Diagnostics of Homocysteine Metabolism

P. M. Ueland, H. Refsum, and M. R. Malinow

Introduction

Homocysteine (Hcy) was discovered in 1932 by Du Vigneaud [27] as a product of demethylation of methionine, and in 1962, Carson and Neill [19] reported on to two siblings with the inborn error homocystinuria detected by a screening program of mentally retarded children. Since then about 600 patients with homocystinuria have been reported [64]. Deficiency of the enzyme cystathionine β-synthase is the most common cause, but other enzymic defects have been described in a minority of these patients. Notably, all forms of homocystinuria, irrespective of enzymic defect, are associated with a high incidence of cardiovascular disease that may occur in early adolescence and even in childhood [64]. The high incidence of vascular disease in these patients led to the Hcy theory of atherosclerosis, formulated by McCully in 1975 [62, 61] and later substantiated by epidemiological data [52, 88].

This chapter reviews the literature on clinical aspects of Hcy metabolism with emphasis on the methodology used for the determination of plasma Hcy levels.

Biochemistry

Hcy is a sulfur amino acid that is formed from methionine as a product of S-adenosylmethionine-dependent transmethylation (Fig. 1). Intracellular Hcy is salvaged to methionine by remethylation. The reaction is catalyzed in most tissues by the enzyme methionine synthase (EC 2.1.1.13) which requires methylcobalamin as cofactor and 5-methyltetrahydrofolate as methyl donor. Hcy is also remethylated by the enzyme betaine-homocysteine transmethylase, which is confined to the liver and possibly kidney. An alternative route of Hcy disposal is conversion to cysteine, and the first step of this pathway is catalyzed by the vitamin B_6-dependent enzyme cystathionine β-synthase, which completes the transsulfuration pathway [31].

Methionine synthase has low K_m for Hcy, and the activity increases at low dietary methionine intake. These features suggest that the enzyme conserves methionine. Cystathionine β-synthase has high K_m for Hcy; the activity increases in response to high intake of methionine, and the enzyme probably controls the catabolism of excess Hcy [31]. Cellular export of Hcy represents an additional mechanism regulating the intracellular Hcy content. This process becomes important under conditions of imbalance between Hcy production and metabolism [87]. Increased Hcy production (induced by methionine loading) or inhibition of Hcy metabolism (during folate or cobalamin deficiencies, or defect of cystathionine β-synthase) cause export of Hcy into the extracellular fluid. This is the biochemical basis for plasma Hcy as a marker of folate or cobalamin deficiency or inborn errors of Hcy metabolism [89].

Homocysteine Species and Concentrations in Plasma or Serum

Hcy is probably released into the extracellular fluid and plasma in its reduced form, and only trace amounts can be detected in plasma under physiological conditions [59, 60]. In plasma, Hcy undergoes oxidation and disulfide exchange reactions. In freshly prepared plasma, about 70% of Hcy exists as albumin-Hcy mixed disulfide, and this fraction is refered to as protein-bound Hcy [48, 70]. When whole plasma or serum is deproteinized with acid, the soluble free Hcy is obtained, and most Hcy in this fraction has been identified as Hcy-cysteine mixed disulfide. The sum of all Hcy species in plasma/serum is termed total Hcy [87]. Total Hcy shows a skewed distribution toward higher values in healthy subjects. The me-

