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Plasma Homocysteine and Cardiovascular Disease

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Homocysteine is a sulfur amino acid with a free sulfhydryl group (Fig. 1). It was first discovered by du Vigneaud in 1932 as a product of demethylation of methionine (1). The interest in homocysteine was greatly enhanced by the discovery of the inborn errors, homocystinuria. *Homocystinuria* means the excretion of large amounts of homocystine, the disulfide of homocysteine, in urine secondary to a high level of homocysteine in blood. This disease was first reported in 1962 by Carson and Neill (2), who identified two siblings, aged 4 and 6 years, among 2081 mentally retarded individuals. Almost simultaneously, Gerritsen et al. (3,4) identified urinary homocystine and documented the absence of cerebral cystathionine in a mentally retarded infant with congenital anomalies and thromboembolism. Mudd and co-workers (5) demonstrated lack of cystathionine β-synthase activity in liver from a typical patient in 1964. Within a few years after the first cases of cystathionine β-synthase deficiency were discovered, additional patients and their susceptibility to life-threatening vascular disease were described (6–8). In 1969, McCully (9) described vascular lesions, similar to those observed in cystathionine β-synthase deficiency, in a 7 1/2-week-old infant dying of homocystinuria caused by a newly discovered defect in cobalamin metabolism. Similar vascular alterations were reported by Kanwar et al. in 1976 (10) in a 10-year-old girl dying of homocystinuria caused by a 5,10-methylenetetrahydrofolate reductase deficiency.

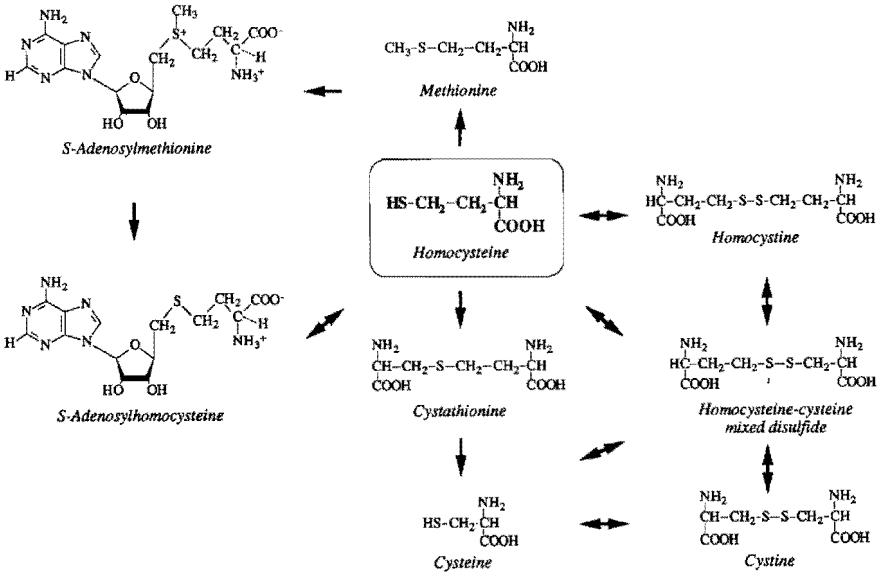


Figure 1 The chemical structure of homocysteine, closely related compounds, and their interconversion.

These and subsequent reports demonstrated the clinical and biochemical diversity of homocystinuria patients. The most common form is the cystathionine β-synthase deficiency, and rare cases are due to defects in cobalamin metabolism or 5,10-methylenetetrahydrofolate reductase deficiency (11,12). The development of precocious vascular disease in different forms of homocystinuria has been established by reports on several additional cases (11).

In 1975, McCully (13) formulated the homocysteine theory of atherosclerosis, which is based on clinical and experimental evidence. He pointed out that vascular lesions develop in homocystinuria caused by different metabolic defects, suggesting that high concentrations of homocysteine itself are responsible for the vascular changes (14). The theory also implies that moderate elevation of homocysteine in blood, caused by subtle abnormalities in homocysteine metabolism, might be associated with increased risk for vascular disease.

Improved techniques for the determination of homocysteine in blood have allowed the investigation of the possible relation between hyperhomocysteinemia homocysteinemia and vascular disease. Since the pioneering work on coronary heart disease by Wilcken and Wilcken in 1976 (15), about 20 clinical studies on homocysteine and vascular disease are known to us, including about 1800 patients. They have established that premature atherosclerosis in the

coronary, cerebral, and peripheral vessels, independently of other risk factors, is associated with increased plasma homocysteine.

This chapter reviews the literature on homocysteine, with emphasis on aspects related to the development, occurrence and treatment of cardiovascular disease.

NOMENCLATURE

In this article, *homocystinuria* refers to genetic diseases characterized by massive urinary excretion of homocystine and its derivatives. A *homocystinuric* is a patient with homocystinuria.

Homocysteine is the sulfhydryl form, but homocysteine is often used in contexts that encompass several (oxidized and reduced) species. Thus, *free homocysteine* in plasma is the acid-soluble, non-protein-bound fraction in which the homocysteine-cysteine mixed disulfide is the predominating species. *Total homocysteine* includes all (free and protein-bound) forms of homocysteine in plasma.

Moderate elevation of plasma and serum homocysteine above normal values has been designated *homocystinemia* (16,17), *homocysteinemia* (18), *moderate homocysteinemia* (19), or *intermediate homocysteinemia* (20). Since homocysteine is normally present in blood, the term *hyperhomocysteinemia* seems more appropriate and is used throughout this chapter.

HOMOCYSTEINE METABOLISM AND ITS REGULATION

Homocysteine is an intermediate in the transsulfuration pathway (i.e., the conversion of methionine to cysteine). Its metabolism is depicted in Figure 2.

Methionine is an essential sulfur-containing amino acid that is supplied through catabolism of dietary proteins. The daily intake of the Western male is about 15–35 mg/kg (18,21), and the daily requirements are 10–40 mg/kg. Methionine that is not incorporated into proteins may be catabolized through transamination (22), but most is converted to *S*-adenosylmethionine, catalyzed by methionine adenosyltransferase (EC 2.5.1.6). Only a small fraction of formed *S*-adenosylmethionine is used for the synthesis of polyamines, and most functions as a methyl donor in various transmethylation reactions. *S*-Adenosylhomocysteine, the demethylated product of *S*-adenosylmethionine, is further hydrolyzed to adenosine and homocysteine, catalyzed by the enzyme *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1.). Notably, this reaction is the only known source of homocysteine in vertebrates (23).

Homocysteine is catabolized to cysteine through two vitamin B_6-dependent reactions that complete the transsulfuration pathway. In the first of these reactions, homocysteine is condensed with serine to form cystathionine by the

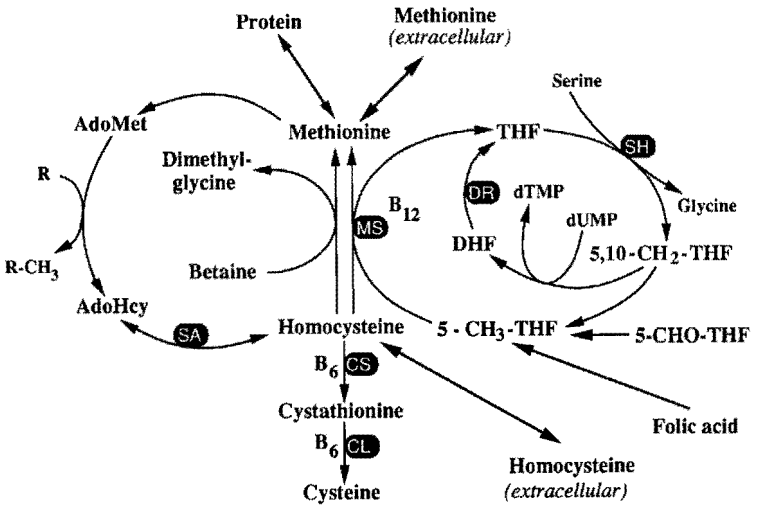


Figure 2 Homocysteine metabolism and enzymes and cofactors involved. AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethione; 5,10-CH_2-THF, 5,10-methylene THF; 5-CH_3-THF, 5-methyltetrahydrofolate; 5-CHO-THF, 5-formyltetrahydrofolate; CL, cystathionine lyase (γ-cystathionase); CS, cystathionine β-synthase; DHF, dihydrofolate; DR, dihydrofolate reductase; Met, methionine, MS, methionine synthase (5-methyl-THF-homocysteine methyltransferase); SA, *S*-adenosylhomocysteine hydrolase; SH, serine hydroxymethyl transferase; THF, tetrahydrofolate. (From Ref. 270.)

enzyme cystathionine β-synthase. The reaction is irreversible under physiological conditions, and at this point, homocysteine is committed to the transsulfuration pathway. The irreversibility also explains the inability of cysteine to serve as a methionine percursor. Cystathionine is further metabolized to cysteine (and α-ketobutyrate), catalyzed by the vitamin B_6-dependent enzyme γ-cystathionase (23).

Remethylation of homocysteine to methionine is catalyzed either by 5-methyltetrahydrofolate-homocysteine methyltransferase (methionine synthase, EC 2.1.1.13.) or betaine-homocysteine methyltransferase (EC 2.1.1.5.). The former enzyme, which is widely distributed, requires 5-methyltetrahydrofolate as a methyl donor and methylcobalamin as a cofactor. Betaine-homocysteine methyltransferase is confined to the liver, and only minor activity has occasionally been found in kidney and adrenal glands (23). Both these reactions conserve methionine. 5-Methyltetrahydrofolate is the circulating form of reduced folate (24). It must be demethylated to tetrahydrofolate through the action of 5-methyltetrahydrofolate-homocysteine methyltransferase to enter the pool of intracellular reduced folates. Impairment of this reaction decreases the avail-

ability of tetrahydrofolate and related folates. This is the basis for the so-called folate trap hypothesis that explains the perturbation of the folate homeostasis in cobalamin deficiency (25,26).

Homocysteine is an important branch-point metabolite that connects the metabolism of diverse compounds like methionine, cysteine, cobalamin, reduced folates, and vitamin B_6, and this system obviously represents an important regulatory locus (23). Homocysteine may be directed into different anabolic or catabolic pathways. The principles governing the distribution of homocysteine between competing pathways have been described in a series of elegant papers by Finkelstein and co-workers (27–29). Important principles have been clarified concerning the metabolic adaptation to variable methionine supply (28).

During methionine excess, methionine is catabolized through the transmethylation–transsulfuration pathway to cysteine and finally sulfate. Such a catabolic sequence requires the utilization of a nonessential methyl acceptor, forming an inert product. The glycine methyltransferase reaction has been suggested to serve such a function. This enzyme is extremely abundant in liver and is stimulated by *S*-adenosylmethionine and inhibited by 5-methyltetrahydrofolate (30,31). Excess methionine increases *S*-adenosylmethionine and decreases 5-methyltetrahydrofolate in liver. Adaptation to methionine excess involves increased flux of homocysteine into cystathionine synthesis, and the rate of remethylation is low. Mechanisms put into immediate action are reduced remethylation through inhibition of 5-methyltetrahydrofolate synthesis by *S*-adenosylmethionine, and a higher K_m for homocysteine of the catabolic enzymes compared with that of the two homocysteine transmethylases. Long-term effects are down-regulation of the transmethylases and up-regulation of cystathionine β-synthase (23).

