factors)</b>				
Elevated total homocysteine†				
Fasting	1.9 (1.4-2.4)	2.0 (1.4-2.8)	1.7 (1.1-2.7)	1.7 (1.0-2.9)
Postload	1.8 (1.4-2.4)	2.0 (1.4-2.8)	1.9 (1.2-2.9)	1.2 (0.7-2.1)
Increase	1.4 (1.1-1.9)	1.4 (1.0-2.0)	1.4 (0.9-2.2)	1.1 (0.6-1.9)

\*CHD indicates coronary heart disease; CVD, cerebrovascular disease; and PVD, peripheral vascular disease.

†For those above the top quintile compared with remainder.

‡Continuous variable: relative risk for individual with a cholesterol level of 7.1 mmol/L (275 mg/dL) compared with an individual with a cholesterol level of 4.9 mmol/L (189 mg/dL).

§Continuous variable: relative risk for smoker of 20 cigarettes per day (or equivalent) compared with a nonsmoker.

||Categorical variable: relative risk for individual with a systolic blood pressure of 160 mm Hg or more or diastolic blood pressure of 95 mm Hg or more or receiving antihypertensive treatment compared with remainder.

en and in many of the individual vascular disease categories.

## COMMENT

This study addresses the strength and possible independence of the relationship between plasma tHcy level and all categories of atherosclerotic vascular disease. The design and power of the study were sufficient to allow examination of interaction effects between tHcy level and conventional risk factors. These relationships may have considerable implications for public health.

In the present study, subjects with plasma tHcy levels in the top fifth of the control distribution, either fasting or after methionine loading, had a 2-fold increase in vascular disease risk compared with the remaining four fifths. This level of risk was equivalent to that of hypercholesterolemia or smoking and applied to all categories of vascular disease. It is lower than earlier estimates of risk because of the lower cutoff point chosen to define elevated tHcy level and for this reason applies to a much larger proportion of the population. The risk estimate was also inde-

pendent of the effect of other risk factors.

Fasting and postload tHcy levels were highly interrelated. The former may reflect cobalamin- and folate-dependent remethylation, and the latter, pyridoxal 5'-phosphate-dependent transsulfuration.<sup>24,25</sup> Reliance on fasting tHcy level alone will result in classifying 27% fewer patients as hyperhomocysteinemic. Boston and colleagues<sup>26</sup> reported that use of fasting tHcy level alone would have led to classification of more than 40% fewer subjects as being hyperhomocysteinemic.

The risk associated with elevated postload tHcy was higher in women, particularly those with coronary heart disease. While not statistically significant, this finding may be clinically relevant if confirmed in larger numbers of subjects. It is possible that the age cutoff point of 60 years selected particularly high-risk women. A similar effect might partially explain the high risk associated with hypertension in women.

The strong, independent association between elevated tHcy level and vascular disease risk is consistent with other