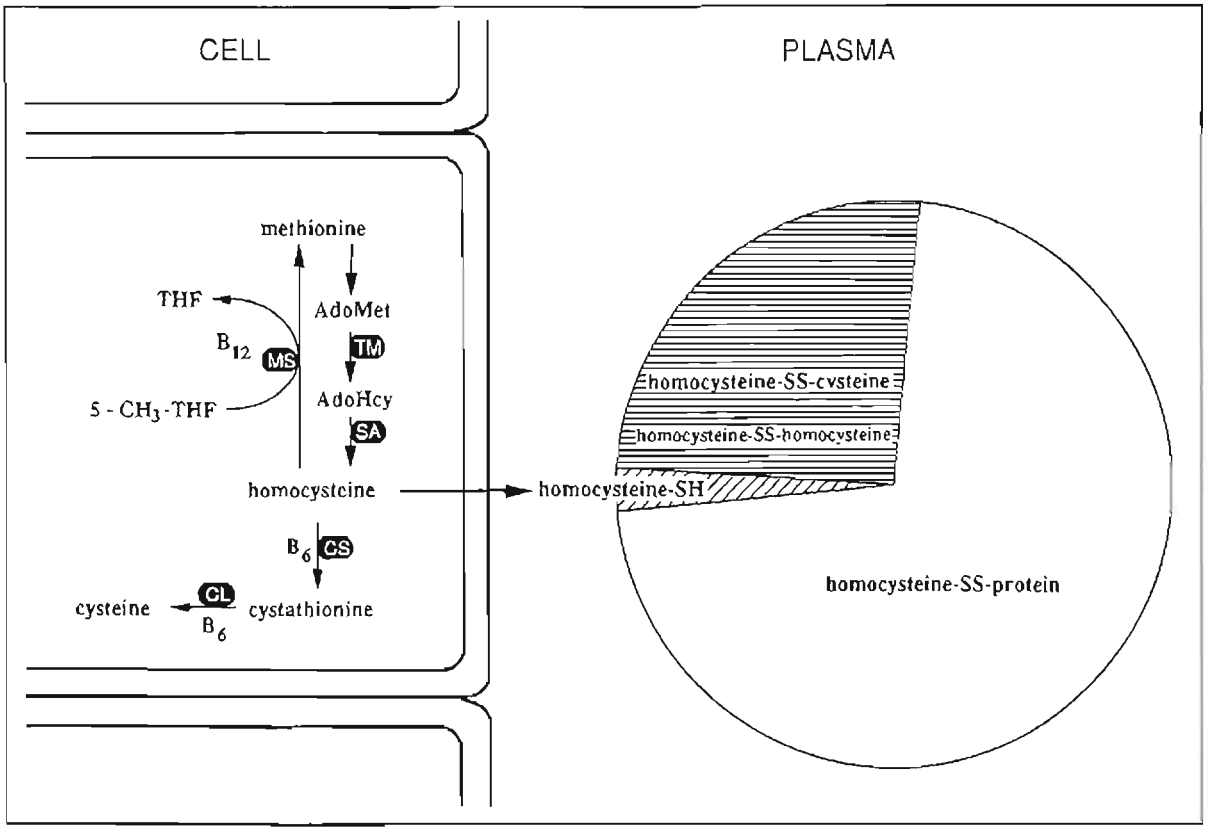

Fig. 1. Intracellular homocysteine metabolism and distribution of various homocysteine species in plasma. *AdoHcy*, *S*-Adenosylhomocysteine; *AdoMet*, *S*-adenosylmethionine; *CL*, cystathionine lyase (γ-cystathionase); *CS*, cystathionine β-synthase; *MS*, 5-methyl-THF-homocysteine methyltransferase (methionine synthase); *TM*, transmethylase; *SA*, *S*-adenosylhomocysteine hydrolase; *THF*, tetrahydrofolate

dian value has been reported as 10.7 μmol/l in 3 000 healthy men aged 40–42 years, and the 95 th percentile as 17.6 μmol/l [89]. Total plasma Hcy seems to be dependent on age, gender, and in women possibly the menopausal status. The normal mean values are about 1 μmol/l lower in premenopausal women than in men or postmenopausal women, and there seems to be a significant increase (about 1–2 μmol/l) in the mean values as a function of age (from 20 to 70 years) in both sexes [3]. Good correlation between total Hcy measured in different laboratories and with different methods has been obtained, and values between 5 and 15 μmol/l are usually considered as normal [89].

Analytical Methods

There is a continuous redistribution of Hcy species in plasma/serum, so that after storage for days (at room temperature) or months (frozen), most Hcy becomes associated with plasma proteins. Reliable measurement of free Hcy therefore requires immediate deproteinization of plasma/serum, which is impractical in the clinical routine and has been largely abandoned [89]. Measurement of total Hcy is now widely recommended since the values remain stable during storage of plasma/serum. Total Hcy is measured by procedures including treatment of whole plasma/serum with a reductant. The Hcy disulfides are then quantitatively converted into reduced Hcy [89]. The methods used for separation and quantitation of reduced Hcy vary. The Hcy assays can be categorized into four types, according to the construction of the method: (a) radioenzymic assays, (b) gas chromatography-mass spectrometry, (c) assays based on precolumn derivatization, HPLC, and fluorescence detection, (d) HPLC and electrochemical detection, and (e) assays based on

liquid chromatography and postcolumn derivatization, including the amino acid analyzer.

Radioenzymic Assays

These methods are based on the conversion of Hcy to *S*-adenosylhomocysteine catalyzed by the enzyme *S*-adenosylhomocysteine hydrolase. *S*-adenosylhomocysteine is quantitated by HPLC [70], paper [20] or thin-layer chromatography [21]. Low instrumental cost of the modification based on thin-layer chromatography separation is the main advantage of this assay. However, these assays are laborious, and the linearity may be limited by consumption of the cosubstrate (adenosine) used for the enzymic derivatization of Hcy.

Gas Chromatography-Mass Spectrometry

In this assay, the sample, supplemented with deuterated Hcy used as internal standard, is subjected to solid-phase extraction, and Hcy is derivatized with *N*-methyl-*N*(*t*-butyldimethylsilyl)trifluoroacetamide. The t-butyldimethylsilyl derivatives are separated and quantitated by capillary gas chromatography and selected ion monitoring [80, 79]. Attractive features of this method are codetermination of other metabolites such as methylmalonic acid [55]. The initial version of this technique has been simplified [79], but it still a cumbersome procedure. Furthermore, expensive equipment and technical skill are required.

Precolumn Derivatization, HPLC, and Fluorescence Detection

Hcy can be derivatized with the thiol-specific fluorogenic reagents monobromobimane (mBrB) [46, 72, 60, 32] or ammonium-7-fluoro-2,1,3-benzoxadiazole-4-sulfonate (SBD-F) [7, 85, 91], followed by separation of the adducts with HPLC. Both tags also label cysteine and cysteinylglycine, which are codetermined in these assays.

mBrB is a reactive agent, and the derivatization is completed within minutes. Assays based on this reagent can be fully automated [72]. The automatization results in high precision. The composition and pH of the mobile phase are critical for the separation of the Hcy adduct from hydrolysis products and other interfering material [32]. Experience in HPLC is necessary to operate this method.

SBD-F has low reactivity and derivatization is carried out at 60 °C for 30–60 min. This prevents automatization. The separation of thiol adducts is simple and fast since the reagent itself is not fluorogenic, and there is no interfering material [7, 85, 91].

Methods have recently been published which are based on derivatization with *o*-phthaldialdehyde [30, 44]. This agent is not a thiol-specific reagent but reacts with primary amino acids. These assays include several manual steps, and the presence of fluorescent material may cause chromatographic interference. They have not gained widespread use.

HPLC and Electrochemical Detection

In this assay, plasma/serum samples are subjected to HPLC immediately after treatment with reductant, and Hcy is detected with an electrochemical detector equipped with a gold-mercury electrode, which affords great specificity towards thiols. Simple sample processing, short run time, and high sample output are attractive features of this technique [78, 56]. However, particular attention must be paid to maintenance of flow cell and the gold-mercury electrode since deterioration of these parts of the detector system may cause baseline fluctuations and variable electrochemical response.

