Annu. Rev. Nutr. 1992. 12:279–98

HYPERHOMOCYST(E)INEMIA AS A RISK FACTOR FOR OCCLUSIVE VASCULAR DISEASE

Soo-Sang Kang, Paul W. K. Wong, and M. Rene Malinow

Department of Pediatrics, Rush Medical College and Presbyterian-St. Luke's Medical Center, Chicago, Illinois, 60612; Laboratory of Cardiovascular Diseases, Oregon Regional Primate Research Center, Beaverton, Oregon 97006

KEY WORDS: homocysteine, coronary artery disease, cerebrovascular disease, thromboembolism, arteriosclerosis, atherosclerosis

CONTENTS

Introduction

Homocysteine is metabolized by transsulfuration to cysteine via cystathionine or by remethylation to methionine. An excessive accumulation of plasma homocysteine and its derivatives is found in homocystinuria, which is caused by inborn metabolic defects in the transsulfuration or remethylation of homocysteine (18, 56). Patients with untreated homocystinuria develop premature occlusive vascular disease (18, 56). The major cause of morbidity and early mortality among these patients, irrespective of the simultaneous occur-

0199-9885/92/0715-0279$02.00

rence of hyper- or hypomethioninemia, is the development of thrombosis and thromboembolism, with resultant strokes, coronary occlusions, and other complications.

Pathological changes similar to those observed in arteriosclerosis have been described in homocystinuric patients (3, 43, 49), and the accumulation of homocyst(e)ine and its derivatives in tissue fluids appears to be the primary factor associated with pathological changes in the intimal cells (49, 56). Thus, it is important to determine whether or not a correlation exists between moderate hyperhomocyst(e)inemia and the development of arterial changes leading to vascular diseases in nonhomocystinuric subjects. Quantification of plasma cysteine-homocysteine disulfide after methionine loading was used initially to investigate this correlation. Subsequently, the determination of total homocyst(e)ine, including protein-bound homocysteine, introduced new insight into this area of research.

In this chapter we review the mechanisms that characterize moderate hyperhomocyst(e)inemia and the results of studies demonstrating the association of elevated plasma concentrations of homocyst(e)ine with the occurrence of occlusive vascular disease. We define the terminology used and describe the etiologies of various types of hyperhomocyst(e)inemias. The role of homocysteine as a risk factor in occlusive vascular disease is discussed based on three lines of evidence: premature thromboembolism and arteriosclerosis in severe hyperhomocyst(e)inemia; experimental production of arterial lesions in hyperhomocyst(e)inemic animals; and epidemiologic studies in patients with occlusive arterial diseases. In addition, homocysteine toxicity to endothelial cells is discussed.

Several reviews addressing the issue of moderate hyperhomocyst(e)inemia in occlusive vascular disease have been published within the last several years (6, 55, 56, 83, 85).

Total Homocyst(e)ine and Classification of Hyperhomocyst(e)inemia

In normal circulating plasma, most free homocysteine molecules are present as oxidized forms in the supernatant after acid precipitation. However, neither homocysteine nor homocystine is detectable in the plasma of nonhomocystinuric subjects by conventional amino acid analysis because concentrations are low. Sardharwalla et al (72) first observed the presence of cysteine-homocysteine disulfide after methionine loading in normal subjects and in subjects heterozygous for cystathionine β-synthase deficiency. Subsequently, the presence of cysteine-homocysteine disulfide without methionine loading was reported (28), but its quantification in nonhomocystinuric subjects is difficult owing to its low concentration.

In 1979, Kang et al (37) demonstrated that protein-bound homocysteine is

the major form of plasma homocyst(e)ine in nonhomocystinuric subjects and that unbound forms convert to protein-bound homocysteine during storage of plasma, even at –70°C. Refsum and co-workers (66) have confirmed these findings by using radioenzymatic methods. Hence, plasma homocysteine and its derivatives are currently classified into free and protein-bound homocysteine. The latter accounts for 70–85% of total homocyst(e)ine in normal individuals. Free homocyst(e)ine includes homocysteine, homocystine, and cysteine-homocysteine disulfide. In plasma, albumin is the major protein that binds homocysteine, mostly through disulfide bonds between homocysteine and protein molecules. However, there is evidence that some homocystine and cysteine-homocysteine disulfide molecules are bound noncovalently to proteins (37).

Since homocyst(e)ine is a normal constituent of tissues and tissue fluids, the condition of increased homocyst(e)ine concentration is designated as hyperhomocyst(e)inemia in this review. In addition, moderate, intermediate, and severe hyperhomocyst(e)inemia are defined as basal values for plasma homocyst(e)ine less than 30, between 31 and 100, and above 100 nmol/ml, respectively. All values are expressed as homocysteine; thus homocystine values are converted to homocysteine, and cysteine-homocysteine disulfide is treated as equivalent to homocysteine. Finally, the condition previously known as homocystinuria is called severe hyperhomocyst(e)inemia.

Homocysteine Metabolism and Etiology of Hyperhomocyst(e)inemia

Homocysteine is formed by the cleavage of S-adenosylhomocysteine, which is produced from the versatile methyl donor S-adenosylmethionine. Normal rat liver contains more than 50 nmol per gram of wet tissue of S-adenosylhomocysteine and an approximately similar amount of S-adenosylmethionine (2, 71). In contrast, the concentration of homocysteine is usually less than one-tenth of S-adenosylmethionine or S-adenosylhomocysteine (i.e. < 5 μM). Homocysteine is readily transsulfurated to cysteine via cystathionine or is remethylated to methionine. In humans on a normal diet, approximately 50% of the available homocysteine is remethylated (57). The amount of methionine intake influences homocysteine synthesis and controls the ratio between transsulfuration and remethylation of homocysteine. The methyl group of methionine in humans on high protein diets disappears twice as fast as when they are on normal diets; and 70% of homocysteine is converted to cystathionine (21, 22). In contrast, only 10% of homocysteine is transsulfurated to cysteine in humans on low protein diets (21, 22).

The remethylation of homocysteine is carried out either by betaine-homocysteine methyltransferase or 5-methyltetrahydrofolate-homocysteine

methyltransferase (methionine synthase). The former is found mainly in the liver, whereas the latter is distributed ubiquitously in all tissues. In humans, the rates of homocysteine remethylation by each of these two enzymes are approximately equal. S-adenosylmethionine and methionine are important modulators for the transsulfuration and remethylation of homocysteine. S-adenosylmethionine inhibits the activities of both betaine-homocysteine methyltransferase and methylenetetrahydrofolate reductase; it also activates cystathionine β-synthase (21, 22). Excess methionine intake increases the concentration of S-adenosylmethionine and also inhibits the activity of methionine synthase. Therefore one can speculate that methionine loading may help to evaluate the pathway of transsulfuration but may hinder the evaluation of homocysteine remethylation.