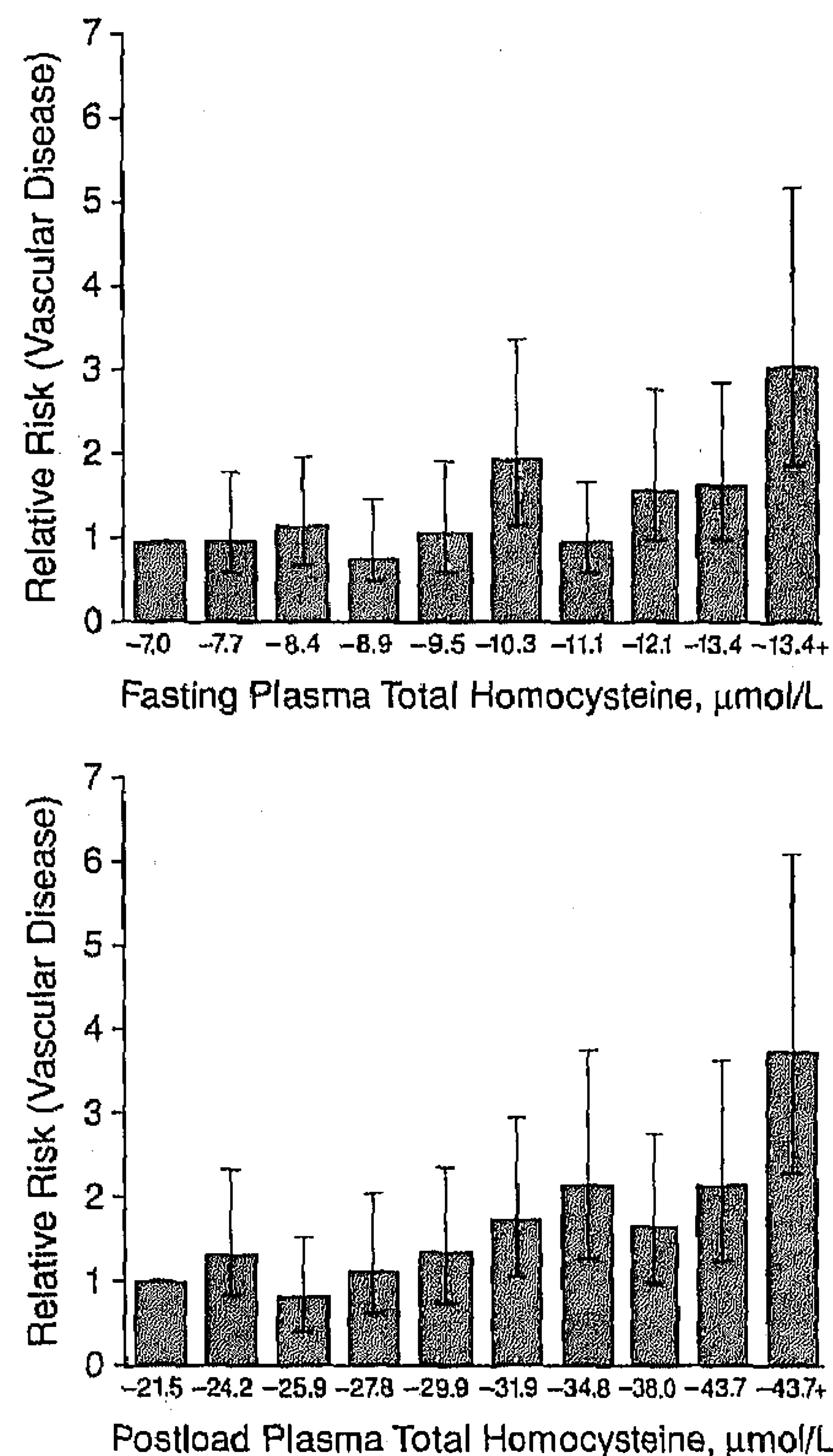


Figure 4.—Relative risks of vascular disease in groups defined by deciles (in controls) of fasting and postload plasma total homocysteine level with 95% confidence intervals (adjusted for age, sex, center, cholesterol, smoking, and blood pressure).

case-control<sup>1</sup> and most<sup>27-32</sup> but not all<sup>33</sup> prospective studies. Boushey et al<sup>1</sup> estimated that the odds ratio for coronary artery disease of a 5-µmol/L increment in plasma homocysteine level is 1.6 (95% CI, 1.4-1.7) for men and 1.8 (95% CI, 1.3-1.9) for women, equivalent to an increase in cholesterol level of 0.5 mmol/L (19 mg/dL). Corresponding risks in the present study are 1.3 (95% CI, 1.1-1.6) for men and 1.4 (95% CI, 1.0-2.0) for women.