Amino Acid Analyzer and Postcolumn Derivatization

Hcy can be determined using an amino acid analyzer, either following reduction [2] or after the sulfhydryl group has been carboxymethylated using iodoacetic acid [51]. The free sulfhydryl is quantitated on a ion-exchange column eluted with the standard program [45] or with a mobile phase optimized for Hcy determination [2]. Even the optimized program results in long retention time (about 25 min) which is followed by column regeneration [2]. This seriously restricts sample output which is low compared to the HPLC methods. Other disadvantages include relatively high imprecision and formation of interfering ninhydrin-positive material upon storage of samples at −20 °C [89]. A method for total Hcy based on HPLC and postcolumn derivatization with thiol

specific chromophor 4,4'-dithiodipyridine has recently been published [5]. Critical measures to reduce baseline noise have been worked out, and the method may turn out to be useful.

Procedures for Sample Collection and Processing

Total plasma Hcy increases slowly to a maximum increase of 15%–20% 6–8 h after a protein-rich meal. The increased Hcy level persists for several hours [89]. This effect from food intake is small relative to the high Hcy levels caused by some acquired or genetic diseases (see below, Table 1) and does not interfere with interpretation of data. However, a recent prospective study reported a 41% increase in risk of myocardial infarction for each 4 μmol/l increase in Hcy level [9]. Thus, using plasma/serum Hcy as a parameter to evaluate cardiovascular risk, measurement of fasting levels is preferable.

Total Hcy is increased in plasma/serum when whole blood is left at room temperature. This is caused by a temperature and time-dependent release of Hcy from the blood cells. After 1 h at 22 °C there is a significant (10%) increase in plasma Hcy [91, 6, 86, 32]. This artificial elevation is markedly reduced when the samples of whole blood are placed on ice. Under these conditions (0°–2 °C), plasma Hcy is stable for at least 4 h [32]. Therefore to stabilize plasma Hcy, it is recommended that whole blood is kept on ice and the blood cells removed from the plasma fraction within 1 h. Serum must be aspirated immediately after clot retraction. A release of Hcy from the blood cell before aspiration explains the observation that total Hcy in serum is slightly higher than in plasma [9].

Total Hcy in serum/plasma is stable for at least 4 days at room temperature, and for several weeks when stored at 0°–2 °C. In frozen samples kept at –20 °C, it is stable for years [89].

Table 1. Conditions or agents causing hyperhomocysteinemia

Condition/agent	Hyperhomocysteinemia	Cause of hyperhomocysteinemia
Genetic		
Homozygous for CS deficiency	I, S	Rare
Homozygous for MTFR deficiency	I, S	Rare
Cabalamin mutations (C, D, E, F, G)	I	Rare
Heterozygous for CS deficiency	M	Common
Heterozygous MTFR deficiency	M	Rare
Thermolabile MTFR	M	Probably common
Compound heterozygosity (MTFR deficiency and thermolabile MTFR)	I	–
Acquired		
Cobalamin deficiency	M, I, S	Fairly common
Folate deficiency	M, I	Common
Vitamin B_6 deficiency	(M)	Probably common
Renal failure	M, I	Common
Malignancy	M	Not common
Psoriasis	M	Fairly common
Hypothyroidism	M	Fairly common
Drugs	M	Fairly common
Methotrexate		
Nitrous oxide		
Antiepileptic agents		
Colestipol plus niacin		
Vitamin B_6 antagonists (azaribine, isoniazide)		

M, Moderate hyperhomocysteinemia, 15–30 μmol/l; I, intermediate hyperhomocysteinemia, 30–100 μmol/l; S, severe hyperhomocysteinemia, >100 μmol/l; CS, cystathionine β-synthase; MTFR, methylenetetrahydrofolate reductase.

Methionine Loading Test

The methionine loading test involves oral intake of a standard dose of methionine (0.1 g/kg or 3.8 g/m^2), and total Hcy is measured after a fixed time interval, usually 4 or 6 h [13, 69, 4, 16, 88]. A typical Hcy response after a standard methionine load is shown in Fig. 2. The methionine loading test has been used to find possible defects in methionine metabolism in patients with cardiovascular disease [88]. Abnormal response has been defined as postload Hcy concentration or postload increase in Hcy exceeding the 95 th percentile, the mean plus 2 standard deviations for controls, or the highest control value [88]. Superfluous methionine is directed into the transsulfuration pathway via cystathionine β-synthase [31], and this agrees with the observation of abnormal response in homozygotes and most heterozygotes for cystathionine β-synthase deficiency [63]. Folate and cobalamin deficiency cause impaired Hcy remethylation, but data on the response to methionine loading in such patients are sparse and not conclusive [69; 16].

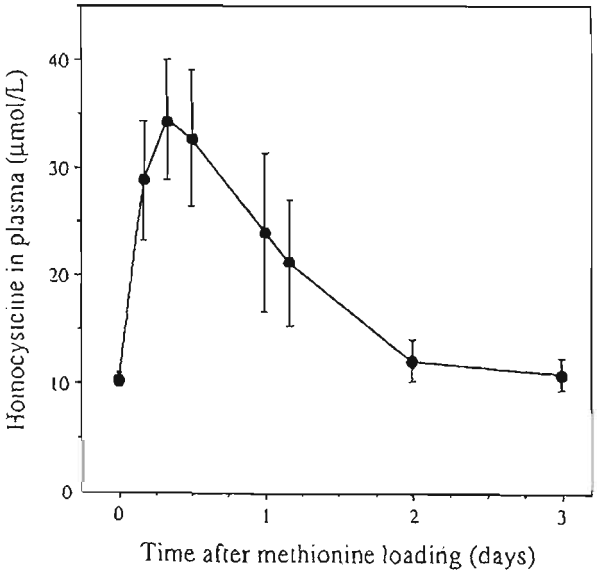

Fig. 2. Total homocysteine in plasma after methionine loading. Seven healthy postmenopausal women were given peroral methionine (0.1 g/kg). Data are given as mean ± standard deviation