Metabolite levels and enzyme activities are changed in essentially opposite direction during methionine deficiency, and this regulatory response ensures efficient methionine conservation through enhanced homocysteine remethylation (28).

Cellular homocysteine egress is an important process, since it is a determinant of the amount of homocysteine in extracellular fluids such as plasma. The intracellular concentration of homocysteine is kept low (1–5 nmol/g) (32). Increased production (33,34) or inhibition of metabolism (35,36) is associated with enhanced homocysteine export. Reduced export is observed during pharmacologic inhibition of homocysteine formation (32,37,38). Thus, homocysteine export reflects the balance between homocysteine production and utilization, and therefore, extracellular homocysteine may be an indicator of function of enzymic processes or availability of cofactors or substrates involved in homocysteine metabolism (39).

DIFFERENT FORMS OF HOMOCYSTEINE IN PLASMA

Homocysteine was first demonstrated in plasma from healthy subjects by Gupta and Wilcken in 1978 (40). By using an amino acid analyzer, they detected the homocysteine–cysteine mixed disulfide in deproteinized plasma and established the normal values for men and women (41). Several clinical studies on plasma homocysteine in cardiovascular disease and renal insufficiency published in the years 1976–1984 were based on determinations of acid-soluble mixed disulfides (15,19,42). In 1979, Kang et al. (43) demonstrated that a significant fraction of homocysteine in plasma is associated with plasma proteins and, in 1985, a precise method for the determination of acid-soluble plus protein-bound homocysteine in plasma was described by Refsum and co-workers (44). Most clinical studies (45–51) performed during the last 5 years are based on assays including protein-bound homocysteine.

Thus, there are two main forms of homocysteine in freshly prepared human plasma. A major fraction (about 70%) is associated with plasma proteins, probably linked to albumin by a disulfide bridge. About 30% exists in the acid-soluble supernatant obtained after whole plasma has been deproteinized with acid. This fraction has been referred to as *free homocysteine* (44). Most of the acid-soluble, free homocysteine has been identified as homocysteine–cysteine mixed disulfide (40). Only trace amounts of homocysteine and homocystine exist (52). The sum of all homocysteine species in plasma (free plus protein-bound) is referred to as *total homocysteine.*

The interaction of homocysteine with plasma protein(s) has been superficially characterized (53). Scatchard analysis of clinical data suggests the existence of a heterogeneous population of homocysteine-binding sites in human plasma, and high-affinity sites seem to become saturated at about 20 μmol/L of free homocysteine. One may speculate whether or not the binding represents a mechanism protecting the endothelium and other tissues from possible detrimental effect from circulating homocysteine. Studies of protein-binding of homocysteine also revealed some sort of specificity, since it was not inhibited by increasing concentrations of free cysteine (53,54). In contrast, homocysteine can inhibit the protein binding of cysteine, which shows no saturation. Cysteine is a possible modulator of homocysteine binding to plasma proteins; both at the level of binding sites and by trapping homocysteine as free homocysteine–cysteine mixed disulfide.

DETERMINATION OF HOMOCYSTEINE IN PLASMA

Ex vivo, free homocysteine becomes progressively associated with plasma protein(s), and in stored plasma, probably all homocysteine is protein bound. Such redistribution takes place at room temperature, but also in plasma sam-

ples frozen at −20°C for some weeks. This is why determination of free, acid-soluble homocysteine gives variable results. In contrast, total homocysteine seems to be stable for years in samples stored in closed vials at −20°C. In the clinical setting and in studies based on stored samples, determination of total homocysteine is recommended (39).

Total homocysteine in plasma is increased upon storage of whole blood for longer than 4 hr at room temperature before removal of the formed elements of blood; after 24 hr, it is increased by about 50%. In line with this observation, total homocysteine in serum is often higher than the concentration in freshly prepared plasma. Artificial increase in total homocysteine is avoided when blood is put on ice immediately after collection, and the plasma is prepared within hours (55,56; Lilletvedt et al., unpublished).

Since 1982, several methods for the determination of total homocysteine in plasma or serum have been described. Most assays can be divided into four steps: (a) reduction of oxidized homocysteine species to thiol, (b) precolumn derivatization, (c) chromatographic separation, and (d) detection of the homocysteine derivative.

The construction of these assays and evaluation of their performance are summarized in Table 1.

REFERENCE VALUES FOR HOMOCYSTEINE IN PLASMA AND SERUM

The normal values for total homocysteine differ somewhat from one laboratory to another (Table 2), but values between 7 and 14 μmol/L in fasting subjects are usually considered normal. The variability may be related to different methodology (see Table 1) or to the selection of subjects under influence of various factors affecting the concentration of fasting plasma homocysteine. Values seem to be dependent on age, gender, and in women, possibly the menopausal status (discussed later). We (57) and others (58) found low concentration (about 6 μmol/L) in children aged 3–14 years.

Total homocysteine is influenced by vitamins and drug intake and is related to serum creatinine (39) and serum uric acid (48). The importance of selection of proper control subjects is illustrated by the finding that patients in the dermatological ward, devoid of conditions known to affect plasma homocysteine level, had plasma levels significantly higher than healthy subjects outside the institution (59).

METHIONINE LOADING

Methionine loading is a test first described by Brenton et al. (60) for the detection of heterozygotes for homocystinuria. This procedure involves oral intake

Table 1 Construction and Evaluation of Assays for Total Homocysteine in Plasma and Serum

Reduction	Derivatization	Separation	Detection	Advantages	Disadvantages	Study	Ref.
2-Mercaptoethanol	*S*-Carboxymethylation with iodoacetic acid	Ion exchange (amino acid analyzer)	Ninhydrin reaction	Equipment available Reliable Assay of other amino acids Autoinjection possible	Laborious sample preparation Low sensitivity Long analysis time and low sample output	Kang et al., 1982	271
DTE	Enzymic conversion to *S*-adenosyl-homocysteine	HPLC	Absorbance at 254 nm or scintillation counting	Specific Sensitive Autoinjection	Laborious sample preparation Sensitive to enzyme inactivation or denaturation Low range Low sample output	Refsum et al., 1985	44
2-Mercaptoethanol	*t*-Butyldimethylsilyl derivatization	Capillary GC	Mass spectrometry (single ion monitoring)	Specific Simultaneous determination of cysteine and methionine Autoinjection	Laborious sample clean-up and preparation Instrument not available in most routine laboratories	Stabler et al., 1987	56
Tri-*n*-butyl-phosphine	SBD-F	HPLC	Fluorescence	Specific Sensitive Measures other thiols Autoinjection	Long incubation (90 min) and heating (60°C) Toxic reducing agent	Araki and Sako, 1987 Ubbink et al., 1991	52 272

DTE	Enzymic conversion to *S*-adenosyl-homocysteine	TLC	Scintillation counting	Specific Sensitive Inexpensive equipment	Laborious sample preparation Scintillation counting necessary Sensitive to enzyme inactivation or denaturation Low range	Chu and Hall, 1988 1989	157
Sodium borohydride	None	HPLC	Electrochemical detection	Specific Sensitive Measures other thiols No derivatization	Careful maintenance of flow-cell and reference electrode required	Smolin and Benevenga, 1982	205
				Autoinjection		Malinow et al., 1988	47,128
Sodium borohydride	Monobromobimane	HPLC	Fluorescence	Fairly specific Sensitive Precise Fully automated High sample-output Measures other thiols	Requires expensive equipment	Refsum et al., 1989	274
Potassium borohydride	Monobromobimane	HPLC	Fluorescence	Fairly specific Sensitive Measures other thiols	Sample clean-up	Jacobsen et al., 1989	275
DTT	None	Ion exchange (amino acid analyzer)	Ninhydrin reaction	Equipment available Autoinjection Measures methionine	Low sensitivity	Andersson et al., 1989	276

Abbreviations: GC, gas chromatagraphy; SBD-F, 7-thiorobenzo-2-oxa-1,3-diazole-4-sulphonate; DTE, dithioerythritol; DTT, dithiothreitol; TLC, thin-layer chromatography.

Table 2 Normal Values for Homocysteine in Human Plasma or Serum

	Age (yr)	*n*	Homocysteine species	Value[a] (μmol/L)	Study	Ref.
Male and female	(23–50)	20	MDS[b] (free[c])	3.25 ±0.85	Gupta and Wilcken, 1978	40
Male	(21–50)	24	MDS (free)	3.3 ±0.8	Wilcken and	41
Female	(21–50)	24		2.4 ±0.7	Gupta, 1979	
Male	(45–61)	10	MDS (free)	3.1 ±0.3	Boers et al.,	42
Male	(22–35)	10		3.5 ±0.5	1983	
Female (postm.)	(45–59)	10		2.6 ±0.4		
Female (prem.)	(14–42)	10		0.9 ±0.3		
Male	(25–55)	18	Free	2.27 ±0.48	Refsum et al.,	44
			Bound	6.51 ±1.35	1985	
Female	(25–55)	16	Free	1.95 ±0.56		
			Bound	7.29 ±2.62		
Male	(<30)	5	Bound = total[d]	6.82 ±1.28	Kang et al.,	45
	(30–39)	14		8.92 ±2.32	1986	
	(40–49)	25		9.44 ±2.00		
	(50–59)	26		8.84 ±2.02		
	(60–69)	23		8.06 ±2.32		
Female	(<30)	9	Bound = total	7.50 ±2.02		
	(30–39)	8		7.26 ±1.64		
	(40–49)	24		7.00 ±1.94		
	(50–59)	30		8.82 ±3.82		
	(60–69)	38		9.20 ±3.62		
Male and female	(18–65)	50	Total	13.0 (7.2–21.7)[e]	Stabler et al.,	56
Male			(20% higher than in female)		1987	
Female						
(normotensive)	(62.9 ±10.8)	45	Total	5.8 ±0.9	Araki et al.,	48
Male					1989	
(normotensive)			Total	8.1 ±3.2		
Female						
(hypertensive)	(63.3 ±10.0)	45	Total	7.8 ±1.4		
Male						
(hypertensive)			Total	10.9 ±4.6		
Male	(20–39)	26	Free	2.1 ±0.7	Andersson et al.,	125
			Total	9.7 ±2.4	1991	
	(40–49)	15	Free	2.5 ±0.9		
			Total	10.4 ±2.6		
	(50–69)	33	Free	2.8 ±0.7		
			Total	11.7 ±2.4		

Table 2 (continued)

	Age (yr)	*n*	Homocysteine species	Value[a] (μmol/L)	Study	Ref.
Female (prem.)	(20–39)	24	Free	2.0 ±0.9		
			Total	8.9 ±3.4		
	(40–49)	22	Free	2.0 ±1.0		
			Total	9.8 ±3.0		
Female (postm.)	(50–69)	37	Free	2.1 ±0.8		
			Total	10.0 ±2.8		
Male	(49 ±6)	255	Total	10.93 ±4.92	Genest et al., 1990	129

[a] Values are given as homocysteine equivalents, mean ± standard deviation.