The interruption of transsulfuration or remethylation of homocysteine produces hyperhomocyst(e)inemia. Genetic defects, inadequate availability of cofactors or substrates, or other mechanisms can interfere with both processes. Severe hyperhomocyst(e)inemia, with an incidence of 1:200,000, is found in individuals that are homozygous for cystathionine β-synthase deficiency. Although some individuals that are heterozygous for cystathionine β-synthase deficiency have moderate hyperhomocyst(e)inemia, approximately 30–50% have normal plasma homocyst(e)ine concentrations (60, 73). Since pyridoxal 5'-phosphate is a coenzyme of cystathionine β-synthase, nutritional deficiency of pyridoxine may also cause hyperhomocyst(e)inemia. Hyperhomocyst(e)inemia has been demonstrated in experimental animals fed diets deficient in pyridoxine (75).

A severe genetic defect of methylenetetrahydrofolate reductase has been documented (18, 59). 5-Methyltetrahydrofolate and homocysteine are substrates for methionine synthesis, and the lack of 5-methyltetrahydrofolate produces severe hyperhomocyst(e)inemia. Homozygotes for a new type of mutation, thermolabile methylenetetrahydrofolate reductase, have recently been found in 5% of the general population (38, 41). Subjects with thermolabile methylenetetrahydrofolate reductase have a 50% reduction in enzyme activity and a tendency toward moderate hyperhomocyst(e)inemia. Some of these subjects exhibit intermediate hyperhomocyst(e)inemia resulting from compound heterozygosity of severe and thermolabile mutations (38).

A deficiency of 5-methyltetrahydrofolate is expected to result from any impairment of folate metabolism. Moderate and intermediate hyperhomocyst(e)inemia is most commonly seen in subjects with low concentrations of serum folate that are due to either inadequate nutrition or to certain medications (40, 66). Plasma homocyst(e)ine greater than 2 SD above the normal mean was observed in 61% of subjects with subnormal serum folate; 19% of those had intermediate hyperhomocyst(e)inemia. Moreover, 53% of the subjects with low normal serum folate (between 2.1 and 4.0 ng/ml) showed moderate hyperhomocyst(e)inemia (40).

Impaired methionine synthase activity is also caused by a deficiency of the cofactor methylcobalamin. Known genetic defects include abnormalities in intestinal absorption of cobalamin, transcobalamin II, cbl C, D, and cbl E, F, G (14, 19). Kang et al (39) have demonstrated the presence of intermediate hyperhomocyst(e)inemia in subjects with subnormal serum levels of B_{12}. A negative correlation is found between serum cobalamin levels and plasma total homocyst(e)ine. Elevation of homocyst(e)ine as a result of cobalamin and/or folate deficiency has been also reported by Brattström et al (8) and Stabler et al (77).

Normal plasma homocyst(e)ine has been observed in pregnant women who had subnormal folate and/or cobalamin levels (39), which suggests that increased betaine-homocysteine methyltransferase activity prevents the occurrence of hyperhomocyst(e)inemia. Plasma homocyst(e)ine levels in pregnant women are less than 60% of the normal mean (42), and this reduction appears to be associated with increased cortisol level (S.-S. Kang, unpublished results). Cortisol is a potent activator of betaine-homocysteine methyltransferase (20). Reduced homocyst(e)ine levels in premenopausal women have previously been interpreted as a result of the direct interaction of homocysteine metabolism with estrogen (7, 83). However, it is plausible that augmented betaine-homocysteine methyltransferase activity is responsible for maintaining low levels of plasma homocyst(e)ine in premenopausal women, as well as during pregnancy.

Hyperhomocyst(e)inemia may occur also through an interference of betaine-homocysteine methyltransferase. Although a genetic defect of this enzyme has not been reported, rats fed choline-deficient diets for 10 days exhibit increased homocyst(e)ine concentration in the spleen (80). The severity of hyperhomocyst(e)inemia that is due to folate deficiency is amplified by the concomitant restriction of choline in the diet (S.-S. Kang, unpublished data). Betaine supplementation effectively corrects most types of hyperhomocyst(e)inemia, irrespective of their etiology (76). Hyperhomocyst(e)inemia that is due to other diseases such as renal failure and certain medications has been discussed in the comprehensive review by Ueland & Refsum (83).

In summary, hyperhomocyst(e)inemia is caused by genetic and/or nutritional defects. Since plasma homocyst(e)ine levels are controlled by various genetic and nongenetic factors, the evaluation of moderate and intermediate hyperhomocyst(e)inemia requires a thorough assessment of parameters involved in homocysteine metabolism. Hence, identification of a genetic defect(s) plays an important role in detecting those subjects susceptible to the development of hyperhomocyst(e)inemia.

Vascular Disease in Severe Hyperhomocyst(e)inemia

Gibson et al (26) published the first pathological description of children with homocystinuria that was due to cystathionine β-synthase deficiency. Subse-

quent reports by other investigators confirmed the pathological findings (9, 10). Thromboembolism is a frequent cause of death. Thrombosis is observed in arteries of various sizes. The consequences of thrombosis depend on the size and locations of the arteries affected; infarctions may result. Venous thrombosis is also common and may occur in the vena cava, cerebral sinuses, and/or peripheral veins. Emboli from these thrombi may be found in various organs. Some thrombi may be organized and recanalized.

The arterial walls may be dilated, stretched thin, or thickened; and the arterial lumen may be narrowed. Aneurysms may also occur. Intimal hyperplasia and fibrosis are evident. The arteries are often affected in a patchy fashion. The internal elastic lamina is prominent and often thick or frayed. The most obvious pathological change is intimal fibrosis, which may be concentric, resulting in ridges and causing severe narrowing, or eccentric in the form of pads. In these intimal pads, layers of elastic fibrils may have split off from the main elastic lamina. Areas of atheromatous plaques may be found. Medial hyperplasia is often found. The muscle fibers may be enlarged and separated from each other. Deposits of interstitial material that have the staining properties of collagen are found. The external elastic lamina is often swollen and frayed. Generally, the adventitia is little affected.

Kanwar et al (43) were the first to provide a detailed description of the pathological findings in a 10-year-old child with methylenetetrahydrofolate reductase deficiency. A later study was reported by Baumgartner et al (3). The pathological changes are very similar to those observed in cystathionine β-synthase deficiency.

Pathologic findings in patients with inherited metabolic defects of cobalamin have been reported by McCully (49, 51) and Baumgartner et al (3). The vascular lesions are very similar to those observed in cystathionine β-synthase deficiency and methylenetetrahydrofolate reductase deficiency. McCully (49) implicated homocysteine as the etiologic factor for the premature vascular disease in these patients. He also suggested that hyperhomocyst(e)inemia might be a factor in the pathogenesis of arteriosclerosis (49, 51).