Causes of Hyperhomocysteinemia

Hyperhomocysteinemia means elevated total Hcy in blood [56]; it is classified as moderate, intermediate, and severe. Mild hyperhomocysteinemia is defined as fasting total Hcy in plasma/serum of less than 30 µmol/l, moderate as concentrations between 30 and 100 µmol/l, and severe as concentrations higher than 100 µmol/l [52].

Hyperhomocysteinemia is caused by either genetic or acquired conditions (Table 1). Genetic diseases include the homozygous form of cystathionine β-synthase deficiency which is the most common cause of homocystinuria [64]. Rare forms are severe genetic defects of methylenetetrahydrofolate reductase, and inborn errors of cobalamin metabolism, classified as cblC, cblD, cblF, cblG, and cblE mutations [75]. Heterozygosity for cystathionine β-synthase deficiency is present in 0.3 %–1 %, or 2 % at the most, of the general population [64, 23], and 50 %–70 % of these subjects have moderate hyperhomocysteinemia [65, 77]. A mutation of the enzyme methylenetetrahydrofolate reductase, characterized by 50 % reduction in enzyme activity and thermolabile enzyme, occurs in 5 % of the general population, and these subjects have a tendency towards moderate hyperhomocysteinemia [49, 53]. Four subjects with compound heterozygosity of methylenetetrahydrofolate reductase deficiency and the thermolabile enzyme have been described; these had intermediate hyperhomocysteinemia [49]. Thus, heterozygosity for various forms of homocystinuria may explain the observation that total plasma Hcy is a genetic trait [68].

Among the acquired conditions causing elevated plasma Hcy, folate [47] or cobalamin deficiency [15, 1] are most often encountered. Folate deficiency is associated with moderate and intermediate hyperhomocysteinemia. In cobalamin-deficient patients, severe hyperhomocysteinemia is occasionally observed [89]. Elevation of plasma Hcy is also observed in disease states such as renal failure [93], acute leukemia, psoriasis, and hypothyroidism [88] and is induced by some drugs [71], i.e. methotrexate, nitrous oxide, antiepileptic agents, colestipol plus niacin [10], and some agent acting as vitamin B_6 antagonists [26, 71].

Hyperhomocysteinemia and Arterial Occlusive Diseases

Results from about 20 studies involving about 2000 patients and a comparable number of controls have established that moderate hyperhomocysteinemia is an independent risk factor for vascular disease in the coronary, cerebral, and peripheral vessels [52, 88]. The overall increase in plasma Hcy in patients with premature cardiovascular disease is about 30% compared to the level in healthy controls. The elevation shows some variability related to the site of the vascular lesions, and the incidence of hyperhomocysteinemia is highest in patients with cerebrovascular disease (about 40%), intermediate in peripheral arterial disease (about 25%) and lowest in coronary heart disease (about 15%) [24, 58, 17, 66].

Coronary Artery Disease

Wilcken and Wilcken [94] reported elevation of cysteine-homocysteine mixed disulfide in coronary patients following methionine load, and this observation was confirmed by Murphy-Chutorian et al. [65] and by Clarke et al. [23] in a larger number of cases. Following the demonstration by Kang et al. [48] that a large proportion of total plasma homocysteine is bound to proteins, most investigators have measured basal total homocysteine. Thus, such levels were elevated in patients with myocardial infarction [45] and coronary artery disease established by coronary angiography [50]. These findings were confirmed by others [58, 34, 95, 84]. Most recently, prospective studies have confirmed that hyperhomocysteinemia is a risk factor for the coronary heart disease [82; 9]. Moreover, statistical analyses of the data demonstrated that hyperhomocysteinemia is independent of other common risk factors for atherosclerosis [23, 33, 82]. The influence of genetic factors in hyperhomocysteinemia in coronary patients was established by Williams et al. [95] and Genest et al. [33].

Cerebrovascular Disease

Brattström et al. [18], Boers et al. [12], and Clarke et al. [23] reported that patients with cerebrovascular disease attain higher levels of plasma homocysteine species after the methionine loading test. These studies were confirmed with measurements on basal plasma levels of total homocysteine [14, 8, 24, 17].

Peripheral Arterial Disease

Boers et al. [12] and Clark et al. [23] reported increased plasma levels of homocysteine species following methionine load in patients with peripheral arterial diseases. Studies on basal levels of total homocysteine confirmed these findings in patients with carotid, aortic, and ileofemoral arterial diseases [56] as well as in subjects with intermittent claudication [66]. Studies on thickness of carotid arteries by ultrasonography have yielded conflicting results. Clarke [22] found no increased frequency of carotid arteries plaques in 25 heterozygotes for cystathionine β-synthase deficiency whereas Rubba et al. [76] reported more frequent vascular lesions in the iliac and internal carotid arteries of 14 heterozygotes for cystathionine β-synthase than in 47 controls. Moreover, Malinow et al. [57] demonstrated that asymptomatic subjects with thickened carotid arteries have higher plasma total homocysteine levels than matched controls.