[b] MDS, homocysteine–cysteine mixed disulfide.

[c] MDS accounts for most free (i.e., acid soluble) homocysteine in plasma.

[d] Bound = total homocysteine in stored samples, because free homocysteine becomes associated with plasma protein(s).

[e] Range, skew distribution of values.

Source: Modified from Ref. 39.

of a standard dose of methionine and determination of plasma homocysteine after a certain period (61).

Oral ingestion of methionine leads to a rapid increase in plasma methionine, which peaks within 1 hr (62). The marked elevation of plasma methionine is associated with an increase in free (16,18,19,42,62–66), protein-bound, and total homocysteine (21,49,59,67). Free homocysteine reaches a maximum after about 4 hr, whereas protein-bound homocysteine may lag a few hours behind, reaching a maximum after 4–6 hr (39,59). The half-life of total homocysteine is 12–24 hr in healthy subjects (Fig. 3).

The methionine-loading test has been used to reveal possible defects in methionine metabolism in patients with vascular disease. In these studies, methionine was administered at doses of 0.1 g/kg body weight (15,18,19,65,66) or 3.8 g/m^2 body surface (21,46,49,68), which in average are equivalent dosages (49). The homocysteine concentration in plasma or serum was determined 4 (15,19,46,49,68), or 6 hr (65) after methionine intake, or at several time points after loading, to obtain the peak values (18,59,64,66). The response to methionine loading is considered abnormal when postload homocysteine concentration (18,46,64–66,69) or the postload increase above preload value (49,68) exceeded the 95th percentile (65), or the mean plus 2 standard deviations for control subjects (46,49,64,68,69), or the highest control value (66).

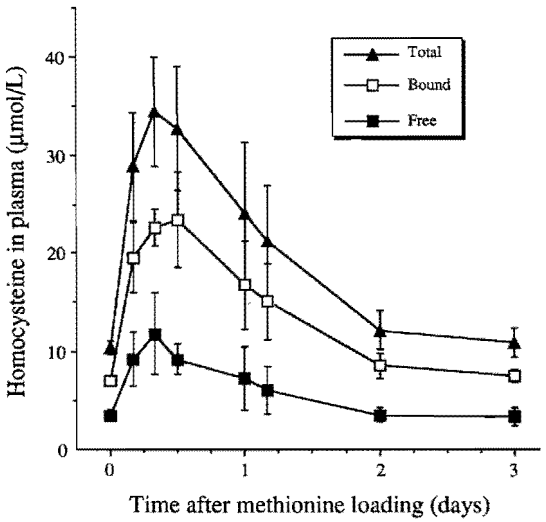


Figure 3 Free and protein-bound homocysteine in plasma after methionine loading. Seven healthy postmenopausal women were given peroral methionine 0.1 g/kg. Data are given as mean ± SD.

Knowledge of factors influencing the response to methionine loading is essential for the interpretation of results. In the rat, a methionine-rich diet induced the activity of enzymes involved in homocysteine catabolism (28,70), but excess dietary intake of methionine for 2 weeks did not affect the homocysteine response to methionine loading in healthy men and women (21).

Excess methionine is catabolized through the transmethylation–transsulfuration pathway (23), which agrees with the observation that the response to the methionine-loading test is abnormal in homozygotes (71) and in most heterozygotes (72,73) for cystathionine β-synthase deficiency. Some controversy exists on whether inefficient homocysteine remethylation because of a low concentration of folate or vitamin B_{12} may cause a pathological homocysteine response (49,59,66).

The plasma homocysteine response following methionine loading probably reflects homocysteine egress from the liver. Other cells and tissues export significant, but small, amounts of homocysteine. This conclusion is based on a study of methionine loading of hepatocytes and other cell types in culture (34).

HOMOCYSTINURIA CAUSED BY CYSTATHIONINE β-SYNTHASE DEFICIENCY

The term *homocystinuria* is often restricted to this defect. The prevalence is about 1:200,000 worldwide; somewhat higher in Ireland (1:10,000) (66) and

New South Wales (1:60,000) (74), but probably lower in Japan (75). The condition is inherited as an autosomal recessive trait (76,77). More than 700 patients have now been reported.

Clinical symptoms of cystathionine β-synthase deficiency are ectopia lentis, which involves the majority (90–100%) of the patients, mental retardation in 40–60% of the patients, seizures and abnormal electroencephalograms, and skeletal abnormalities, including disproportional growth and osteoporosis. Vascular disease is the major cause of death and will be detailed in a following section in this chapter.

The cystathionine β-synthase activity is deficient in various tissues and cells including cultured skin fibroblasts from homozygous patients. Enzymologic studies show that marked genetic heterogeneity exists. The residual activity, the affinity for serine and the cofactor pyridoxal 5′-phosphate, health stability, and immunological reactivity of the synthase vary markedly between patients (11).

A central biochemical feature of cystathionine β-synthase deficiency is a high concentration of homocysteine in plasma. Levels up to 250 μmol/L for free homocystine corresponding to more than 500 μmol/L of total homocysteine have been reported (53,78,79). Protein binding seems to become saturated and seldom exceeds 100 μmol/L (53). More than 1 mmol of homocystine may be excreted daily into the urine (78), in which it can be detected by a modified cyanide-nitroprusside test (80). A positive test should be verified by chromatographic analysis of plasma or urine.

In untreated patients, fasting plasma methionine may reach 2000 μmol/L (78,79). The elevation of methionine in plasma may be related to increased amounts of homocysteine available for remethylation. Plasma methionine varies among patients and may become particularly high in the newborn period, possible because of high activity of methionine synthase in the fetus. In some adult patients, plasma methionine increases following administration of betaine, choline, or folic acid, compounds that may enhance remethylation of homocysteine (11).

Treatment of cystathionine β-synthase–deficient patients aims at reducing the concentration of homocysteine in blood, since a relation between homocysteine level and severity of the disease has been demonstrated (81).

Methionine restriction is effective, and the results have been encouraging (81), but on such a diet, cysteine supplementation may become essential.

About 50% of patients with cystathionine β-synthase deficiency receiving high doses (250–1200 mg daily) of pyridoxine, a precursor of pyridoxal 5′-phosphate, have partial or complete normalization of their fasting plasma homocysteine level (16,82). The responders are not restored to biochemical normality, and an abnormal increase in plasma homocysteine after methionine loading persists (16). The view is held that the responsiveness is determined by properties of the mutant enzyme, and a correlation with residual enzyme

activity has been demonstrated (83). Notably, the vitamin B_6-responsive patients have a better overall prognosis than the nonresponsive patients (81).

Concurrent folate deficiency may prevent the beneficial effect from vitamin B_6 administration, and most patients receive folate supplements (84,85). Supplementing folic acid decreases the plasma serine level in these patients, probably owing to increased consumption of serine in the serine hydroxymethyltransferase reaction (86).

There have been reservations against the treatment of homocystinurics with the methyl donor, betaine, because of the possible detrimental effects from the resulting hypermethioninemia. Recently, there has been renewed interest in betaine, which seems particularly useful in pyridoxine nonresponding patients. Betaine reduces both the fasting plasma homocysteine level and the homocysteine response after methionine loading, and there are no significant side effects (87).

HOMOCYSTINURIA CAUSED BY DISORDERS OF HOMOCYSTEINE REMETHYLATION

More rare forms of homocystinuria are characterized by impaired remethylation of homocysteine to methionine, catalyzed by methionine synthase. The inborn defects in this pathway reported to date are either due to inadequate supply of 5-methyltetrahydrofolate or the cofactor, methylcobalamin.

5,10-Methylenetetrahydrofolate reductase deficiency was first described by Mudd and co-workers in 1972 (12), and about 30 patients have been described (88–91). The age of symptom debut varies, and central clinical features are neurological dysfunction and psychiatric symptoms, but anemia is usually lacking. Vascular lesions have been demonstrated upon autopsy (88).

Typical laboratory findings in these patients are elevation of homocysteine concentration in plasma and urine, normal or subnormal plasma methionine levels, and low serum and erythrocyte folate levels (88). Lack of 5,10-methylenetetrahydrofolate reductase activity is demonstrated in cultured skin fibroblasts and leukocytes (12,92).

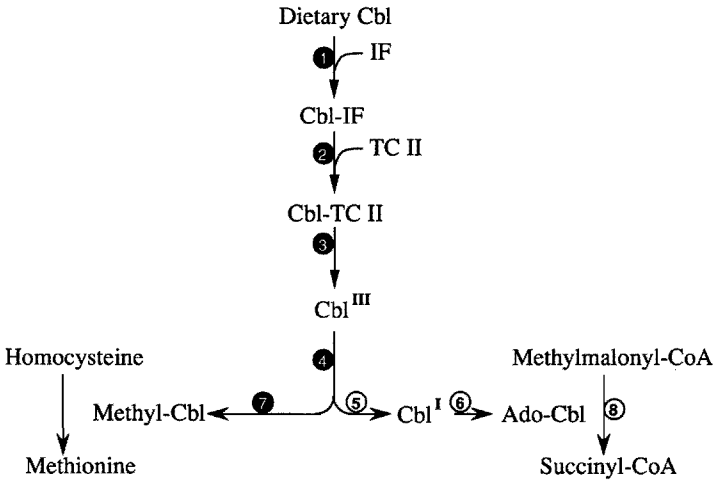
Several therapeutic strategies have been tried, including supplementing folate, methionine, cobalamin, and betaine (88). The therapeutic outcome has been variable (93), but betaine had a beneficial effect in all patients tested (89,94).

Recently, an infant with transcobalamin II deficiency and low absorption of cobalamin, who presented with methylmalonic aciduria and homocystinuria, has been described. She had a life-threatening pancytopenia (95).

Inherited disorders of cobalamin metabolism may affect either or both of the cobalamin-dependent enzymes, methionine synthase and methylmalonyl-CoA mutase. Involvement of methionine synthase results in elevated plasma homo-

cysteine and homocystinuria, whereas disorders involving methylmalonyl-CoA mutase are characterized by elevation of methylmalonic acid in plasma and urine. Seven complementation classes have been defined and designated mutations A–F (*cblA–cblF*) according to the chronology of their discovery (96). The sites of impairment of cobalamin metabolism or function are indicated in Figure 4.

The patients with *cblA* and *cblB* mutations have methylmalonic acidura, but neither megaloblastosis nor homocystinuria. The *cblE* and *cblG* mutations are characterized by homocystinuria, but no methylmalonic aciduria, whereas *cblC, cblD,* and *cblF* mutations have both homocystinuria and methylmalonic aciduria (97).