Experimental Hyperhomocyst(e)inemia and Vascular Damage

Early experiments were conducted by subcutaneous or oral administration of *dl*-homocysteine thiolactone in rabbits for 7 to 12 weeks (50). Although arteriosclerotic plaques were reported in the animals, these results should not be interpreted as the direct action of homocyst(e)ine. Exogenous homocysteine is readily metabolized or excreted through the kidney in a short period of time. Thus, this model would be unable to maintain an abnormal plasma homocysteine level.

Harker et al (31) used a continuous infusion method in baboons for three months. The ensuing endothelial desquamation and early proliferative lesions

observed in certain arteries and the shortening of platelet survival supported the "reaction to injury" hypothesis of atherogenesis (31, 32). The arterial lesions were prevented by the antiplatelet agent dipyridamole, suggesting that platelets mediated intimal proliferation of smooth muscle cells (31). Reddy & Wilcken (64) found no arterial changes in young pigs infused with *dl*-homocysteine thiolactone over a two-month period. Because patients with severe hyperhomocyst(e)inemia usually exhibit 0.2 mM *l*-homocystine levels, the finding of less than 0.04 mM concentration of *l*-homocystine in their study was probably too low to produce vascular damage within two months. Species difference may also account for the discrepancy.

Hyperhomocyst(e)inemia has been induced in several species of animals fed diets deficient in folate (36), pyridoxine (75), or choline (80). Hence, the vascular pathology found in animals fed diets deficient in one of these nutrients may be interpreted as the result of nutritional deficiency or hyperhomocyst(e)inemia.

Prior to the discovery of hyperhomocyst(e)inemia, several investigators studied the development of arterial lesions in animals fed diets deficient in choline, pyridoxine, or folate. Hartcroft et al (33) observed pathological changes resembling those of atheroma in the major arterial trunks and coronary arteries of rats maintained up to seven months on diets low in choline. The initial lesions consisted of microscopic deposits of lipid in the endothelial cells. In later stages, proliferation of intimal cells took place and they appeared as plaques. In rhesus monkeys, pyridoxine deficiency caused lesions resembling arteriosclerosis in humans; similar lesions were also produced by feeding monkeys a "suboptimal" pyridoxine diet (67). Recently, hyperhomocyst(e)inemia and arteriosclerotic changes were observed in rats fed folic acid–deficient diets (88).

In summary, certain nutritional hyperhomocyst(e)inemias may be associated with the development of arteriosclerotic plaques, irrespective of which nutrient is involved. Hyperhomocyst(e)inemia is most likely the common denominator, although the contribution of the nutritional deficiency to the vascular pathology can not be excluded in these models. Since betaine supplement largely corrects any type of hyperhomocyst(e)inemia, deficiency of pyridoxine, folate, or cobalamin without hyperhomocyst(e)inemia can be established by concomitant betaine supplementation. Whether hyperhomocyst(e)inemia is an indispensable condition or nutritional deficiency is sufficient for the production of vascular damage may be clarified in these models.

Moderate Hyperhomocyst(e)inemia and Occlusive Arterial Disease

Only within the last 15 years have abnormalities in the metabolism of homocysteine been investigated in adults with occlusive arterial disease.

Wilcken & Wilcken (86) first demonstrated that abnormally elevated plasma cysteine-homocysteine disulfide levels after methionine loading were three times more common in patients with coronary artery disease than in controls. Similar observations were made by many investigators using various methods. These studies are summarized in Tables 1 and 2.

Before 1986, all studies were conducted by the determination of unbound homocysteine. Because of its low concentration in plasma, most studies during this period were performed after methionine loading. Brattström et al (5) reported, for the first time, comparisons of pre- and post-methionine loading plasma cysteine-homocysteine disulfide levels in patients with cerebrovascular disease. An approximately threefold greater level of plasma cysteine-homocysteine disulfide was noted in the patient group, both before and after methionine loading. Murphy-Chutorian et al (60) reported a similar positive association between plasma homocyst(e)ine values and coronary artery disease. In contrast, an elevated cysteine-homocysteine disulfide level was found by Boers et al (4) in patients with occlusive peripheral artery and cerebrovascular diseases but not in patients with myocardial infarctions. A recent report by Clarke et al (13) demonstrated abnormally high plasma cysteine-homocysteine disulfide and homocystine values after methionine loading in 30–40% of patients with cerebrovascular, peripheral, or coronary artery diseases.

After the discovery of protein-bound homocysteine, most investigators determined plasma total homocyst(e)ine without methionine loading as shown in Tables 1 and 2. Methionine loading does not increase the ratio of the patient homocysteine value to the control value. This is probably due to the fact that the inhibition of homocysteine remethylation is balanced by the activation of cystathione β-synthase following methionine loading. Although values of plasma total homocyst(e)ine are slightly different depending on the methods used, these studies arrived at the same conclusion (Table 1 and 2). In coronary artery disease, the ratio for mean homocyst(e)ine of patients to controls varied from 1.2 to 1.3, whereas in peripheral and cerebrovascular disease the ratio ranged from 1.5 to 1.8 (Table 1). The ratio of the incidence of hyperhomocyst(e)inemia in patients versus controls ranged from 2.6 to 5.3 in coronary artery disease and from 2.2 to 9 in cerebrovascular disease (Table 2). These results suggest a more pronounced correlation of hyperhomocyst(e)inemia with peripheral and cerebrovascular arterial disease than with coronary artery disease. When all available data on plasma total homocyst(e)ine from various investigators were taken together, hyperhomocyst(e)inemia was found in 41.8% of patients with peripheral and cerebrovascular arterial diseases and in 11.9% of those with coronary artery disease (44).

Since Sardharwalla et al (72) first observed elevated concentrations of plasma cysteine-homocysteine disulfide in subjects heterozygous for cys-

tathionine β-synthase deficiency, investigators have speculated that hyperhomocyst(e)inemia found in patients with vascular diseases is exclusively due to heterozygosity for cystathionine β-synthase deficiency. Moreover, Boers et al (4) reported that 13 out of 14 patients with abnormal accumulation of cysteine-homocysteine disulfide had decreased enzyme activity that was comparable to the range of activity found in individuals that were obligate heterozygotes for cystathionine β-synthase deficiency. This proposition has been strengthened by the recent observations of Clarke et al (13), who confirmed the presence of cystathionine β-synthase deficiency in 18 of 23 hyperhomocyst(e)inemic patients with vascular disease. However, defining heterozygosity by enzyme activity determination is not a simple matter in many genetic disorders (38, 52, 73). It has been well established that several genetic and nongenetic factors, singly or in combination, may control plasma homocyst(e)ine concentration.