Mechanisms

Experimental studies have focused on a variety of mechanisms for the Hcy-induced atherogenesis. In vitro [35] and in vivo [39, 38] experiments suggest that Hcy promotes aggregation of platelets, but this conclusion has been contested [90; 43; 81]. Endothelial damage mediated by H_2O_2 production has been suggested by several authors, but most studies have been performed with high concentrations of D, L-Hcy or Hcy-thiolactone [92, 25, 37, 83]; cysteine induces similar effects, and the validity of these observations for the in vivo atherogenesis has been questioned [28]. Two reports have described oxidative modification of low-density lipoproteins by Hcy in vitro [42, 67], but increased lipid peroxidation has not been demonstrated in patients with hyperhomocysteinemia [11, 29].

Several recent reports describe stimulation of procoagulant activities and impairment of endothelial cell thromboresistance by Hcy. High concentrations of Hcy in vitro activate factor V [74],

reduce protein C activation [73, 54], inhibit surface expression [54] and inactivate cofactor activity of thrombomodulin [41], and block tissue plasminogen activator binding to human endothelial cells [36]. The latter effect seems to be specific for Hcy, since the inhibition was reversed by cysteine. Harpel et al [40] recently demonstrated that physiological levels of Hcy enhance the binding of lipoprotein (a) to fibrin. Cysteine and other thiols have a similar effect. This indicates a link between sulfhydryl compounds and thrombosis and atherhogenesis. Stamler et al. [81] showed that low concentrations of Hcy also rapidly react with endothelium-derived relaxing factor/nitric oxide (NO) to form *S*-nitroso-Hcy, which acts as a potent antiplatelet agent and vasodilator. The formation of this adduct may attenuate H_2O_2 production from Hcy and thereby protect against the atherogenic properties of Hcy. According to this model, vascular injuries result under conditions of imbalance between NO production from dysfunctional endothelial cells and the levels of Hcy [81]. In conculusion, most experimental work on the adverse vascular properties of Hcy describes short-term effects provoked in the presence of very high concentrations of Hcy, and the effects are also produced by other thiols. Thus, no convincing or unifying hypothesis explaining the vascular injuries caused by hyperhomocysteinemia has yet been presented.

References

1. Allen RH, Stabler SP, Savage DG, Lindenbaum J (1990) Diagnosis of cobalamin deficiency. I. Usefulness of serum methylmalonic acid and total homocysteine concentrations. Am J Hematol 34: 90–98
2. Andersson A, Brattström L, Isaksson A, Israelsson B, Hultberg B (1989) Determination of homocysteine in plasma by ion-exchange chromatography. Scand J Clin Lab Invest 49: 445–450
3. Andersson A, Brattström L, Israelsson B, Isaksson A, Hamfelt A, Hultberg B (1992) Plasma homocysteine before and after methionine loading with regard to age, gender, and menopausal status. Eur J Clin Invest 22: 79–87
4. Andersson A, Brattström L, Israelsson B, Isaksson A, Hultberg B (1990) The effect of excess daily methionine intake on plasma homocysteine after a methionine loading test in humans. Clin Chim Acta 192: 69–76
5. Andersson A, Isaksson A, Brattström L, Hultberg B (1993) Homocysteine and other thiols determined in plasma by HPLC and a thiol-specific postcolumn derivatization. Clin Chem 39: 1590–1597
6. Andersson A, Isaksson A, Hultberg B (1992) Homocysteine export from erythrocytes and its implication for plasma sampling. Clin Chem 38: 1311–1315
7. Araki A, Sako Y (1987) Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescene detection. J Chromatography 442: 43–52
8. Araki A, Sako Y, Fukushima Y, Matsumoto M, Asada T, Kita T (1989) Plasma sulfhydryl-containing amino acids in patients with cerebral infarction and in hypertensive subjects. Atherosclerosis 79: 139–146
9. Arnesen E, Refsum H, Bønaa KH, Ueland PM, Førde OH, Nordrehaug JE (1993) The Tromsø study; a population based prospective study of serum total homocysteine and coronary heart disease. (submitted)
10. Bankenhorn DH, Malinow MR, Mack WJ (1991) Colestipol plus niacin therapy elevates plasma homocysteine levels. Coron Art Dis 2: 357–360
11. Blom HJ, Engelen DPE, Boers GHJ, Stadhouders AM, Sengers RCA, De Abreu R, TePoele-Pothoff MTWB, Triebels JMF (1992) Lipid peroxidation in homocysteinaemia. J Inher Metab Dis 15: 419–422
12. Boers GHJ, Smals AGH, Trijbels FJM, Fowler B, Bakkeren JAJM, Schoonderwaldt HC, Kleijer WJ, Kloppenborg PWC (1985) Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. N Engl J Med 313: 709–715
13. Brattström L, Israelsson B, Hultberg B (1988) Impaired homocysteine metabolism – a possible risk factor for arteriosclerotic vascular disease. In: Smith U, Eriksson S, Lindgärde F (eds) Genetic susceptibility to environmental factors a challenge for public intervention. Almqvist & Wiksell International, Stockholm, Sweden, pp 25–34
14. Brattström L, Israelsson B, Hultberg B (1988) Impaired homocysteine metabolism – a possible risk factor for arteriosclerotic vascular disease. In: Smith U, Eriksson S, Lindgärde F (eds) Genetic susceptibility to environmental factors a challenge for public intervention. Almqvist & Wiksell International, Stockholm, Sweden, pp 25–34
15. Brattström L, Israelsson B, Lindgärde F, Hultberg B (1988) Higher total plasma homocysteine in vitamin B12 deficiency than in heterozygosity for homocystinuria due to cystathionine β-synthase defiency. Metabolism 37: 175–178
16. Brattström L, Israelsson B, Norrving B, Bergqvist D, Thörne J, Hultberg B, Hamfelt A (1990) Impaired homocysteine metabolism in early-onset cerebral and peripheral occlusive arterial disease-effects of pyridoxine and folic acid treatment. Atherosclerosis 81: 51–60
17. Brattström L, Lindgren A, Israelsson B, Malinow MR, Norrving B, Upson B (1992) Hyperhomocysteinemia in stroke. Prevalence, cause and relationship to other risk factors or type of stroke. Eur J Clin Invest 22: 214–221