Site	Mutant class	Site of defect
❶	—	Intrinsic factor
❷	—	Transcobalamin II
❸	cblF	Lysosomal cobalamin (Cbl) efflux
❹	cblC, cblD	Reduction of cbl^{III} to cbl^{II} in cytosol
⑤	cblA	Reduction of cbl^{II} to cbl^{I} in mitochondria
⑥	cblB	Cob(I)alamin adenosyltransferase
❼	cblE, cblG	Methionine synthase
⑧	mut	Methylmalonyl-CoA mutase apoenzyme

Figure 4 Sites of defects in cobalamin metabolism. The defects marked with a dark circle are associated with hyperhomocysteinemia. Ado-Cbl, adenosylcobalamin; Cbl, cobalamin; IF, intrinsic factor; methyl-Cbl, methylcobalamin; TCII, transcobalamin II.

Patients with cobalamin mutations involving homocysteine remethylation (C, D, F, E, and G; see Fig. 4) usually have psychomotor retardation, lethargy, failure to thrive, and megaloblastic anemia. Most patients come to medical attention in infancy or later in childhood, but onset may be delayed until adolescence (*cblC, cblG*) with predominantly neurological symptoms (97). Thromboembolic disease occurs, and vascular lesions have been demonstrated upon autopsy. These lesions will be described in detail in the following.

CARDIOVASCULAR DISEASE IN HOMOCYSTINURIA

Cystathionine β-Synthase Deficiency

The cardinal vascular sign in cystathionine β-synthase deficiency is thrombosis, occurring in both the arterial and venous system, and affecting most arteries and veins (6–8). Vascular occlusions, even leading to death, may occur at any age. On the basis of the early series comprising the most severely affected cases, it was assumed that more than 50% of cystathionine β-synthase–deficient patients died of vascular disease before aged 20 years (98). However, this conclusion was refined when the heterogeneity in the clinical expression of the metabolic defect was recognized by investigating also mildly affected patients.

In 1982–1983, Mudd et al. (81) conducted a large international questionnaire survey on 629 patients with homocystinuria resulting from cystathionine β-synthase deficiency. Of these patients, 158 had a total of 253 thromboembolic events; 81 (32%) were cerebrovascular accidents; 130 (51%) affected peripheral veins, with 32 resulting in pulmonary embolism; 10 (4%) produced myocardial infarction; and 28 (11%) affected peripheral arteries. In the untreated patients, 25% had at least one clinically apparent event by aged 16 years, and 50% by aged 29. A time-to-events graph for the first thromboembolic episode is shown in Figure 5. Once a patient had one thromboembolic event, he or she passed into a statistically higher-risk category. Pathological examination revealed that considerable vascular disease remained clinically silent (81).

Of the 629 cases enrolled, 64 had died. Thromboembolism was the main cause of death in 42 patients and was probably a contributing factor in additional 5 patients. By aged 20 years, mortality was less than 5% among those patients who were responsive to pyridoxine treatment, and about 20% in nonresponsive patients (81). Time-to-events graph for deaths is shown in Figure 6.

Several reports describe the vascular pathology in cystathionine β-synthase deficient patients (6–9,98–102). In many cases, extensive, often multifocal vascular changes in form of multiple arterial and venous thromboses and widespread arteriosclerotic lesions in most large and medium-sized arteries have been found at autopsy (6,8,102). In the first decade of life, death has

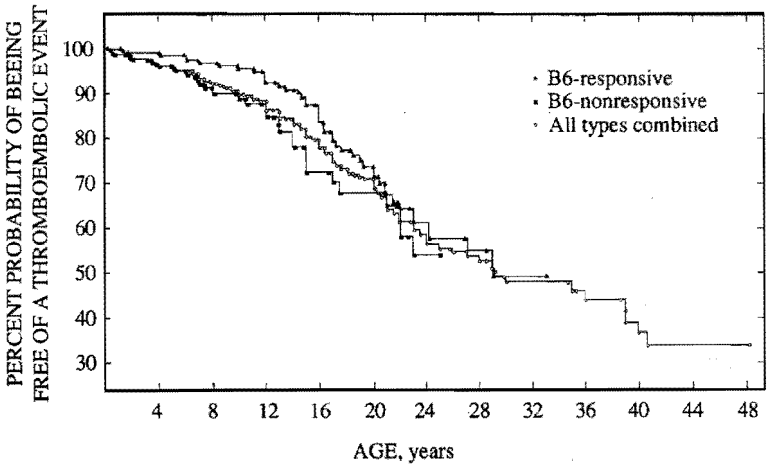


Figure 5 Time-to-event graphs for first thromboembolic event in untreated patients with cystathionine β-synthase deficiency. Data for 627 patients were used for the "all-types" curve. (From Ref. 81.)

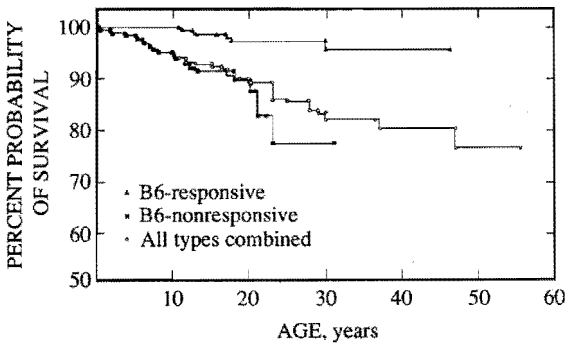


Figure 6 Time-to-event graphs for deaths. Patients were not removed from the at-risk groups upon initiation of therapy. Data for 629 patients were used for the "all-types" curve. (From Ref. 81.)

occurred of carotide artery occlusion leading to ischemic stroke (9), thrombosis of intracranial sinuses and veins (103), myocardial infarction caused by coronary artery disease (102,104), thrombophlebitis with secondary pulmonary embolism (4,98,99), thrombotic occlusion of renal arteries (6) or veins (100) or vena porta (98), and pancreatitis with multiple thromboses (105). Patients are

described with hypertension owing to renal artery stenoses or occlusion (98,106) and with cor pulmonale caused by pulmonary embolism (98). Infarction of kidneys and the myocardium has been described (102,106,107). Multiple small and large infarcts of different ages have been observed in both gray and white matter of the central nervous system (6,8,100).

Microscopic examinations have revealed similar vascular changes in several patients (9,98,100,102). There is intimal thickening caused by fibrosis and proliferation of connective tissue, which focally may obliterate the lumen. The elastic membrane is often thickened, frayed, split, reduplicated, and disorganized. The tunica media may be thinned and shows increased amounts of unidentified ground substance, disorganization of elastic wave, and cystic material resembling media necrosis (7,98). Fatty atheromatous plaques have only occasionally been observed in these young patients (6).

Inborn Errors of Homocysteine Remethylation

Of 23 patients with 5,10-methylenetetrahydrofolate reductase deficiency, 12 have died, and autopsy data have been reported for 7 cases, aged from 9 months to 10 years (10,88,94,108–110). Of the 7 cases, 2 had no vascular abnormalities or only minor changes, whereas prominent vascular pathology was found in 5 patients. In 3 cases, cerebral venous thromboses were possible cause of death (88). Thromboses in pulmonary arteries and lung infarcts were reported in 2 patients (10,88,94). Three patients had widespread arterial lesions, characterized by intimal hyperplasia and fibrosis with focal fragmentation and disruption of elastic lamellae in the media and partial destruction of the inner elastic membrane (10,88,109). Cerebral arterioles in 2 patients showed prominent endothelial cell proliferation and thick hyalinized walls, sometimes resembling fibrinoid necrosis. The vascular changes were considered to be similar to those observed in homocystinuria caused by cystathionine β-synthase deficiency or cobalamin defects (10,109).

About 30 cases of homocystinuria caused by inherited defects of cobalamin metabolism have been described. Five patients have died, and results of postmortem examination of 4 patients aged 7 1/2 weeks, 3 months, 4 months, and 7 years, have been published (9,109,111–113). Vascular lesions were found in all these patients and was a contributing cause of death in at least 1 case who died of acute cor pulmonale secondary to diffuse pulmonary thromboembolism (113). Two patients had focal arteriosclerotic lesions in the large, medium-sized, and small arteries, with intimal proliferation and splitting, fraying, and disruption of the internal elastic membrane, leading to narrowing of the arterial lumen (9,109,112). These lesions resembled those found in cystathionine β-synthase–deficient patients. In the remaining patient, intimal swelling and endothelial cell proliferation, "fibrinoid necrosis"-like lesions were observed in

arterioles in the white matter of the brain (111). A patient with elevated plasma homocysteine caused by a cobalamin D mutation suffered four thromboembolic episodes between aged 18 and 20 years (114).

Most of the patients with elevated plasma homocysteine caused by 5,10-methylenetetrahydrofolate reductase deficiency or defects in cobalamin metabolism are very young and receive homocysteine-lowering therapy. This complicates the evaluation of homocysteine as a pathogenic factor.

CARDIOVASCULAR DISEASE AND HYPERHOMOCYSTEINEMIA

During the last decade, the question of whether or not mild hyperhomocysteinemia is also associated with increased risk for vascular disease, has gained increasing attention. This issue was first addressed by Wilcken and Wilcken in 1976 (15). They found that the concentration of homocysteine–cysteine mixed disulfide in plasma after methionine loading was higher in patients with coronary heart disease, compared with controls. The pioneering work was followed by numerous clinical studies indicating that minor genetic defects in methionine metabolism, such as heterozygosity for cystathionine β-synthase deficiency, or acquired anomalies caused by lack of cofactors, may increase the risk for vascular disease. These studies are reviewed in the following.

Identification of Heterozygotes for Cystathionine β-Synthase Deficiency

Heterozygosity for cystathionine β-synthase deficiency is estimated to be present in 0.3–1%, or 2% at most, of the general population (11,66,115). Attempts to identify heterozygotes have been made by determination of cystathionine β-synthase activity in liver, in phytohemagglutinin-stimulated or long-term cultured lymphocytes, or in cultured skin fibroblasts (11,73). McGill et al. (73) reviewed 12 articles describing cystathionine β-synthase activity in obligate heterozygotes. The mean enzyme activity was about one-third normal, instead of one-half as expected. The interindividual variation was considerable, with marked overlap between heterozygotes and controls.

Determination of homocysteine in plasma or serum during fasting or following a standard methionine load has been evaluated as an approach to discriminate between heterozygotes and normal controls. Fasting values for free, protein-bound, or total homocysteine in plasma or serum of obligate heterozygotes are either not significantly higher (49,116) or only moderately increased (43,53,117), as compared with controls. Most investigations show a considerable overlap between the two groups (73).