One significant challenge is posed by the results from a survey of families with cystathionine β-synthase deficiency. Mudd et al (58) found no increase of vascular disease in individuals heterozygous for cystathionine β-synthase deficiency. Since there is considerable overlap of homocyst(e)ine values between these obligate heterozygotes and controls, one can speculate that a positive correlation is confined to hyperhomocyst(e)inemic heterozygotes for cystathionine β-synthase deficiency.

Note that the distribution pattern of plasma homocyst(e)ine values in patients with coronary artery disease is similar to that of controls, except for the values above the 95th percentile (39, 87). This pattern is consistent with a multifactorial disorder. Reed et al (65) found a significantly greater intraclass correlation coefficient of homocyst(e)ine levels in monozygotic twins than in dizygotic twins, which suggests that the level of plasma homocyst(e)ine is influenced by genetic factor(s). A strong family correlation in homocyst(e)ine values was also observed by Williams et al (87) and Genest et al (24). High homocyst(e)ine values may be the result of a combination of a major genetic defect and other minor genetic and nongenetic factors.

Evidence indicates that moderately elevated plasma homocyst(e)ine level is an independent risk factor in the development of occlusive arterial disease (13, 24, 60). Other major risk factors including smoking, hypertension, diabetes mellitus, hyperlipidemia, and elevated levels of fibrinogen or apolipoprotein AI or B showed no positive correlation with hyperhomocyst(e)inemia in patients with vascular disease. However, Swift & Shultz (81) reported a higher plasma homocyst(e)ine value in a high risk group of patients than in a low risk group. Genest et al (23) also found that patients with four or more risk factors had higher homocyst(e)ine levels than patients with two risk factors.

The association of hyperhomocyst(e)inemia and coronary heart disease was

Table 1 Mean homocysteine levels (nmol/ml) in human plasma or serum of patients with vascular disease and controls[a]

Disease studied	Homocysteine determined as	Basal levels			Postmethionine loading levels			Refs.
		Controls	Patients	Ratio	Controls	Patients	Ratio	
Coronary artery disease	Cysteine-homocysteine disulfide				6 ± 2	18 ± 8	3.0	86
Arteriosclerotic cerebrovascular disease	Cysteine-homocysteine disulfide	3.5 ± 0.2	5.1 ± 0.6	1.5	11.9 ± 1.0	16.0 ± 3.6	1.3	5
Premature occlusive artery disease (male)	Homocystine plus cysteine-homocysteine disulfide	3.3 ± 1.3	4.7 ± 1.0	1.4	13.3 ± 3.6	23.8 ± 1.4	1.9	4
Coronary artery disease	Homocystine	0.06 ± 0.12	0.03 ± 0.11	0.5	0.59 ± 0.37	0.70 ± 0.68	1.6	60
	Cysteine-homocysteine disulfide	2.73 ± 1.11	3.22 ± 2.07	1.2	9.39 ± 3.67	10.47 ± 5.17	1.1	60
Coronary artery disease	Total homocyst(e)ine	8.50 ± 2.80	10.96 ± 3.44	1.3				39
Myocardial infarction	Total homocyst(e)ine	13.5 ± 3.6	16.4 ± 6.9	1.2				35
Cerebral infarction	Total homocyst(e)ine	7.3 ± 2.9	13.1 ± 5.6	1.8				1

Peripheral occlusive artery	Total homocyst(e)ine	9.80 ± 3.44	16.6 ± 6.94	1.7				45
Cerebral infarction	Total homocyst(e)ine	10.7 ± 3.2	15.8 ± 5.4	1.5				15
Coronary heart disease	Total homocyst(e)ine							
Male		11.3 ± 3.7	13.1 ± 4.3	1.2				46
Female		10.1 ± 5.0	13.0 ± 7.4	1.3				
Coronary artery disease	Total homocyst(e)ine	10.9 ± 4.9	13.7 ± 6.4	1.3				23
Familial Coronary artery disease	Total homocyst(e)ine							
Male		11.1 ± 2.6	14.3 ± 7.1	1.3				87
Aortoiliac disease	Total homocyst(e)ine	11.0 ± 3.4	18.7 ± 14.9	1.7	21.1 ± 7.3	32.9 ± 2.03	1.6	6
Cerebral thrombosis	Total homocyst(e)ine	11.0 ± 3.4	12.5 ± 7.8	1.1	21.1 ± 7.3	26.0 ± 14.2	1.2	
Coronary artery disease	Total homocyst(e)ine	13.7 ± 4.8	18.1 ± 7.5	1.3				82

[a] Differences between patients and controls are statistically significant ($p < 0.05$) except cysteine-homocysteine disulfide studies by Murphy-Chutorian (60).

Table 2 Hyperhomocyst(e)inemia in controls and patients with occlusive vascular disease

Disease studied	Homocysteine determined as	Basal levels			Postmethionine loading			Refs.
		Controls	Patients	Ratio	Controls	Patients	Ratio	
Coronary artery disease	Cysteine-homocysteine disulfide				5/22 (23%)	17/25 (68%)	3.1	86
Arteriosclerotic cerebrovascular disease	Cysteine-homocysteine disulfide	2/17 (12%)	5/19 (26%)	2.2	1/16 (6%)	4/19 (21%)	3.5	5
Occlusive peripheral artery	Homocystine and cysteine-homocysteine disulfide				0/40 (0%)	7/25 (28%)	(5.6)[a]	4
Occlusive cerebrovascular disease						7/25 (28%)	(5.6)[a]	4
Myocardial infarction						0/25 (0%)		4
Coronary artery disease	Homocystine and cysteine-homocysteine disulfide				1/39 (3%)	16/99 (16%)	5.3	60
					3/39 (8%)	12/99 (12%)	1.5	60

Coronary artery disease	Total homocyst(e)ine	8/202 (4%)	25/240 (10%)	2.6					39
Myocardial infarction	Total homocyst(e)ine	2/36 (6%)	5/21 (24%)	4.0					35
Peripheral artery disease	Total homocyst(e)ine	0/29 (0%)	22/47 (47%)	(9.4)[a]					45
Cerebral infarction	Total homocyst(e)ine	1/31 (3%)	11/41 (27%)	9.0					15
Coronary artery disease	Total homocyst(e)ine								
Male			12/64 (19%)	(3.8)[a]					46
Female			3/35 (9%)	(1.8)[a]					46
Cerebrovascular disease	Total homocyst(e)ine	2/46 (4%)	20/72 (28%)	7.0					6
Cerebrovascular disease	Homocystine and cysteine-homocysteine disulfide				0/27 (0%)	16/38 (42%)	(8.4)[a]		13
Peripheral artery disease						7/25 (28%)	(5.6)[a]		13
Coronary artery disease						18/60 (30%)	(6.0)[a]		13

[a] Frequency of hyperhomocyst(e)inemic is 5% in general population; hyperhomocyst(e)inemia was defined as total homocyst(e)ine level above 2 SD of normal mean.

investigated using a prospective design in 14,916 participants in the Physician's Health Study (78). In a five-year follow-up, 271 cases of acute myocardial infarction were reported and were matched to 271 controls. Results showed that the patients had a higher mean level of homocyst(e)ine than did the controls (47). Moreover, hyperhomocyst(e)inemia was observed in 31 patients, but only in 13 controls (47).