If the top 10th of the tHcy distribution is compared with the bottom 10th, the corresponding relative risks were 3.1 for elevated fasting and 3.7 for elevated postload tHcy levels. Figure 4 suggests a dose-response effect, an observation supported by case-control<sup>34</sup> and prospective<sup>29-31</sup> data. Severe, genetic hyperhomocysteinemia, irrespective of the enzyme defect causing it, is almost invariably associated with premature and aggressive vascular disease.<sup>9</sup> Biologically plausible mechanisms of vascular damage have been suggested,<sup>1</sup> including effects on the endothelium, platelet function, coagulation factors, and lipoprotein oxidation. Many of these studies require confirmation, and some used nonphysiologically high doses of homocysteine.<sup>2</sup> While these observations strongly suggest a causal relationship be-



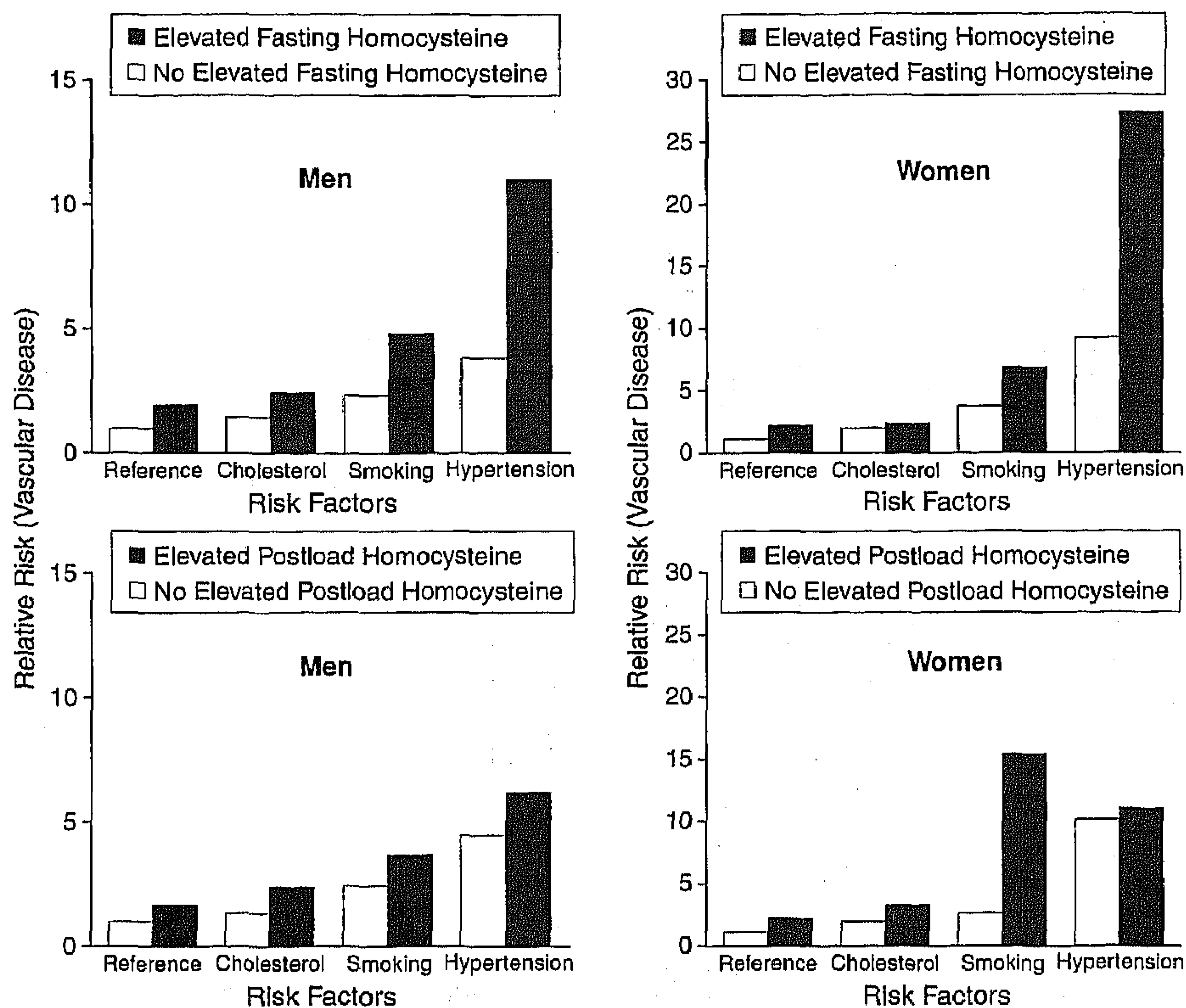


Figure 5.—Relative risks of vascular disease in groups defined by the presence or absence of classic risk factors and elevated plasma total homocysteine levels (adjusted for age, sex, and center; see text).

Table 4.—Observed and Expected Relative Risks of All Vascular Disease in All Subjects With Elevated Plasma Total Homocysteine and 1 Other Conventional Risk Factor\*

	Relative Risk		
	Observed	Expected	
		Multiplicative Model	Additive Model
Elevated fasting total homocysteine and Hypercholesterolemia	2.1	2.6	2.2
Smoking	4.6	4.5	3.3
Hypertension	11.3	8.2	5.4†
Elevated postload total homocysteine and Hypercholesterolemia	2.5	2.4	2.1
Smoking	5.1	4.4	3.2‡
Hypertension	7.8	9.1	5.9

\*Adjusted for sex, age, center, and the single and combined effects of the other risk factors and the relevant total homocysteine level.

† $P < .05$  comparing observed with expected.

‡ $P < .1$  comparing observed with expected.

tween elevated plasma homocysteine levels and vascular disease, randomized controlled trial evidence of benefit arising from homocysteine reduction is lacking.

Plasma homocysteine concentration relates inversely to blood levels of folate, cobalamin, and pyridoxine and to intakes of these vitamins.<sup>2,36</sup> The present study confirms this observation (data not shown). In this study, users of vitamin preparations containing these nutrients appear to experience substantial protection from vascular disease, with a relative risk of 0.38 (95% CI, 0.2-