Determination of homocysteine in plasma or serum after methionine loading improves the discrimination between heterozygotes and normal subjects, but also under this condition, there is overlap between the groups (49,66,73,118). In two recent studies, 18 of 20 and 23 of 25 obligate heterozygotes, respectively, were discriminated from normal subjects by postload values for free homocysteine in serum (66,118). When the methionine-loading test was combined with determination of cystathionine β-synthase, all 20 obligate heterozygotes could be segregated from the controls (118). In another study, the increase in total plasma homocysteine at 4 hr after methionine loading identified 16 of 20 obligate heterozygotes, and probably 1 heterozygote among 46 control subjects (49,69). Thus, no single screening procedure allows the unequivocal identification of heterozygosity for cystathionine β-synthase deficiency.

DNA analysis is an obvious alternative, but allelic and genetic heterogeneity complicates that approach (73).

Abnormal Methionine-Loading Test and Heterozygosity for Cystathionine β-Synthase Deficiency in Patients with Cardiovascular Disease

Wilcken and Wilcken (15) found a significant difference in the amounts of plasma homocysteine–cysteine mixed disulfide after methionine loading between male patients aged below 50 years with coronary heart disease and matched controls. Detectable levels were found in 17 of 25 patients and only in 5 of 22 control subjects. Seven patients (28%) and 1 control (5%) had postload values within the range previously reported in obligate heterozygotes for cystathionine β-synthase deficiency (63). These results prompted Mudd et al. (119) to assess by questionnaire the frequencies of heart attacks and stroke in parents (n = 394, 100% heterozygotes) and grandparents (n = 776, 50% heterozygotes) of children with cystathionine β-synthase deficiency. This study failed to detect a significantly increased risk for heart attacks or strokes in heterozygotes for cystathionine β-synthase deficiency, and virtually excluded an increase in the cardiovascular risk of as much as fivefold compared with controls. Swift and Morrell (120) questioned the validity of some methods and the conclusion of Mudd et al. and stated that the data may indicate a relation between heterozygosity for cystathionine β-synthase deficiency and death of cardiovascular disease.

Since then, in the period from 1982 to 1991, ten additional studies have addressed the risk for cardiovascular disease in the carrier state for cystathionine β-synthase deficiency (18,19,46,49,64–66,68,121,122). In two of these studies, obligate heterozygotes and controls were investigated by noninvasive techniques for the presence of subclinical occlusive arterial disease

(121,122). Clarke (121) found no evidence of increased frequency of endothelial plaques in the neck arteries of 25 heterozygotes (mean age, 42 years) compared with 21 control subjects, whereas the finding of Rubba et al. (122) indicated more frequent early vascular lesions in the iliac and internal carotid arteries in 14 heterozygotes (mean age, 46 years) than in 47 controls.

In the remaining eight studies, defects in methionine metabolism in patients with premature (onset before 55 or 60 years) vascular disease were evaluated by the methionine-loading test (Table 3). In six of these studies, a reference group of obligate heterozygotes for cystathionine β-synthase deficiency was also investigated and the response to methionine loading was compared with that observed in controls and the patients with vascular disease (18,46,49,64–66). Two studies also included the measurement of cystathionine β-synthase activity in cultured skin fibroblasts from those patients who responded abnormally to methionine (64,66). Results of the methionine-loading test were based on measurement of homocysteine–cysteine mixed disulfide (19), homocystine (65), or both species (18,64,66), or on total homocysteine (46,49,68). The results, summarized in Table 3, show that an abnormal homocysteine response to methionine loading was more frequent among patients with vascular disease than in controls. The percentage of patients with abnormal methionine-loading test was higher in the categories for cerebral or peripheral arterial disease (28–42%) than in patients with coronary artery disease (0–30%; see Table 3).

When results from nine studies are pooled, abnormal homocysteine response to methionine was observed in 121 of 495 patients with vascular disease (24%), but only in 7 of 289 control subjects (2%). This difference is highly significant. From the pooled data, an odds ratio of 13.0 (95% confidence interval 5.9–28.1) can be calculated. This ratio expresses the relative cardiovascular risk in persons who have an abnormal response versus those who respond normally.

All patients with abnormal homocysteine response showed postload levels within the range of, or even higher than, that found in obligate heterozygotes for cystathionine β-synthase deficiency (18,46,49,64–66). This was a consistent finding with few exceptions (118). Boers et al. (64,71) found reduced cystathionine β-synthase activity in cultured skin fibroblasts in all of 60 patients with vascular disease who responded abnormally to methionine loading. However, in a recent study by Clarke et al. (66), reduced cystathionine β-synthase activity in cultured skin fibroblasts was demonstrated in 18 of 23 patients with vascular disease and abnormal methionine loading test, suggesting that the cystathionine β-synthase activity is not the only determinant of the postload homocysteine level. They found that the homocysteine response was inversely related to red cell folate and vitamin B_{12} levels (66). This suggests that the postload homocysteine level may also be affected by folate and vitamin B_{12}-dependent remethylation of homocysteine.

Table 3 Frequencies of Abnormal Homocysteine Response to Methionine Loading in Patients with Vascular Disease and in Controls

Study	Ref.	Vascular disease[a]	Patients (*n*)	Controls (*n*)	Abnormal response[b]				Significance[c]
					Patients		Controls		
					(*n*)	(%)	(*n*)	%	
Wilcken and Wilcken, 1976	15	CHD	25	22	7	(28)	1	(5)	$p = 0.09$
Wilcken et al., 1983	18	CHD	20	20	2	(10)	0		$p = 0.47$
Brattström et al., 1984	19	CVD	18	17	5	(28)	0		$p = 0.06$
Boers et al., 1985	19	CHD	25		0				
		CVD	25	40	7	(28)	1	(2)	$p < 0.01$
		PAD	25		9	(36)			$p < 0.001$
Murphy-Chutorian et al., 1985	65	CHD	99	39	16	(16)	1	(3)	$p = 0.06$
Israelsson et al., 1988	46	CHD	21	36	3	(14)	1	(3)	$p = 0.27$
Brattström et al., 1990	49	CVD	35	46	12	(34)	1	(2)	$p < 0.001$
		PAD	37		14	(37)			$p < 0.001$
Brattström et al., 1991	68	VTD	42	42	5	(14)	2	(5)	$p = 0.26$
Clarke et al., 1991	66	CHD	60		18	(30)			$p < 0.01$
		CVD	38	27	16	(42)	0		$p < 0.001$
		PAD	25		7	(28)			$p < 0.02$
Pooled results			495	289	121	(24)	7	(2)	$p < 0.0001$

[a] CHD, coronary artery disease; CVD, cerebrovascular disease; PAD, peripheral arterial disease; VTD, venous thromboembolic disease.

[b] Abnormal response = higher than control mean plus 2 SD, over 95th percentile for controls, or higher than any control value.

[c] Chi-square test with continuity correction factor.

The fasting plasma homocysteine level but not the postload increase in homocysteine, was elevated in subjects with low or low-normal serum folate or vitamin B_{12} values (49,69). Therefore, the postload increase in homocysteine concentration was considered to be a more specific marker for cystathionine β-synthase activity than the postload concentration (49,69).

Factors other than vitamin B_{12} or folate levels may affect the methionine-loading test. High methionine doses given to overweight patients with coronary heart disease have been suggested to cause increased homocysteine response (18), but such a relation has not been found by others (19,49,65,68). Humans receiving a vitamin B_6-deficient diet show increased urinary excretion of homocysteine after methionine loading (123,124), but no relation between low plasma pyridoxal 5′-phosphate concentration and the results of methionine loading was found in 72 patients with vascular disease (49). The presence of heterozygotes for cystathionine β-synthase deficiency among these patients may have obscured such a relation.

In the studies on methionine loading of patients with cardiovascular disease (see Table 3), abnormal responses were observed in 2% of the control subjects. This figure fits the highest estimate for the prevalence of heterozygotes for cystathionine β-synthase deficiency in the general population (66). Recently, Andersson et al. (125) found that 8% of 169 healthy subjects responded abnormal to methionine loading, and this figure far exceeds the estimated prevalence of heterozygotes. Moreover, very high frequencies of abnormal methionine-loading test have been found among psoriatic patients (59) and patients treated with the antiepileptic drug phenytoin (126). There is an age-related decrease in the cystathionine β-synthase activity in cultured skin fibroblasts, and markedly lower values are obtained in the middle-aged and elderly subjects, compared with children and young adults (126). These data suggest that conditions other than heterozygosity for cystathionine β-synthase deficiency may also result in an abnormal response to methionine loading or low cystathionine β-synthase activity in cultured skin fibroblasts.

Basal Plasma Homocysteine in Patients with Cardiovascular Disease

Data from 18 studies on basal plasma homocysteine in patients with vascular disease are known to us (18,19,45–49,51,65,68,127–134). These include more than 1500 patients and 1400 controls (Table 4). In some of these studies (listed in Table 3), the patients were also subjected to a methionine loading, the results of which were presented in the foregoing. The first 3 studies (18,19,65) were based on measurement of free, non-protein-bound homocysteine, whereas, in the remaining 15 studies (45–49,51,68,127–134), total plasma homocysteine was determined. All but 1 study showed higher fasting plasma

concentration of homocysteine in patients with cardiovascular disease than in controls, and in 14 studies, this difference was statistically significant. In the studies of patients with cerebrovascular disease or peripheral arterial disease, the ratio between the mean plasma homocysteine concentration in patients versus controls were on average higher than in the studies of patients with coronary artery disease or venous thromboembolic disease (see Table 4). The (overall) frequencies of fasting plasma homocysteine above normal (i.e., hyperhomocysteinemia) were between 23 and 47% in patients with cerebral or peripheral occlusive arterial disease (47,49,51,69,127,131), between 10 and 24% in patients with coronary artery disease (18,46,65,128–130), and up to 7% in controls (49). This difference among patient categories, which resembles that observed with the methionine-loading test (see Table 3), led Boers et al. (64) to propose that it was reminiscent of the propensity sites of vascular damage induced by homocysteine in homozygotes for cystathionine β-synthase deficiency (81). However, a recent study could not demonstrate a relation between hyperhomocysteinemia and venous thromboembolism (68), which constitute half of the vascular events in homozygotes (81), and this observation may confound the analogy.

In addition to the studies just cited, McCully and Vezeridis (135) recently reported high homocysteine thiolactone concentrations in plasma from patients with cardiovascular disease. Others (136,137) could not confirm these results, and the authors have withdrawn the data (138). Likewise, Olszewski and Szostak (139) found very high homocysteine levels in plasma hydrolysates from patients with myocardial infarction, but not from controls. This finding has not been confirmed by others (140,141).

Hyperhomocysteinemia and Conventional Risk Factors for Cardiovascular Disease

The relations between plasma homocysteine and established risk factors for vascular disease have been examined. In several studies (19,47,49,65, 128,129,131,133,134), no relation was found between plasma homocysteine level and cholesterol or triglycerides in serum. There are occasional reports on weak but statistically significant correlations between plasma homocysteine and serum cholesterol in patients with coronary artery disease (45), intermittent claudication (51), and in control subjects, but not in patients with cerebrovascular disease (48). In the recent study conducted by Mölgaard et al. (51), plasma homocysteine significantly correlated with both LDL cholesterol and apolipoprotein B levels, but these relations were due to covariation with serum folate, and could not be confirmed with multiple linear regression analysis (51).