In summary, whether cysteine-homocysteine disulfide or total homocyst(e)ine was determined with or without methionine loading, the results have been consistent. In every series (with one exception, Ref. 4), patients with coronary artery disease, cerebrovascular disease, or peripheral arterial disease had mean values significantly higher than those found in controls. The difference between the mean values of basal homocyst(e)ine in patients with vascular disease and in controls was small. The patients usually had mean values 30–50% above those of the controls. Moreover, hyperhomocyst(e)inemia was demonstrated to be independent of other risk factors.

Pathogenic Mechanisms of Vascular Damage and Thromboembolism in Hyperhomocyst(e)inemia: The in vitro Studies

At least three lines of in vitro evidence suggest that excessive homocyst(e)ine is associated with the development of vascular disease. These include endothelial injury, platelet adhesion to endothelium, and release of mitogenic factors leading to intimal smooth muscle cell proliferation.

Chemical endothelial injury has been proposed as the initiating event, followed by patchy desquamation and focal proliferation of intimal smooth muscle cells, similar in appearance to early atherosclerotic lesions in humans (31). This hypothesis was further examined by the evaluation of adhesion of human umbilical vein endothelial cells to glass or plastic surfaces. Wall et al (84) found that 0.1–1.0 mM *dl*-homocysteine thiolactone induced endothelial cell detachment in direct proportion to its concentration. The cytotoxicity of homocysteine on cultured endothelial cells from an individual who was an obligate heterozygote for cystathionine β-synthase deficiency was also explored by this method (16). Cultured cells from the heterozygote were more susceptible to injury induced by sulfur-containing amino acids than were normal endothelial cells. These observations support the hypothesis that cystathionine β-synthase is expressed in normal endothelium.

Homocysteine-induced endothelial cell detachment was prevented by catalase but not by superoxide dismutase, which would suggest that hydrogen peroxide plays a role in mediating injury (62, 70, 84). It seems that homocysteine participates in the generation of hydrogen peroxide, which interacts with the synthesis of arterial prostacyclin (PGI_2). Thus the effect of homocysteine on PGI_2 synthesis may be dependent on the oxidation of homocysteine and the subsequent generation of hydrogen peroxide.

Normal hemostasis depends in part on the balance achieved between platelet thromboxane A_2 and endothelial PGI_2 production. Graeber et al (27) examined the effect of homocysteine on platelet arachidonic acid metabolism by studying thromboxane B_2, which is the stable end-product of thromboxane A_2. In their studies, they were unable to find any stimulatory or inhibitory effect of homocysteine on PGI_2 synthesis, but they found increased platelet thromboxane production in the presence of 1 mM homocysteine. In contrast, cystine, cysteine, or methionine did not have a similar effect. The conflicting observations of the two studies in regard to PGI_2 syntheses were thought to result from the employment of different assay systems (62).

Because copper-catalyzed oxidation of thiol compounds can lead to the reduction of oxygen and the generation of hydrogen peroxide (12, 89), Starkebaum & Harlan (79) have further studied the effect of homocysteine and copper on endothelial cells. Cultured human and bovine endothelial cells were lysed in the presence of 66–500 μM homocysteine and copper ions, in a dose-dependent manner. Although the generation of hydrogen peroxide was not measured directly, these results suggested a mechanism whereby elevated levels of homocysteine could injure endothelial cells through copper-catalyzed generation of hydrogen peroxide.

Several investigators have demonstrated superoxide (54), hydrogen peroxide (89), and hydroxyl radical generation (70) during autooxidation of thiol compounds, including protein-bound thiols. A wide spectrum of both oxygen- and sulfur-derived free radicals can be produced in the presence of thiols and trace metals, thus initiating lipid peroxidation. Parthasarathy (63) showed that homocysteine promoted the oxidation of LDL. Other thiol compounds including cysteine and reduced glutathione also showed these effects. In contrast, oxidized thiols were ineffective in oxidizing LDL. Homocystine, therefore, is unlikely to produce these effects by a superoxide-mediated mechanism (34). However, this does not exclude the possibility that smooth muscle cells are able to reduce homocystine, since homocysteine autooxidizes by a two-electron transfer reaction that results in the production of hydrogen peroxide (34).

Features of blood coagulation in homocystinuric patients have been recently reviewed by Palareti & Coccheri (61). Reduced antithrombin III and factor VII levels were observed in patients with severe hyperhomocyst(e)inemia (12, 25, 48, 53). An increased homocyst(e)ine level appeared to be directly linked to low antithrombin III and factor VII activity levels, which were correctable by the administration of pyridoxine and folate. Rodgers & Kane (69) demonstrated that 0.5–10 mM homocysteine increased factor V activity and prothrombin activation in bovine aortic endothelial cell cultures. These results indicated that homocysteine-treated vascular endothelium induced the activation of factor V. Their studies explored further the mechanism of factor V activation by which homocysteine reduced endothelial cell protein C activa-

tion (68). These data suggested that coagulation abnormalities induced by homocysteine may have contributed to the thrombotic tendency seen in patients with severe hyperhomocyst(e)inemia.

In summary, homocysteine-induced chemical injury to cultured endothelial cells is probably due to the modification of arachidonic acid metabolism. Oxidation of homocysteine causes copper-catalyzed generation of hydrogen peroxide, leading to the inhibition or stimulation of PGI_2 synthesis. An increased platelet thromboxane production is also involved. Oxidation of low density lipoprotein is another effect of thiol compounds. Coagulation abnormalities, such as reduced antithrombin III and factor VII, and increased factor V and prothrombin activities are observed in the presence of homocysteine, suggesting its association with thrombotic tendency. These in vitro effects are initiated by homocysteine, whereas the major portion of plasma homocyst(e)ine is present as disulfides. Hence, investigators have speculated that cells take up homocystine and reduce it to homocysteine intracellularly; subsequently, reoxidation of homocysteine leads to the generation of hydrogen peroxide (34, 79).