18. Brattström LE, Hardebo JE, Hultberg BL (1984) Moderate homocysteinemia – possible risk factor for arteriosclerotic cerebrovascular disease. Stroke 15: 1012–1016
19. Carson NAJ, Neill DW (1962) Metabolic abnormalities detected in a survey of mentally backward individuals in Northern Ireland. Arch Dis Child 37: 505–513
20. Chadefaux B, Coude M, Hamet M, Aupetit J, Kamoun P (1989) Rapid determination of total homocysteine in plasma. Clin Chem 35: 2002
21. Chu RC, Hall CA (1988) The total serum homocysteine as an indicator of vitamin B 12 and folate status. Am J Clin Pathol 90: 446–449
22. Clarke R (1990) The Irish experience. In: Robinson K (ed) Homocysteinaemia and vascular disease. Commission of the European Communities, Luxembourg, pp 41–48
23. Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, Graham I (1991) Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 324: 1149–1155
24. Coull BM, Malinow MR, Beamer N, Sexton G, Nordt F, Garmo P (1990) Elevated plasma homocysteine concentration as a possible independent risk factor for stroke. Stroke 21: 572–576
25. De Groot PG, Willems C, Boers GHJ, Gonsalves MD, Van Aken WG, Van Mourik JA (1983) Endothelial cell dysfunction in homocystinuria. Eur J Clin Invest 13: 405–410
26. Drell W, Welch AD (1989) Azaribine-homocystinemia-thrombosis in historical perspectives. Pharmac Ther 41: 195–206
27. du Vigneaud V, Ressler C, Rachele JR (1950) The biological synthesis of „labile methyl groups." Science 112: 267–271
28. Dudman NPB, Hicks C, Wang J, Wilcken DEL (1991) Human arterial endothelial cell detachment in vitro – its promotion by homocysteine and cysteine. Atherosclerosis 91: 77–83
29. Dudman NPB, Wilcken DEL, Stocker R (1993) Circulating lipid hydroperoxide levels in human hyperhomocysteinemia – relevance to development of arteriosclerosis. Arterioscler Thromb 13: 512–516
30. Fermo I, Arcelloni C, Devecchi E, Vigano S, Paroni R (1992) High-performance liquid chromatographic method with fluorescence detection for the determination of total homocysteine in plasma. J Chromatography 593: 171–176
31. Finkelstein JD (1990) Methionine metabolism in mammals. J Nutr Biochem 1: 228–237
32. Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM (1993) Homocysteine and other thiols in plasma and urine: automated determination and sample stability. Clin Chem 39: 263–271
33. Genest JJJr, McNamara JR, Upson B, Salem DN, Ordovas JM, Schaefer EJ, Malinow MR (1991) Prevalence of familial hyperhomocysteinemia in men with premature coronary artery disease. Arterioscler Thromb 11: 1129–1136
34. Genest JJJ, McNamara JR, Salem DN, Wilson PWF, Schaefer EJ, Malinow MR (1990) Plasma homocysteine levels in men with premature coronary artery disease. J Am Coll Cardiol 16: 1114–1119
35. Graeber JE, Slott JH, Ulane RE, Schulman JD, Stuart MJ (1982) Effect of homocysteine and homocystine on platelet and vascular arachidonic acid metabolism. Pediatr Res 16: 490–493
36. Hajjar KA (1993) Homocysteine-induced modulation of tissue plasminogen activator binding to its endothelial cell membrane receptor. J Clin Invest 91: 2873–2879
37. Harker LA, Harlan JM, Ross R (1983) Effect of sulfinpyrazone on homocysteine-induced endothelial injury and arteriosclerosis in baboons. Circ Res 53: 731–739
38. Harker LA, Ross R, Slichter SJ, Scott CR (1976) Homocysteine-induced arteriosclerosis. The role of endothelial cell injury and platelet response in its genesis. J Clin Invest 58: 731–741
39. Harker LA, Slichter SJ, Scott CR, Ross R (1974) Homocystinemia. Vascular injury and arterial thrombosis. N Engl J Med 291: 537–543
40. Harpel PC, Chang VT, Borth W (1992) Homocysteine and other sulfhydryl compounds enhance the binding of lipoprotein (a) to fibrin: a potential biochemical link between thrombosis, atherogenesis, and sulfhydryl compound metabolism. Proc Natl Acad Sci USA 89: 10193–10197
41. Hayashi T, Honda G, Suzuki K (1992) An atherogenic stimulus homocysteine inhibits cofactor activity of thrombomodulin and enhances thrombomodulin expression in human umbilical vein endothelial cells. Blood 79: 2930–2936
42. Heinecke JW, Rosen H, Suzuki LA, 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
43. Hill-Zobell RL, Pyeritz RE, Scheffel U, Malpica O, Engin S, Camargo EE, Abbott M, Guilarte TR, Hill J, McIntyre PA, Murphy EA, Tsan M-F (1982) Kinetics and distribution of 111 indium-labeled platelets in patients with homocystinuria. N Engl J Med 307: 781–786
44. Hyland K, Bottiglieri T (1992) Measurement of total plasma and cerebrospinal fluid homocysteine by fluorescence following high-performance liquid chromatography and precolumn derivatization with orthophthaldialdehyde. J Chromatography 579: 55–62
45. Israelsson B, Brattström LE, Hultberg BJ (1988) Homocysteine and myocardial infarction. Atherosclerosis 71: 227–234
46. Jacobsen DW, Gatautis VJ, Green R (1989) Determination of plasma homocysteine by high-performance liquid chromatography with fluorescence detection. Anal Biochem 178: 208–214
47. Kang S-S, Wong PWK, Norusis M (1987) Homocysteinemia due to folate deficiency. Metabolism 36: 458–462
48. Kang S-S, Wong PWK, Becker N (1979) Protein-bound homocysteine in normal subjects and in patients with homocystinuria. Pediatr Res 13: 1141–1143
49. Kang S-S, Wong PWK, Bock H-GO, Horwitz A, Grix A (1991) Intermediate hyperhomocysteinemia resulting from compound heterozygosity of methylenetetrahydrofolate reductase mutations. Am J Hum Genet 48: 546–551