Table 4 Results of Plasma Homocysteine Measurements in Patients with Vascular Disease and in Controls

Study	Ref.	Vascular disease[a]	Patients (*n*)	Controls (*n*)	Patient/control homocysteine ratio[b]	Significance
Wilcken et al., 1983	18	CHD	20	20	0.97	ns
Brattström et al., 1984	19	CVD	19	17	1.46	$p < 0.5$
Murphy-Chutorian et al., 1985	65	CHD	99	39	1.18	ns
Kang et al., 1986	45	CHD	241	202	1.29	$p < 0.001$
Israelsson et al., 1988	46	CHD	21	36	1.21	$p < 0.05$
Araki et al., 1989	48	CVD	65	90	1.40	$p < 0.001$
Malinow et al., 1989	47	CVD/PAD	47	29	1.60	$p < 0.05$
Brattström et al., 1990	49	CVD	35	46	1.17	$p < 0.05$
		PAD	37		1.70	$p < 0.001$
Coull et al., 1990	127	CVD	68	31	1.44	$p < 0.001$
Malinow et al., 1990	128	CHD	99	259	1.20	$p < 0.05$
Brattström et al., 1991	68	VTD	42	42	1.19	ns
Genest et al., 1990	129	CHD	170	225	1.26	$p < 0.001$
Williams et al., 1991	130	CHD	37	48	1.29	$p < 0.05$
Brattström et al., 1991	131	CVD	70	66	1.41	$p < 0.001$
Mölgaard et al., 1991	51	PAD	78	98	1.21	$p < 0.001$
Mereau-Richard et al., 1991	132	CVD	92	25		
Taylor et al., 1991	133	PAD	214	103	1.42	$p < 0.05$
Ubbink et al., 1991	134	CHD	129	34[c]	1.23	$p < 0.01$
Pooled results			1583	1410	1.31 ±0.17	

[a] CHD, coronary artery disease; CVD, cerebrovascular disease; PAD, peripheral arterial disease; VTD, venous thromboembolic disease.

[b] Ratio between mean plasma homocysteine concentrations in patients and controls.

[c] Patients with angiographically normal coronary arteries are taken as controls.

In ten studies (19,45,48,49,51,65,127,129,131,133), no relation was found between plasma homocysteine and blood pressure or hypertension in patients with vascular disease. In one study (47), hypertension was more common in patients with vascular disease and elevated plasma homocysteine than in patients with normal homocysteine, and there is one report (129) on a trend toward lower plasma homocysteine in patients taking beta-blockers than in patients who did not. In another study (48), total plasma homocysteine was significantly increased in hypertensive compared with normotensive controls, and plasma homocysteine tended to be lower in those receiving antihypertensive treatment, and was positively related to serum creatinine. The difference in plasma homocysteine between hypertensives and normotensive controls persisted after adjustment for serum creatinine, indicating that elevated blood pressure per se may affect the concentration of homocysteine in plasma (48).

Tobacco smoking does not seem to influence plasma homocysteine levels (19,47–49,51,65,127–129,131), and in only 1 (130) of 11 studies, a relation has been found between plasma homocysteine and smoking habits.

In patients with vascular disease, plasma homocysteine was no different in diabetic versus nondiabetic subjects, and it was not related to the blood glucose level (47,51,65,127–131).

MULTIPLE DETERMINANTS OF PLASMA HOMOCYSTEINE LEVEL AND THEIR RELATION TO VASCULAR DISEASE

The cause of increased fasting plasma homocysteine in patients with vascular disease is probably multifactorial. In two large studies (45,129), the frequency distributions of plasma homocysteine values were shifted to the right for patients with coronary artery disease compared with controls. The increase in mean plasma homocysteine for the patient group was not explained by the presence of few patients with a marked elevation in homocysteine, which might be expected if the difference was due to a monogenic trait.

Genetic, pathological, and environmental conditions that may influence the plasma homocysteine level, are reviewed in the following.

Genetics

Wilcken et al. (18) found a marked hyperhomocysteinemia during fasting and after methionine loading in a pair of identical male twins, both with premature coronary artery disease. Recent studies report on a significant higher correlation for the plasma homocysteine concentration within pairs of 96 monozygote twins, compared with 92 dizygote twins (142), and a strong familial correlation of plasma homocysteine among 26 male sibling pairs, including individuals both with and without early coronary heart disease. Notably, there was no such

correlation among spousal pairs (130). These findings show that the plasma homocysteine level is influenced by genetic factors.

The plasma homocysteine level in frozen samples stored for up to 14 years strongly correlated with the level in fresh plasma samples from the same individuals, and the plasma levels both in healthy subjects and in patients with vascular disease are relatively stable over years (49,125,131). This finding supports the possibility that plasma homocysteine levels are influenced by genetic factors.

Kang et al. (20,143) have recently described the occurrence in patients with vascular disease of a mutant variant of methylenetetrahydrofolate reductase, characterized by 50% of normal activity and thermolability. Compound heterozygotes for the classic methylenetetrahydrofolate reductase deficiency and the thermolabile enzyme have also been described (144). This thermolabile variant is inherited as an autosomal recessive trait, and was found in 17% of 212 patients with coronary heart disease, but in only 5% of 202 controls (145). Both patients and controls with mutant enzyme had significantly elevated plasma homocysteine. It was suggested that this enzymic defect may render these subjects susceptible to the development of hyperhomocysteinemia provoked by several nongenetic factors (145).

Healthy male South African blacks, with a low incidence of coronary artery disease, have about 30% lower plasma total homocysteine than healthy South African Caucasian men, with a high incidence of coronary artery disease (134). This difference may be due to genetic or nutritional factors.

Down Syndrome

The gene for cystathionine β-synthase has been assigned to chromosome 21 (146), and patients with Down syndrome have an increased gene dose and increased activity (166%) of cystathionine β-synthase (147).

Murdoch et al. described the absence of atherosclerotic disease in five patients aged 40 to 66 years, whereas mentally retarded patients from the same institution had mild to severe atheromatosis (148). They suggested that Down syndrome may represent an atheroma-free model. The absence of atheromatosis in these patients has been contested by others (149), but recently confirmed in two studies of 30 adult patients with Down syndrome (150,151).

Patients with Down syndrome have low blood pressure (148), but normal blood lipid levels (148,152,153), and the cause of low incidence of atheromatosis is enigmatic. Brattström et al. (151) suggested that Down syndrome may protect against atherosclerosis because of a more efficient homocysteine catabolism. Fasting and postload plasma homocysteine significantly below normal levels was demonstrated in eight patients aged 6–10 years (58), whereas the plasma levels in nine adult patients were no different from controls (154).

The controversies about the occurrence of atheromatosis and hypohomocysteinemia in patients with Down syndrome may reflect variability between patients. Prospective clinical studies and further biochemical investigations are warranted.

Age and Gender

Men have higher fasting plasma homocysteine than women (41,45,52,56,125). Data from some (42,62), but not all (125), studies suggest that men also have higher postload homocysteine than women, and that the fasting homocysteine level in women increases after the menopause, so that postmenopausal women attain the level found in men (45). In a comprehensive study, including 169 healthy subjects, it was found that the fasting level in men increased as a function of age, and a small subpopulation of postmenopausal women showed high postload homocysteine levels (125).

The age- and sex-related differences in plasma homocysteine may reflect that homocysteine metabolism is induced by sex hormones (42). It has also been suggested that the age-dependent increase in men may be related to a decrease in cofactor levels with age (125). Plasma homocysteine shows variations related to age and gender that paralleled the risk of cardiovascular disease (155), and efficient methionine metabolism may protect women against vascular disease (42).

Nutritional Factors

Nutritional factors leading to hyperhomocysteinemia include deficiencies of vitamin B_{12}, folate, or vitamin B_6 (39). Folate or cobalamin deficiency may result in elevated plasma homocysteine to the levels observed in inherited forms of homocystinuria (116,156–161). Even in subjects with low-normal serum or blood folate or serum cobalamin, plasma homocysteine may be elevated above normal (46,49,156).

In one study, patients with megaloblastic anemias had a significantly higher mortality from cardiovascular causes than controls with iron deficiency anemia (162). There is one report on cardiovascular lesions resembling those observed in early arteriosclerosis in sheep fed a vitamin B_{12}-deficient diet for 34 weeks (163). However, no differences in the extent or severity of arteriosclerosis or signs of thromboembolism were found between a small number of autopsied patients with pernicious anemia and matched controls (164).

There are several possible explanations why a clear relation between hyperhomocysteinemia caused by folate and cobalamin deficiencies and premature vascular disease has not been described. First, this relation has not been carefully investigated. Second, nutritional vitamin B_{12} deficiency usually develops at advanced age (165). Third, some signs associated with megaloblas-

tic anemias, including thrombocytopenia, defects of platelet function, and hypocholesterolemia (165), may protect against thromboembolism.

In several studies of patients with vascular disease, serum or blood folate and serum vitamin B_{12} have been no different in patients and controls (19,46,49,51,68,69,131). Notably, in patients with vascular disease, but not in healthy controls, plasma homocysteine is strongly and negatively related to serum folate, but also to serum vitamin B_{12} (49,51,69,131,134). This points to the interesting possibility that, in patients with vascular disease, the presence of genetic or acquired conditions, such as heterozygosity for cystathionine β-synthase deficiency, thermolabile methylenetetrahydrofolate reductase, or reduced renal function, may increase the demand for folate (49,130,131).

Results from several epidemiological studies relate vitamin B_6 deficiency to atherosclerosis (49,166–169), and risk factors for vascular disease, such as smoking (170) and intake of oral contraceptives (171–173), induce low plasma vitamin B_6.

A strong, negative correlation between plasma pyridoxal 5′-phosphate and plasma homocysteine in patients with vascular disease has been found in one, but not in another, study (49,131). Urinary homocysteine excretion and plasma homocysteine were increased and inversely correlated with blood folate and pyridoxal 5′-phosphate in 142 patients with stroke (131).

Renal Function

A significant, positive correlation between fasting plasma homocysteine and serum creatinine has been found in six studies on patients with vascular disease but without renal failure (45,46,48,51,127,131). This may suggest that renovascular arteriosclerosis may be a cause of increased plasma homocysteine in patients with vascular disease (127,131). However, the relation between homocysteine and creatinine in serum or plasma may reflect that most homocysteine in humans is formed in conjunction with creatine–creatinine synthesis (174). Plasma homocysteine, but not urinary homocysteine, excretion was related to serum creatinine, suggesting that prerenal factors may contribute to the hyperhomocysteinemia in these patients (131). There are reports on significant positive correlations between plasma homocysteine and serum uric acid in patients with vascular disease and in controls (45,48,51,127).