Concluding Remarks

In the past several years, significant progress has been achieved in our understanding of various hyperhomocyst(e)inemias and their association with the development of occlusive arterial diseases. Homocysteine and its derivatives are reliably measured as total homocyst(e)ine in nonhomocystinuric individuals. Not only is plasma homocyst(e)ine level affected by genetic defects of homocysteine metabolism but it is also controlled by various nongenetic factors such as nutritional inadequacy of folate, cobalamin, pyridoxine, or choline, endocrinological status, other diseases, and some medications.

Many retrospective studies have substantiated the positive correlation between moderate hyperhomocyst(e)inemia and occlusive arterial diseases. Recently, Malinow et al (47) reported a prospective study in which moderately high levels of homocyst(e)ine were associated with increased risk of myocardial infarction. Although definite proof is still lacking, it is reasonable to assume that the accumulation of excessive homocyst(e)ine causes damage to endothelial and smooth muscle cells and alters the activity of coagulation factor(s). No ideal animal models are available to demonstrate homocysteine-induced vascular pathology. Chronic infusion studies are technically difficult because of the high metabolic turnover of homocysteine molecules. In nutritionally induced hyperhomocyst(e)inemias, it has not been possible to rule out the direct effect of nutritional deficiency itself on the vascular system.

Irrespective of the etiology, moderate and intermediate hyperhomocyst(e)inemia is readily correctable by supplementation with folate and/or

betaine. Whether or not normalization of hyperhomocyst(e)inemia will improve morbidity and mortality is a question that must await the outcome of clinical trials.

ACKNOWLEDGMENTS

We express our appreciation to S. Harvey Mudd, J. B. Ubbink, and W. J. H. Vermack for communicating papers prior to publication. We also thank Delilah Delgado for her excellent typing of the manuscript. Work in the authors' laboratories was supported by grants from the National Heart, Lung and Blood Institute (HL 36135) and the National Institute of Health (RR00163-32).

Literature Cited

1. Araki, A., Sako, Y., Fukushima, Y., Matsumoato, M., Asada, T., Kita, T. 1989. Plasma sulfhydryl-containing amino acids in patients with cerebral infarction and in hypertensive subjects. *Atherosclerosis* 79:139–46
2. Baldessarini, R. J., Kepin, I. J. 1963. Assay of tissue levels of S-adenosylmethionine. *Anal. Biochem.* 6:289–92
3. Baumgartner, E. R., Wick, H., Linnell, J. C., Gaull, G. E., Bachmann, C., Steinmann, B. 1979. Congenital defect in intracellular cobalamin metabolism resulting in homocystinuria and methylmalonic aciduria. *Helv. Paediatr. Acta* 34:483–96
4. Boers, G. H. J., Smals, A. G. H., Trijbels, F. J. M., Fowler, B., Bakkeren, J. A., et al. 1985. Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. *New Engl. J. Med.* 313:709–15
5. Brattström, L. E., Hardebo, J. E., Hultberg, B. L. 1984. Moderate homocysteinemia—a possible risk factor for arteriosclerotic cerebrovascular disease. *Stroke* 14:1012–16
6. Brattström, L., Israelsson, B., Hultberg, B. 1988. Impaired homocysteine metabolism—a possible risk factor for arteriosclerotic vascular disease. In *Genetic Susceptibility to Environmental Factors: A Challenge for Public Intervention,* ed. U. Smith, S. Ericksson, F. Lindgarde, pp. 25–34. Stockholm: Almqvist & Wiksell Int.
7. Brattström, L., Israelsson, B., Jeppsson, J. O. 1986. Heterozygosity for homocystinuria in premature arterial disease. *New Engl. J. Med.* 314:849–50
8. Brattström, L., Israelsson, B., Lindgarde, F., Hultberg, B. 1988. Higher total plasma homocysteine in vitamin B_{12} deficiency than in heterozygosity for homocystinuria due to cystathionine B-synthase deficiency. *Metabolism* 37:175–78
9. Carey, M. C., Donovan, D. E., FitzGerald, O., McAuley, F. D. 1968. Homocystinuria. I. A clinical and pathological study of nine subjects in six families. *Am. J. Med.* 45:7–25
10. Carson, N. A. J., Neill, D. W. 1962. Metabolic abnormalities detected in a survey of mentally backward individuals in Northern Ireland. *Arch. Dis. Child.* 37:505–13
11. Deleted in proof
12. Charlot, J. C., Haye, C., Chaumien, J. P. 1982. Homocystinuric et deficit en facteur VII. *Bull. Soc. Ophthalmol. Fr.* 82:787–89
13. Clarke, R., Daly, L., Robinson, K., Naughten, E., Cahalane, S., et al. 1991. Hyperhomocysteinemia: An independent risk factor for vascular disease. *New Engl. J. Med.* 324:1149–55
14. Cooper, B. A., Rosenblatt, D. S. 1987. Inherited defects of vitamin B_{12} metabolism. *Annu. Rev. Nutr.* 7:291–320
15. Coull, B. M., Malinow, M. R., Beamer, N., Saxton, G., Nordt, F., deGarmo, P. 1990. Elevated plasma homocyst(e)ine concentration as a possible independent risk factor for stroke. *Stroke* 21:572–76
16. De Groot, P. G., Willems, C., Boers, G. H. J., Gonsalves, M. D., Van Aken, W. G., Van Mourik, J. A. 1983. Endothelial cell dysfunction in homocystinuria. *Eur. J. Clin. Invest.* 13:405–10
17. Deleted in proof