50. Kang S-S, Wong PWK, Cook HY, Norusis M, Messer JV (1986) Protein-bound homocysteine. A possible risk factor for coronary artery disease. J Clin Invest 77: 1482–1486
51. Kang S-S, Wong PWK, Curley K (1982) The effect of D-penicillamine on protein-bound homocysteine in homocystinurics. Pediatr Res 16: 370–372
52. Kang S-S, Wong PWK, Malinow MR (1992) Hyperhomocysteinemia as a risk factor for occlusive vascular disease. Annu Rev Nutr 12: 279–298
53. Kang S-S, Wong PWK, 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: 536–545
54. Lentz SR, Sadler JE (1991) Inhibition of thrombomodulin surface expression and protein-C activation by the thrombogenic agent homocysteine. J Clin Invest 88: 1906–1914
55. Lindenbaum J, Stabler SP, Allen RH (1988) New assays for cobalamin defiency getting better spezificity. Lab Manag 26: 41–44
56. Malinow MR, Kang SS, Taylor LM, Wong PWK, Inahara T, Mukerjee D, Sexton G, Upson B (1989) Prevalence of hyperhomocysteinemia in patients with peripheral arterial occlusive disease. Circ Res 79: 1180–1188
57. Malinow MR, Nieto FJ, Szklo M, Chambless LE, Bond G (1993) Carotid artery intimal-medial wall thickening and plasma homocysteine in asymptomatic adults – the Atherosclerosis Risk in Communities study. Circulation 87: 1107–1113
58. Malinow MR, Sexton G, Averbuch M, Grossman M, Wilson DL, Upson B (1990) Homocysteinemia in daily practice: levels in coronary heart disease. Coron Art Dis 1: 215–220
59. Mansoor MA, Svardal AM, Schneede J, Ueland PM (1992) Dynamic relation between reduced, oxidized and protein-bound homocysteine and other thiol components in plasma during methionine loading in healthy men. Clin Chem 38: 1316–1321
60. Mansoor MA, Svardal AM, Ueland PM (1992) Determination of the in vivo redox status of cysteine, cysteinylglycine, homocysteine and glutathione in human plasma. Anal Biochem 200: 218–229
61. McCully KS (1992) Homocystinuria, arteriosclerosis, methylmalonic aciduria, and methyltransferase deficiency – a key case revisited. Nutr Rev 50: 7–12
62. McCully KS, Wilson RB (1975) Homocysteine theory of arteriosclerosis. Atherosclerosis 22: 215–227
63. McGill JJ, Mettler G, Rosenblatt DS, Scriver CR (1990) Detection of heterozygotes for recessive alleles. Homocysteinemia: paradigm of pitfalls in phenotypes. Am J Med Genet 36: 45–52
64. Mudd SH, Levy HL, Skovby F (1989) Disorders of transsulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited Disease. McGraw-Hill, New York, pp 693–734
65. Murphy-Chutorian DR, Wexman MP, Grieco AJ, Heininger JA, Glassman E, Gaull GE, Ng SKC, Feit F, Wexman K, Fox AC (1985) Methionine intolerance: a possible risk factor for coronary artery disease. J Am Coll Cardiol 6: 725–730
66. Mölgaard J, Malinow MR, Lassvik C, Holm A-C, Olsson AG (1992) Hyperhomocysteinemia: an independent risk factor for intermittent claudicatio. J Intern Med 231: 273–279
67. Parthasarathy S (1987) Oxidation of low density lipoprotein by thiol compounds leads to its recognition by the acetyl-LDL receptor. Biochim Biophys Acta 917: 337–340
68. Reed T, Malinow MR, Christian JC, Upson B (1991) Estimates of heritability for plasma homocysteine levels in aging adult male twins. Clin. Pharmacol. Ther. Genetics 39: 425–428
69. Refsum H, Helland S, Ueland PM (1989) Fasting plasma homocysteine as a sensitive parameter to antifolate effect. A study on psoriasis patients receiving low-dose methotrexate treatment. Clin Pharmacol Ther 46: 510–520
70. Refsum H, Helland S, Ueland PM (1985) Radioenzymic determination of homocysteine in plasma and urine. Clin Chem 31: 624–628
71. Refsum H, Ueland PM (1990) Clinical significance of pharmacological modulation of homocysteine metabolism. Trends Pharmacol Sci 11: 411–416
72. Refsum H, Ueland PM, Svardal AM (1989) Fully automated fluorescence assay for determining total homocysteine in plasma. Clin Chem 35: 1921–1927
73. Rodgers GM, Conn MT (1990) Homocysteine, an atherogenic stimulus, reduces protein-C activation by arterial and venous endothelial cells. Blood 75: 895–901
74. Rodgers GM, Kane WH (1986) Activation of endogenous factor V by a homocysteine-induced vascular endothelial cell activator. J Clin Invest 77: 1909–1916
75. Rosenblatt DS, Cooper BA (1990) Inherited disorders of vitamin-B12 utilization. BioEssays 12: 331–334
76. Rubba P, Faccenda F, Pauciullo P, Carbone L, Mancini M, Strisciuglio P, Carrozzo R, Sartorio R, Delgiudice E, Andria G (1990) Early signs of vascular disease in homocystinuria – a noninvasive study by ultrasound methods in eight families with cystathionine-beta-synthase deficiency. Metabolism 39: 1191–1195
77. Sartorio R, Carrozzo R, Corbo L, Andria G (1986) Protein-bound plasma homocysteine and identification of heterozygotes for cystathionine-synthase deficiency. J Inher Metab Dis 9: 25–29
78. Smolin LA, Benevenga NJ (1982) Accumulation of homocysteine in vitamin B-6 deficiency: a model for the study of cystathionine β-synthase deficiency. J Nutr 112: 1264–1272
79. Stabler SP, Lindenbaum J, Savage DG, Allen RH (1993) Elevation of serum cystathionine levels in patients with cobalamin and folate deficiency. Blood 81: 3404–3413
80. Stabler SP, Marcell PD, Podell ER, Allen RH (1987) Quantitation of total homocysteine, total cysteine, and methionine in normal serum and urine using capillary gas chromatography-mass spectrometry. Anal Biochem 162: 185–196
81. Stamler JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D, Loscalzo J (1993) Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. J Clin Invest 91: 308–318

82. Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, Tishler PV, Hennekens CH (1992) A prospective study of plasma homocysteine and risk of myocardial infarction in United States physicians. J Am Med Ass 268: 877–881
83. Starkebaum G, Harlan JM (1986) Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. J Clin Invest 77: 1370–1376.
84. Ubbink JB, Vermaak WJH, Bennett JM, Becker PJ, van Staden DA, Bissbort S (1991) The prevalence of homocysteinemia and hypercholesterolemia in angiographically defined coronary heart disease. Klin Wochenschr 69: 527–534
85. Ubbink JB, Vermaak WJH, Bissbort S (1991) Rapid high-performance liquid chromatographic assay for total homocysteine levels in human serum. J Chromatography 565: 441–446
86. Ubbink JB, Vermaak WJH, Vandermerwe A, Becker PJ (1992) The effect of blood sample aging and food consumption on plasma total homocysteine levels. Clin Chim Acta 207: 119–128
87. Ueland PM, 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
88. Ueland PM, Refsum H, Brattström L (1992) Plasma homocysteine and caardiovascular disease. In: Francis RB Jr (ed) Atherosclerotic cardiovascular disease, hemostasis, and endothelial function. Marcel Dekker, New York, pp 183–236
89. Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH (1993) Total homocysteine in plasma or serum. Methods and clinical applications. Clin Chem 39: 1764–1779
90. Uhlemann ER, TenPas JH, Lucky AW, Schulman JD, Mudd SH, Shulman NR (1976) Platelet survival and morphology in homocystinuria due to cystathionine synthase deficiency. N Engl J Med 295: 1283–1286
91. Vester B, Rasmussen K (1991) High performance liquid chromatography method for rapid and accurate determination of homocysteine in plasma and serum. Eur J Clin Chem Clin Biochem 29: 549–554
92. Wall RT, Harlan JM, Harker LA, Striker GE (1980) Homocysteine-induced endothelial cell injury in vitro: a model for the study of vascular injury. Thrombosis Res 18: 113–121
93. Wilcken DEL, Gupta VJ, Betts AK (1981) Homocysteine in the plasma of renal transplant recipients: effects of cofactors for methionine metabolism. Clin Sci 61: 743–749
94. Wilcken DEL, Wilcken B (1976) The pathogenesis of coronary artery disease. A possible role for methionine metabolism. J Clin Invest 57: 1079–1082
95. Williams RR, Malinow MR, Hunt SC, Upson B, Wu LL, Hopkins PN, Stults BN, Kuida H (1990) Hyperhomocysteinemia in Utah siblings with early coronary disease. Coron Artery Dis 1: 681–685

P. Lanzer J. Rösch (Eds.)

Vascular Diagnostics

Noninvasive and Invasive Techniques
Periinterventional Evaluations

Foreword by W. W. Parmley and G. Schettler

With Contributions by
C. J. G. Bakker, R. E. Barton, G. Biamino, P. Bösinger, E. G. Cape, J. Carreira, L. E. Crooks, P. de Jaegere, C. M. Gross, E. Gussenhoven, K. Haag, M. J. Hartkamp, J. Haase, D. Hausmann, M. Hennerici, J. Hennig, J. B. Hermiller, R. T. Higaschida, N. Hylton, D. Keane, F. S. Keller, M. Kouwenhoven, W. Krause, P. C. Lakin, P. Lanzer, W. Li, W. März, W. P. Th. M. Mali, M. R. Malinow, M. Maynar, K. Mizuno, G. L. Moneta, M. Nauck, J. Niepel, J. Noordzij, C. M. Orr, T. Peters, H. Pieterman, C. A. Pinkerton, L. J. Polman, J. M. Porter, J. M. Pulido-Duque, W. Rautenberg, H. Refsum, N. Reifart, R. Reyes, J. Rösch, P. J. Rubio, R. R. Saxon, M. Scheidegger, P. W. Serruys, W. Steinke, B. H. Strauss, G. P. Teitelbaum, S. H. K. The, P. M. Ueland, V. A. W. M. Umans, A. van der Lugt, F. C. van Egmond, M. S. van Leeuwen, J. van Tassel, H. van Urk, B. Velthuis, I. Wendelhag, K. U. Wentz, H. Wieland, J. Wikstrand, P. G. Yock, A. P. Yoganathan, E. Zeitler

Springer