0.72) compared with nonusers of vitamins. However, this observation applies to a small number of subjects who may have been more health conscious in other ways and cannot be taken as proof of benefit of the known homocysteine-lowering effect of these nutrients.

Interactions between plasma tHcy level and conventional risk factors may have implications for risk management and for our understanding of the causes of vascular disease. The joint effect of smoking and elevated plasma tHcy level was compatible with a multiplicative in-

teraction effect in both sexes combined. An even stronger interaction between elevated fasting tHcy level and hypertension was noted, especially in women. It is conceivable that tHcy may augment smoking-related platelet and clotting effects or exert a toxic effect on the endothelium, and these might be more relevant to the genesis of vascular disease than reported effects of homocysteine and lipoprotein oxidation.<sup>36,37</sup> Control of smoking and hypertension may be particularly important in subjects with elevated tHcy levels, and estimation of plasma tHcy should now be considered as part of total vascular disease risk assessment.

We conclude that an elevated plasma tHcy level is now established as a strong and independent factor associated with all categories of atherosclerotic disease in both men and women. An elevated plasma tHcy level interacts strongly with smoking and hypertension. It is known that folate supplementation reduces homocysteine levels both in the fasting state and after methionine loading,<sup>12-14,24</sup> and that pyridoxal 5'-phosphate can lower postmethionine homocysteine levels. Users of these vitamins have lower homocysteine levels than nonusers do,<sup>38</sup> and there is a suggestion of reduced risk in vitamin users in the present study. We believe it is time to consider whether existing recommended daily allowances of the vitamins that modulate homocysteine metabolism are adequate, and to undertake randomized controlled trials of the effects of folic acid and perhaps pyridoxine in the secondary prevention of cardiovascular disease.

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