18. Erbe, R. W. 1986. Inborn errors of folate metabolism. In *Folates and Pterins,* ed. R. L. Blakely, pp. 413–65. Boston: Wiley
19. Fenton, W. A., Rosenberg, L. E. 1989. Inherited disorders of cobalamin transport and metabolism. In *The Metabolic Basis of Inherited Disease,* ed. C. R. Scriver, A. L. Beadet, W. S. Sly, D. Valle, pp. 2065–82. New York: McGraw-Hill
20. Finkelstein, J. D., Kyle, W. E., Harris, B. J. 1971. Methionine metabolism in mammals. Regulation of homocysteine methyltransferases in rat tissue. *Arch. Biochem. Biophys.* 146:84–92
21. Finkelstein, J. D., Martin, J. J. 1984. Methionine metabolism in mammals. Distribution of homocysteine between competing pathways. *J. Biol. Chem.* 259:9508–13
22. Finkelstein, J. D., Martin, J. J. 1986. Methionine metabolism in mammals. Adaptation to methionine excess. *J. Biol. Chem.* 261:1582–87
23. Genest, J. J., McNamara, J. R., Salem, D. N., Wilson, P. W. F., Schaefer, E. J., Malinow, M. R. 1990. Plasma homocyst(e)ine levels in men with premature coronary artery disease. *J. Am. Coll. Cardiol.* 16:1114–19
24. Genest, J. J., McNamara, J. R., Upson, B., Salem, D. N., Ordovas, J. M., et al. 1991. Prevalence of familial hyperhomocyst(e)inemia in men with premature coronary artery disease. *Arterioscler. Thromb.* 11:1129–36
25. Giannini, M. J., Coleman, M., Innerfield, I. 1975. Antithrombin activity in homocystinuria. *Lancet* 1:1094
26. Gibson, J. B., Carson, N. A. J., Neill, D. W. 1964. Pathological findings in homocystinuria. *J. Clin. Pathol.* 17: 427–37
27. Graeber, J. E., Slott, J. H., Ulane, R. E., Schulman, J. D., Stuart, J. J. 1982. Effect of homocysteine and homocystine on platelet and vascular arachidonic acid metabolism. *Pediatr. Res.* 16:490–93
28. Gupta, V. J., Wilcken, D. E. L. 1978. The detection of cysteine-homocysteine mixed disulphide in plasma of normal fasting man. *Eur. J. Clin. Invest.* 8:205–7
29. Deleted in proof
30. Deleted in proof
31. Harker, L. A., Ross, R., Slichter, S. J., Scott, C. R. 1976. Homocysteine-induced arteriosclerosis. The role of endothelial cell injury and platelet response in its genesis. *J. Clin. Invest.* 58:731–41
32. Harker, L. A., Slichter, S. J., Scott, C. R., Ross, R. 1974. Homocystinemia: Vascular injury and arterial thrombosis. *New Engl. J. Med.* 291:537–43
33. Hartcroft, W. S., Ridout, J. H., Sellers, E. A., Best, C. H. 1952. Atheromatous changes in aorta, carotid and coronary arteries of choline-deficient rats. *Proc. Soc. Exp. Biol. Med.* 81:384–93
34. Heinecke, J. W., Rosen, H., Suzuki, L. A., Chait, A. 1987. The role of sulfur-containing amino acids in superoxide production and modification of low density lipoprotein by arterial smooth muscle cells. *J. Biol. Chem.* 262:10098–10103
35. Israelsson, B., Brattstrom, L. E., Hultberg, B. L. 1988. Homocysteine and myocardial infarction. *Atherosclerosis* 71:227–33
36. Lin, J. Y., Kang, S. S., Zhou, J., Wong, P. W. K. 1989. Homocysteinemia in rats induced by folic acid deficiency. *Life Sci.* 44:319–25
37. Kang, S. S., Wong, P. W. K., Becker, N. 1979. Protein-bound homocyst(e)ine in normal subjects and in patients with homocystinuria. *Pediatr. Res.* 13:1141–43
38. Kang, S. S., Wong, P. W. K., Bock, H. G., Horwitz, A., Grix, A. 1991. Intermediate hyperhomocysteinemia resulting from compound heterozygosity of methylenetetrahydrofolate reductase mutation. *Am. J. Hum. Genet.* 48:546–51
39. Kang, S. S., Wong, P. W. K., Cook, H. Y., Norusis, M., Messer, J. V. 1986. Protein-bound homocyst(e)ine. A possible risk factor for coronary artery disease. *J. Clin. Invest.* 77:1482–86
40. Kang, S. S., Wong, P. W. K., Norusis, M. 1987. Homocysteinemia due to folate deficiency. *Metabolism* 36:458–62
41. Kang, S. S., Wong, P. W. K., Susmano, A., Sora, J., Norusis, M., Ruggie, N. 1991. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. *Am. J. Hum. Genet.* 48:536–45
42. Kang, S. S., Wong, P. W. K., Zhou, J., Cook, H. Y. 1986. Preliminary report: total homocyst(e)ine in plasma and amniotic fluid of pregnant women. *Metabolism* 35:889–91
43. Kanwar, Y. S., Manaligod, J. R., Wong, P. W. K. 1976. Morphologic studies in a patient with homocystinuria due to 5,10-methylenetetrahydrofolate reductase deficiency. *Pediatr. Res.* 10: 598–609
44. Malinow, M. R. 1991. Homocyst(e)ine and vascular occlusive disease. *Nutr. Metab. Cardiovasc. Dis.* 1:166–69
45. Malinow, M. R., Kang, S. S., Taylor,

L. M., Wong, P. W. K., Coull, B., et al. 1989. Prevalence of hyperhomocyst(e)inemia in patients with peripheral arterial occlusive disease. *Circulation* 79:1180–88