Multiple linear regression analyses show that serum folate or blood folate and serum creatinine in particular, but also age and levels of serum vitamin B_{12} and plasma pyridoxal 5′-phosphate, are predictors of plasma homocysteine concentration. These factors may account for 30–40% of the values for plasma homocysteine in patients with vascular disease (49,51,131).

Patients with chronic renal failure (175–177), including those regularly treated with dialysis (178), have plasma homocysteine levels two- to fourfold

above normal. Plasma homocysteine is positively correlated with serum creatinine (175,178); it is somewhat reduced following dialysis (176) and after folic acid administration, even in patients with normal serum folate (179). This may suggest shortage of intracellular folate. The cause of elevated homocysteine in plasma from patients with renal insufficiency could be related to increased homocysteine formation, or to inhibition of renal excretion or metabolism. Plasma serine is low in patients with renal failure (86,180) and is further reduced after folic acid intake (180), suggesting efficient homocysteine metabolism through remethylation and conversion to cystathionine (see Fig. 2) in patients with renal failure. The mechanism of elevated plasma homocysteine should await elucidation of the role of the kidneys in homocysteine homeostasis.

Chronic renal failure imposes an increased risk of atherosclerosis (181,182), and cardiovascular disease is the major cause of death of patients receiving hemodialysis (183). The pathogenesis of vascular disease in renal failure is probably multifactorial, since it is associated with several predisposing conditions, such as hypertriglyceridemia with low HDL cholesterol (184), hypertension, and increased levels of factor VIII/von Willebrand's factor (181). An increased concentration of homocysteine in plasma may be an additional contributing factor. Thus, folic acid may protect against vascular disease in patients with renal failure. This exciting possibility requires substantiation.

Diabetes Mellitus

Hultberg et al. measured total plasma homocysteine in 79 patients with type I diabetes mellitus (185). They found no evidence that diabetes mellitus per se or the presence of diabetic retinopathy affects plasma homocysteine concentration. In contrast, patients with an increased albumin/creatinine clearance ratio or serum creatinine had markedly increased homocysteine levels. Thus, elevated homocysteine in diabetic patients is probably related to reduced renal function (i.e., diabetic nephropathy).

Patients with diabetes mellitus often develop premature cardiovascular disease. Diabetes is associated with established cardiovascular risk factors, such as hypertension, elevated LDL cholesterol and low HDL, obesity, and smoking (186,187). However, these factors do not fully explain the predisposition to cardiovascular disease, since patients free of these major cardiovascular risk factors still have an increased risk (155). Nephropathy in type I diabetes mellitus often develops before cardiovascular disease (186,188). The association between diabetic nephropathy and elevated plasma homocysteine (185) suggests that hyperhomocysteinemia may contribute to the diabetic atherogenesis.

Psoriasis

Patients with severe psoriasis have significantly higher fasting plasma homocysteine concentrations than matched controls, and a few had postload levels above normal. The psoriasis patients had serum folate within the normal range, but significantly below the serum folate in the controls (59). The elevated plasma homocysteine may be related to the large burden of rapidly proliferating cells (189).

A retrospective study involving 323 psoriasis patients compared with matched controls has demonstrated increased incidence of vascular disease in psoriatics, especially those with other predisposing factors. In the older-aged group, there seems to be a relation between percentage body coverage and increased incidence rates (190). This adds psoriasis to the conditions indicating a relation between elevated plasma homocysteine and vascular disease.

Drugs

Azaribine (6-azauridine triacetate) was effective against refractory cases of psoriasis, but was prohibited by the Food and Drug Administration in 1976 because its use was associated with increased incidence of thromboembolism (17). Azaribine induced a hyperhomocysteinemia that was related to the occurrence of vascular disease (191,192). Experimental studies have provided evidence that azaribine causes increased plasma homocysteine by acting as a vitamin B_6 antagonist, thereby inhibiting the enzyme cystathionine β-synthase (17).

Methotrexate, given in doses from 25 mg to 33 g, induces a transient increase in plasma homocysteine (57,59,193,194). We have speculated (195) on whether this effect may contribute to the thrombotic diathesis in patients receiving a chemotherapeutic regimen containing methotrexate (196).

There are indications that contraceptives with an estrogenic component may affect the metabolism of sulfur amino acids, including homocysteine. Subjects taking contraceptives seem to excrete less cystathionine following a methionine load than do controls (172). Wong et al. observed a marked reduction in plasma homocysteine in most women taking oral contraceptives, whereas some had high levels (197). Low excretion of cystathionine and high plasma homocysteine levels may be explained by decreased activity of the vitamin B_6-dependent enzyme cystathionine β-synthase. Contraceptives have been suggested to induce vitamin B_6 deficiency in a small subgroup of women. Low levels of plasma vitamin B_6 may be related to redistribution of the vitamin by stimulation of some vitamin B_6-dependent enzymes by estrogens (173).

The increased risk of thromboembolism in users of contraceptives is established. The estrogen component seems to induce "hypercoagulability" and

increases the risk of both arterial and venous events. Progesterone may affect blood pressure and cause arterial disease (198). It should be considered whether a small subpopulation of women who use contraceptives develop a hyperhomocysteinemia, and whether this population accounts, at least in part, for the increased risk of thromboembolic disease imposed by these drugs (155,199).

MILD HYPERHOMOCYSTEINEMIA: CAUSE OR INDICATOR?

Marked elevation in plasma homocysteine is a central feature of various inborn errors of homocysteine metabolism. As reviewed in detail earlier, patients afflicted with different metabolic defects involving homocysteine catabolism or remethylation suffer from premature vascular disease. On this basis McCully suggested that the vascular changes are induced by homocysteine itself, and not by a particular metabolic deletion or some remote metabolic, epigenetic, or phenotypic effect (14). An important question is whether the ability of homocysteine to provoke vascular lesions is confined to rare inborn disease, or is it an important mechanism for vascular disease in the general population?

Several authors have suggested that mild hyperhomocysteinemia is an independent risk factor for vascular disease (47,51,66,127–129,131). The combined results of the studies hitherto performed, unequivocally establish an association between vascular disease and mild hyperhomocysteinemia. This association is independent of other risk factors. The studies are retrospective and, therefore, do not establish a causal relationship.

If the hyperhomocysteinemia is due to a defined genetic defect, a causal relationship might be considered. An increased frequency of heterozygotes for cystathionine β-synthase deficiency among patients with vascular disease has not unequivocally been demonstrated. Moreover, controversy exists over whether or not heterozygotes have an increased risk of vascular disease (119–122). If heterozygotes have an increased risk, the involvement of homocysteine as the atherogenic agent implies that hyperhomocysteinemia should prevail in these subjects while on a normal diet. This has not been investigated.

The inherited thermolabile methylenetetrahydrofolate reductase was found more frequently among patients with vascular disease than in controls, but some patients with this mutant enzyme had normal plasma homocysteine (145). Whether or not these subjects may develop hyperhomocysteinemia under conditions of reduced intake of cofactors or increased intake of protein is an intriguing question that remains to be answered.

The evidence that mild hyperhomocysteinemia caused by genetic and environmental factors is associated with increased risk of premature cardiovascular disease is convincing. Several lines of evidence suggest that homocys-

teine is the pathogenic factor and not merely an indicator of increased cardiovascular risk. We feel confident that future prospective studies will clarify this question, which is of major concern to public health.

MECHANISMS AND EXPERIMENTAL STUDIES

Clinical and experimental studies relating homocysteine to the thrombotic diathesis and atherosclerotic lesions point to abnormalities of endothelial cells, platelets, function of clotting factors, or blood lipids. However, no unifying hypothesis explaining the vascular damage in hyperhomocysteinemia has been proposed. A discussion of data related to the possible mode of action of homocysteine follows.

Experimental Arteriosclerosis

There are reports published 40 years ago that lack of cofactors or substrates involved in homocysteine metabolism may produce atherosclerotic lesions in experimental animals. About 1950, choline deficiency was reported to produce atheromatous changes in the arteries of rats (200), and pyridoxine deficiency induced similar lesions in monkeys (201,202). There is one recent report on vascular lesions resembling early atherosclerosis in vitamin B_{12}-deficient sheep (163). These deficiency states have later been found to cause hyperhomocysteinemia. Choline deficiency increased homocysteine levels in rats (203), and lack of vitamin B_6 caused marked hyperhomocysteinemia in pigs (204), rats (205) and rat pups (206), and enhanced homocysteine excretion in rabbits (207) and humans (123,124). Marked elevation of plasma homocysteine has been demonstrated in vitamin B_{12}-deficient patients (116,161).

Vitamin B_6 deficiency may cause vascular lesions by inhibiting cross-linking of collagen and elastin in the vessels (208). This may result directly from low vitamin B_6 levels, since pyridoxal 5′-phosphate probably serves as a cofactor of the cross-linking enzyme lysyl oxidase (209,210). However, several lines of evidence suggest that homocysteine may be the responsible agent. Lysyl oxidase is inhibited by high concentrations of homocysteine (208,211), and the cross-linking profiles of elastin during vitamin B_6 deficiency resemble those observed after penicillamine treatment and in homocystinuria, more than in conditions in which lysyl oxidase is inhibited. This suggests a direct involvement of homocysteine (206). Notably, collagen fibril formation in vitro is blocked by homocysteine (212).

The atherosclerotic lesions observed in various classes of homocystinuria (14), resemble the experimental atherosclerosis induced by homocysteine infusion of rabbits (13,213), baboons (214,215), and rats (216). Hyperhomocysteinemia may cause intimal injury, proliferation of smooth-muscle cells,

depositions of sulfated proteoglycosaminoglycans (217), collagen, and calcium; and degeneration of elastin within the arterial wall, leading to atherosclerotic plaques.

The experimental atherosclerosis has not been produced by homocysteine in rabbits (218) and pigs (219) by other workers. However, in pigs fed a vitamin B_6-deficient diet, there was an increase in plasma homocysteine. Microscopic examination after 12 weeks on such a diet revealed foci of intimal degeneration, mural thickening in the renal arterioles, and, in one animal, medial necrosis (204).

The different forms of homocysteine used for the in vivo experiments, may offer a clue to the somewhat incoherent results. Homocysteine is freely soluble and undergoes thiol disulfide exchange reactions with sulfur compounds. Homocysteine thiolactone is readily converted to diketopiperazine and is probably spontaneously and enzymatically hydrolyzed in plasma to homocysteine at physiological pH. The thiolactone itself may acylate amino groups and, thereby, alter the structure and function of proteins, peptides, and amino acids (220).