46. Malinow, M. R., Sexton, G., Averbuch, M., Grossman, M., Wilson, D., Upson, B. 1990. Homocyst(e)inemia in daily practice: levels in coronary artery disease. *Coron. Artery Dis.* 1:215–20
47. Malinow, M. R., Stampfer, M. J., Willett, W. C., Newcomer, L., Upson, B., et al. 1991. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction. *Arterioscler. Thromb.* 11:1480A
48. Maruyama, I., Fukuda, R., Kazama, M. 1977. A case of homocystinuria with low antithrombin activity. *Acta Haematol. Jpn.* 40:267–71
49. McCully, K. S. 1969. Vascular pathology of homocysteinemia: Implications for the pathogenesis of arteriosclerosis. *Am. J. Pathol.* 56:111–28
50. McCully, K. S., Ragsdale, B. D. 1970. Production of arteriosclerosis by homocysteinemia. *Am. J. Pathol.* 61:1–8
51. McCully, K. S., Wilson, R. B. 1975. Homocysteine theory of arteriosclerosis. *Atherosclerosis* 22:215–27
52. McGill, J. J., Mettler, G., Rosenblatt, D. S., Scriver, C. R. 1990. Detection of heterozygotes for recessive alleles. Homocyst(e)inemia: Paradigm of pitfalls in phenotypes. *Am. J. Med. Genet.* 36:45–52
53. Mercky, J., Kuntz, F. 1981. Deficit en facteur VII et homocytinurie. Association fortuite ou syndrome? *Nouv. Presse Med.* 10:3796
54. Misra, H. P. 1974. Generation of superoxide free radical during the autoxidation of thiols. *J. Biol. Chem.* 249:2151–55
55. Mudd, S. H. 1988. Vascular disease and homocysteine metabolism. See Ref. 6, pp. 11–24
56. Mudd, S. H., Levy, H. L., Skovby, F. 1989. Disorders of transsulfuration. See Ref. 19, pp. 693–734
57. Mudd, S. H., Poole, J. R. 1975. Labile methyl balances for normal humans on various dietary regimens. *Metabolism* 24:721–35
58. Mudd, S. H., Skovby, F., Levy, H. L., Pettigrew, K. D., Wilcken, B., et al. 1985. The natural history of homocystinuria due to cystathionine β-synthase deficiency. *Am. J. Hum. Genet.* 37: 1–31
59. Mudd, S. H., Uhlendorf, B. W., Freeman, J. M., Finkelstein, J. D., Shih, V. E. 1972. Homocystinuria associated with decreased methylenetetrahydrofolate reductase activity. *Biochem. Biophy. Res. Commun.* 46:905–12
60. Murphy-Chutorian, D. R., Wexman, M. P., Grieco, A. J., Heiringer, J. E., Glassman, E., et al. 1985. Methionine intolerance: a possible risk factor for coronary artery disease. *J. Am. Coll. Cardiol.* 6:725–30
61. Palareti, G., Coccheri, S. 1989. Lowered antithrombin III activity and other clotting changes in homocystinuria: Effects of a pyridoxine-folate regimen. *Haemostasis* 19(Suppl. 1):24–28
62. Panganamala, R. V., Karpen, C. W., Merola, A. J. 1986. Peroxide-mediated effects of homocysteine on arterial prostacyclin synthesis. *Prostaglandins Leukot. Med.* 22:349–56
63. Parthasarathy, S. 1987. Oxidation of low density lipoprotein by thiol compounds leads to its recognition by the acetyl LDL receptor. *Biochim. Biophys. Acta* 917:337–40
64. Reddy, G. S. R., Wilcken, D. E. L. 1982. Experimental homocysteinemia in pigs: comparison with studies in sixteen homocystinuric patients. *Metabolism* 31:778–83
65. Reed, T., Malinow, M. R., Christian, J. C., Upson, B. 1991. Estimates of heritability for plasma homocyst(e)ine levels in aging adult male twins. *Clin. Genet.* 39:425–28
66. Refsum, H., Helland, S., Ueland, P. M. 1985. Radioenzymic determination of homocysteine in plasma and urine. *Clin. Chem.* 31:624–28
67. Rinehart, J. F., Greenberg, L. D. 1956. Vitamin B_6 deficiency in the rhesus monkey with particular reference to the occurrence of atherosclerosis, dental caries and hepatic cirrhosis. *Am. J. Clin. Nutr.* 4:318–25
68. Rodgers, G. M., Conn, M. T. 1990. Homocysteine, an atherogenic stimulus reduces protein C activation by arterial and venous endothelial cells. *Blood* 75:895–901
69. Rodgers, G. M., Kane, W. H. 1986. Activation of endogenous factor V by a homocysteine-induced vascular endothelial cell activator. *J. Clin. Invest.* 77:1909–16
70. Saez, G., Thornalley, P. J., Hill, H. A. O., Hems, R., Bannister, J. V. 1982. The production of free radicals during autoxidation of cysteine and their effect on isolated rat hepatocytes. *Biochim. Biophys. Acta* 719:24–31
71. Salvatore, F., Zappia, V., Shapiro, S. K. 1968. Quantitative analysis of S-

adenosylhomocystein in liver. *Biophys. Biochim. Acta* 158:461–64
72. Sardharwalla, I. B., Fowler, B., Robins, A. J., Komrower, G. M. 1974. Detection of heterozygotes for homocystinuria. Study of sulphur-containing amino acids in plasma and urine after L-methionine loading. *Arch. Dis. Child.* 49:553–59
73. Sartorio, R., Carrozzo, R., Corbo, L., Andria, G. 1986. Protein-bound plasma homocyst(e)ine and identification of heterozygotes for cystathionine-synthase deficiency. *J. Inherit. Metab. Dis.* 9:25–29
74. Deleted in proof
75. Smolin, L. A., Benevenga, N. J. 1982. Accumulation of homocyst(e)ine in Vitamin B-6 deficiency: A model for the study of cystathionine β-synthase deficiency. *J. Nutr.* 112:1264–72
76. Smolin, L. A., Benevenga, N. J., Berlow, S. 1981. The use of betaine for the treatment of homocystinuria. *J. Pediatr.* 99:467–72
77. Stabler, S. P., Marcell, P. D., Podell, E. R., Allen, R. H., Savage, D. G., Lindenbaum, J. 1988. Elevation of total homocysteine in the serum of patients with cobalamin or folate deficiency detected by capillary gas chromatography-mass spectrometry. *J. Clin. Invest.* 81:466–74
78. Stampfer, M. R., Buring, J., Willett, W., Rosner, B., Eberlein, K., et al. 1985. The 2 × 2 factorial design: its application to a randomized trial of aspirin and caretone among US physicians. *Stat. Med.* 4:111–16
79. Starkebaum, G., Harlan, J. M. 1986. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. *J. Clin. Invest.* 77:1370–76
80. Svardal, A., Ueland, P. M., Berge, R. K., Aarsland, A., Aarsaether, N., et al. 1988. Effect of methotrexate on homocysteine and other sulfur compounds in tissues of rats fed a normal or a defined, choline-deficient diet. *Cancer Chemother. Pharmacol.* 21:313–18
81. Swift, M. E., Shultz, T. D. 1986. Relationship of vitamin B_6 and B_{12} to homocysteine levels: Risk for coronary artery disease. *Nutr. Rep. Int.* 34:1–14
82. Ubbink, J. G., Vermack, W. J. H., Bennett, J. M., Becker, P. G., VanStaden, D. A., Bissbort, S. 1991. The prevalence of homocysteinemia and hypercholesterolemia in angiographically defined coronary heart disease. *Klin. Wochenschr.* 69:527–34
83. Ueland, P. M., Refsum, H. 1989. Plasma homocysteine, a risk factor for vascular disease: Plasma levels in health, disease, and drug therapy. *J. Lab. Clin. Med.* 114:473–501
84. Wall, R. T., Harlan, J. M., Harker, L. A., Striker, G. E. 1980. Homocysteine-induced endothelial cell injury in vitro: a model for the study of vascular injury. *Thromb. Res.* 18:113–21
85. Wilcken, D. E. L., Dudman, N. P. B. 1989. Mechanism of thrombogenesis and accelerated atherogenesis in homocysteinemia. *Haemostasis* 19(Suppl. 1):14–23
86. Wilcken, D. E. L., Wilcken, B. 1976. The pathogenesis of coronary artery disease. A possible role for methionine metabolism. *J. Clin. Invest.* 57:1079–82
87. Williams, R. R., Malinow, M. R., Hunt, S. C., Upson, B., Wu, L. L., et al. 1990. Hyperhomocyst(e)inemia in Utah siblings with early coronary disease. *Coron. Artery Dis.* 1:681–85
88. Wong, P. W. K., Kang, S. S., Kanwar, Y. W. 1990. Folic acid induced homocysteinemia and atherosclerosis. *Clin. Res.* 38:806A
89. Zwart, J., Van Wolput, H. M. C., Van der Cammen, J. C. M., Konigsberger, D. C. 1981. Accumulation and reactions of hydrogen peroxide during the copper ion catalyzed auto oxidation of cysteine in alkaline medium. *J. Mol. Catal.* 11:69–82