Endothelium

Endothelial injury induced by homocysteine was first proposed by Harker et al. in 1974 and 1976 (214,215). They observed patchy loss of arterial endothelium and shortening of platelet survival time in baboons infused for 5 days with homocysteine thiolactone. Sustained infusion (3 months) produced eccentric fibromuscular lesions containing intracellular lipids and foam cells. These changes resemble early atherosclerotic lesions in humans (214). The chronic preatherosclerotic lesions, but not the acute endothelial damage from hyperhomocysteinemia, were prevented by the antiplatelet drug, dipyridamole (215). Sulfinpyrazone, which is also regarded as an antiplatelet drug, decreased aortic endothelial injury and normalized platelet survival time in chronic hyperhomocysteinemic baboons (221). The authors suggested that the endothelial injury is the initiating event leading to platelet-mediated intimal proliferation of smooth-muscle cells which, in turn, produces atherosclerosis in these animals.

Hladovec and co-workers observed endothelial damage, platelet sequestration, and venous thrombosis in rats injected with a single dose of homocysteine (216). The endothelemia observed after methionine administration was reduced by pyridoxine (222) and by drugs with antiplatelet or antiatherosclerotic effect (223), and was increased by estrogen (171).

The cytotoxicity of homocysteine against endothelial cells in culture has been investigated (137). Homocysteine thiolactone produces endothelial damage, as measured by radiochromium release and cell detachment. The effect may be mediated by homocysteine, since it was decreased by pencillamine,

which may block the thiol group through disulfide formation (224). However, interference from thiolactone-dependent acylation of cellular components cannot be excluded. High concentrations of homocysteine damaged cultured endothelial cells from the umbilical vein, and cells from obligate heterozygotes for cystathionine β-synthase deficiency seemed to be more vulnerable than control cells (225). Similar results have been obtained with endothelial cells from umbilical arteries exposed to homocysteine which induced cell detachment from tissue culture dishes (137).

The mechanism behind the toxic effect of homocysteine on endothelial cells was addressed by Starkebaum and Harlan (226). They demonstrated an oxygen-dependent oxidation of homocysteine to homocystine that was catalyzed by micromolar amounts of copper or ceruloplasmin. The endothelial cell injury induced by homocysteine was dependent on copper and was prevented by catalase, a hydrogen peroxide scavenger. This suggests that lysis of endothelial cells is induced by hydrogen peroxide formed during copper-catalyzed oxidation of homocysteine (226). Notably, patients with cystathionine β-synthase deficiency have increased plasma copper (227).

Platelets

Both increased adhesiveness (78,207,228–232) and normal response (7,214,229,232–235) of platelets have been demonstrated in patients with homocystinuria. Some, but not all, cystathionine β-synthase–deficient patients show normalization of platelet adhesiveness following treatment with vitamin B_6 (207,229,230). Whether this inconsistency reflects different methodologies or interpatient variability is an open question.

In his classic work, Harker described decreased platelet survival time in baboons subjected to long-term homocysteine thiolactone infusion, and in four cystathionine β-synthase–deficient patients, compared with control. Treatment of the patients with pyridoxine or dipyridamole normalized platelet survival (214,215). Others have reported on normal platelet half-life in patients with homocystinuria (236,237). There are also incoherent results concerning platelet morphology (236,238) in cystathionine β-synthase–deficient patients and in the platelet response to ADP in vitro (239,240).

Exposure of platelets to homocysteine or homocystine (1 mM) in vitro increased the formation of the cyclooxygenase product thromboxane B_2. This may be important, since its precursor thromboxane A_2 is the most potent platelet aggregator known (241). Its proaggregatory action is opposed by prostacyclin (PGI_2), a platelet inhibitor derived from the endothelial cells. The effect of homocysteine on arterial prostacyclin synthesis in vitro has been investigated (241,242). Low concentrations may stimulate and high concentrations may inhibit prostacyclin formation, and both effects seem to be mediated by

H_2O_2 formed during oxidation of homocysteine (242). The excretion of the major prostacyclin metabolite 2,3-dinor-6-keto-PGF_{1a} is regarded a marker of increased prostacyclin synthesis in response to platelet–vascular interaction in patients with atherosclerotic or thrombotic disease (243,244). A marked increase in 2,3-dinor-6-keto-PGF_{1a} excretion has recently been demonstrated in a cystathionine β-synthase–deficient patient (245). Altered arachidonic acid metabolism as a mechanism of vascular disease in hyperhomocysteinemia is an interesting area for future research.

Clotting Factors

Hilden et al. found a moderately decreased level of factor V in one patient with homocystinuria. The level was normalized upon supplementing vitamins (235).

Homocysteine enhanced factor V activity in cultured endothelial cells from bovine aorta and human umbilical vein (246). Factor V is required for prothrombin activation, and its activity is regulated by the protein C mechanism. The activity of protein C, in turn, is regulated by the endothelial membrane-associated protein thrombomodulin. Homocysteine has also been shown to reduce protein C activation, probably by acting as an inhibitor of the interaction of thrombin with thrombomodulin (247).

Factor VII is reduced to 20–45% of normal (248–252) in homocystinurics. Levels of this factor were dissociated from variations in plasma homocysteine following different treatment modalities in some (253), but not all patients (250). A low factor VII concentration is not inconsistent with the occurrence of thromboembolism (254,255), but its role in the clotting diathesis in patients with homocystinuria is not readily apparent.

Reduced antithrombin III values have been reported for obligate heterozygotes for cystathionine β-synthase deficiency (256), whereas normal values for such individuals have been reported by others (154,252). Antithrombin III is reduced to 50–75% of normal in patients with homocystinuria (252,253,256,257). This may contribute to the thrombotic diathesis in homocystinurics, since those with inherited deficiencies of antithrombin III with levels of about 50% of normal have a marked tendency of thrombosis (258,259).

Low antithrombin III activity in patients with homocystinuria was unaffected by a low methionine diet that reduced homocysteine in plasma and urine (256), but the activity was normalized after pyridoxine or folate treatment (252,253), after which blood levels of homocysteine were still high (252,253). Thus, decreased antithrombin III does not seem to be related to blood levels of homocysteine. Nor was it related to the duration of disease, thromboembolic episodes, anticoagulant therapy, or impaired liver function. In homocystinurics, pyridoxine or folate supplementation may affect metabolic processes closely linked to coagulation abnormalities. This correction may not be depen-

dent on reduction of the concentration of homocysteine in extracellular medium such as plasma (253).

There is one early report, yet to be confirmed, that high concentrations of homocysteine activates factor XII (Hageman factor) in vitro (260). No activation of this factor has been demonstrated in patients (115).

Low-Density Lipoproteins

Low-density lipoprotein (LDL), the major cholesterol transport protein in human plasma, is an established risk factor for atherosclerosis (155). Native LDL is not ingested by macrophages or smooth-muscle cells in sufficient amounts to cause intracellular lipid accumulation. When LDL is modified, through oxidation or other chemical modification, it is recognized by the so-called scavenger receptor, which is distinct from the LDL receptor. The scavenger receptor resides on endothelial cells and macrophages. Macrophages taking up oxidized LDL by this receptor are converted into foam cells. Intimal accumulation of foam cells is an early event in atherogenesis (261,262).

Oxidation of LDL in vitro occurs in the presence of endothelial cells and other cell types. The oxidation is dependent on the presence of copper (263), the plasma level of which is elevated in cystathionine β-synthase–deficient patients (227). More importantly, modification of LDL by cultured cells requires the presence of sulfur-containing amino acids in the medium. It has been postulated that the sulfur amino acids are taken up, reduced and finally released by the cells. The production of reactive oxygen species modifying LDL can then occur by a thiol-dependent mechanism (263,264). This model is supported by the demonstration of a cell-independent oxidation of LDL in the presence of thiols, including homocysteine (264,265) (Fig. 7).

Homocysteine has been shown to cause damage to endothelial cells, probably by generation of reactive oxygen species, like hydrogen peroxide (226). Oxidized LDL, but not native LDL, is highly cytotoxic to endothelial cells (266,267). Endothelial lesions induced by modified LDL may contribute to the atherogenesis (see Fig. 7).

THERAPEUTIC INTERVENTION

Treatment of patients with cystathionine β-synthase deficiency with pyridoxine and folic acid has significantly reduced both plasma homocysteine and the occurrence of thromboembolic events (81). Furthermore, essentially no thromboembolic episodes are reported in patients with homocystinuria caused by genetic defects in homocysteine remethylation when these patients are given homocysteine-lowering therapy (i.e., vitamin B_{12}, folic acid, or betaine; 88). These observations suggest a protective effect of such intervention.

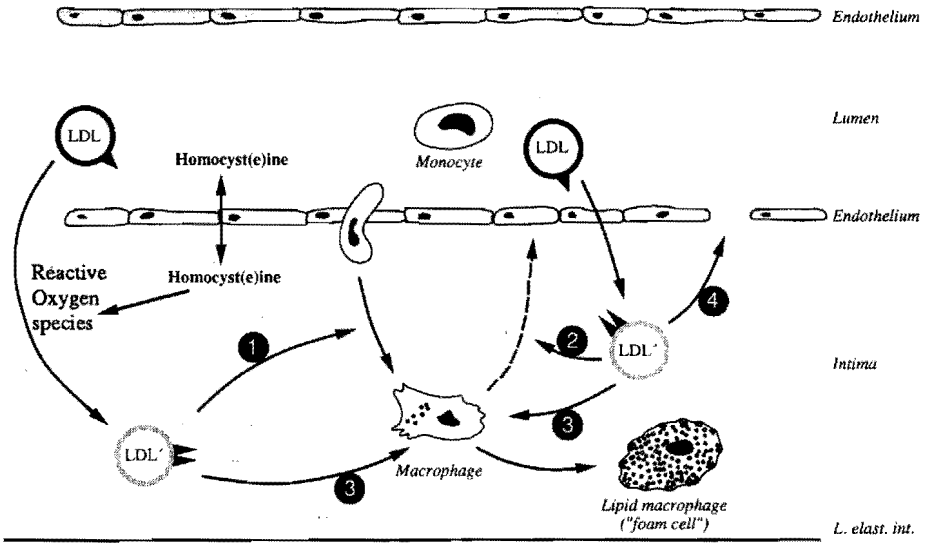


Figure 7 Modification of LDL by homocysteine, and the role of modified LDL (LDL′) in atherogenesis. Homocysteine causes formation of reactive oxygen species that modify LDL to LDL′. LDL′ may contribute to atherogenesis by (1) a chemotactic effect on circulating monocytes which migrate into the intima, (2) inhibiting the motility of resident macrophages, (3) uptake into resident macrophages which are converted into "foam cells", (4) cytotoxic effect on endothelial cells. (Modified from Ref. 261.)

There are consistent reports that pharmacologic doses of folic acid almost invariably reduce elevated plasma homocysteine in patients with renal failure, vascular disease, or thermolabile methylenetetrahydrofolate reductase (49,145,179,180). Folic acid also reduces fasting plasma homocysteine and postload levels in healthy subjects without overt folate deficiency (268,269). Pyridoxine supplementation often normalizes the response to methionine loading in patients with vascular disease (49,64,71). Thus, effective and innocuous means are available for the treatment of hyperhomocysteinemia. Intervention studies should be carried out to evaluate the effect of such regimens on the occurrence of vascular disease.

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