Journal of Internal Medicine 1999; **246**: 425–454

REVIEW

Total homocysteine and cardiovascular disease

O. NYGÅRD[1], S. E. VOLLSET[1], H. REFSUM[2], L. BRATTSTRÖM[3] & P. M. UELAND[2]
From the [1]Division for Medical Statistics, Department of Public Health and Primary Health Care, and [2]Department of Pharmacology, University of Bergen, Norway; [3]Department of Medicine, County Hospital, Kalmar, Sweden

Abstract. Nygård O, Vollset SE, Refsum H, Brattström L, Ueland PM (University of Bergen, Norway; County Hospital, Kalmar, Sweden). Total homocysteine and cardiovascular disease (Review). *J Intern Med* 1999; **246**: 425–454.

Recent data have shown that an elevated plasma level of the amino acid homocysteine (Hcy) is a common, independent, easily modifiable and possibly causal risk factor for cardiovascular disease (CVD) which may be of equal importance to hypercholesterolemia, hypertension and smoking. This paper reviews the biochemical, clinical, epidemiological and experimental data underlying this conclusion and is critically questioning whether elevated tHcy is a causal factor.

Keywords: homocysteine, atherosclerosis, cardiovascular disease, pathogenesis.

History

The sulphur amino acid homocysteine (Hcy) was first described by Butz and du Vigneaud in 1932 [1]. One year later, a case report in the New England Journal of Medicine described an 8-year-old mentally retarded boy who died from a massive infarction of the right cerebral hemisphere and atherothrombosis of the internal carotid arteries [2]. This is believed to be the first description of homocystinuria. More than 30 years later the brother and two nieces of the patient were then confirmed to have this rare metabolic error characterized by severely elevated Hcy levels [3].

The biochemical finding of homocystinuria was first described in mentally retarded children in 1962 [4, 5]. Only two years later, Mudd *et al.* reported that the metabolic defect was due to cystathionine β-synthase (CBS) deficiency. Later, it was recognized that defects in other enzymes, including methionine synthase and methylenetetrahydrofolate reductase (MTHFR) also cause homocystinuria [6].

Clinical and pathological observations in patients with homocystinuria suggested a pathogenic role of elevated Hcy: First, the homocystinuria patients have a high incidence of premature cardiovascular episodes, in adolescence and even in childhood. Secondly, the vascular lesions occur independently of the site of the metabolic lesion, suggesting that Hcy itself and not a remote metabolic defect is responsible for the CVD [3, 6–10]. Based on these observations, McCully in 1969 presented his homocysteine theory of atherosclerosis [8, 11], suggesting that an elevated Hcy level may be a risk factor for CVD in the general population.

In 1976, the first clinical study supporting this theory was published by Wilcken and Wilcken who found that patients with angiographically verified coronary artery disease (CAD) had higher levels of Hcy-cysteine mixed disulphide after a methionine

load than the controls [12]. Since then, about 100 clinical and epidemiological studies on the relationship between Hcy and occlusive disease in the coronary, cerebral and peripheral arteries, and in the veins have been published. These studies demonstrate that an elevated Hcy level is a strong risk factor for CVD [13–16].

Biochemistry

Metabolism

The sulphur amino acid Hcy is not a dietary constituent and is not incorporated into proteins [6] but is exclusively formed as an intermediary product of methionine metabolism (Fig. 1). Through the action of methionine adenosyltransferase (E.C. 2.5.1.6), methionine is converted to S-adenosylmethionine, which is the major biological methyl donor required for numerous cellular processes, including the formation of creatinine and methylation of phospholipids [6, 17–20]. These reactions are catalysed by various methyltransferases that demethylate S-adenosylmethionine to S-adenosylhomocysteine, which is the immediate precursor of Hcy [6].

Once Hcy is formed, it may be salvaged to methionine by methylation, or degraded to cysteine by transsulphuration. Remethylation to methionine is in most tissues catalysed by the ubiquitous enzyme, methionine synthase (E.C. 2.1.1.13). This enzyme uses vitamin B_{12} as cofactor, and 5-methyltetrahydrofolate as methyl donor. 5-Methyltetrahydrofolate is formed by the vitamin B_2-dependent enzyme MTHFR (E.C.1.1.99.15). Hcy may also be converted to methionine by betaine-homocysteine methyltransferase (E.C. 2.1.1.5) using betaine as a methyl donor. This reaction is probably confined to the liver and possibly the kidney [6, 21].

Two vitamin B_6-dependent enzymes are involved in the transsulphuration pathway. The enzyme CBS (E.C.4.2.1.22) first condenses Hcy with serine to form cystathionine, which is then cleaved into cysteine and a-ketobutyrate by cystathionine γ−lyase (E.C.4.4.1.1) [6, 22]. Cysteine may be utilized in the synthesis of proteins or as a precursor of the antioxidant glutathione. The transsulphuration of Hcy to cysteine is irreversible, and therefore directs Hcy to catabolism via cysteine to sulphates as the final product.

Under normal metabolic circumstances, there is a strict balance between Hcy formation and elimination. Usually about 50% of the Hcy formed is remethylated to methionine. When protein or methionine intake is in excess, a larger proportion is catabolized by the transsulphuration pathway [19, 22]. If there is an increased formation of Hcy relative to its consumption, Hcy is excreted from the cells. This can be detected as an increased level of Hcy in plasma/serum or in the urine [23].

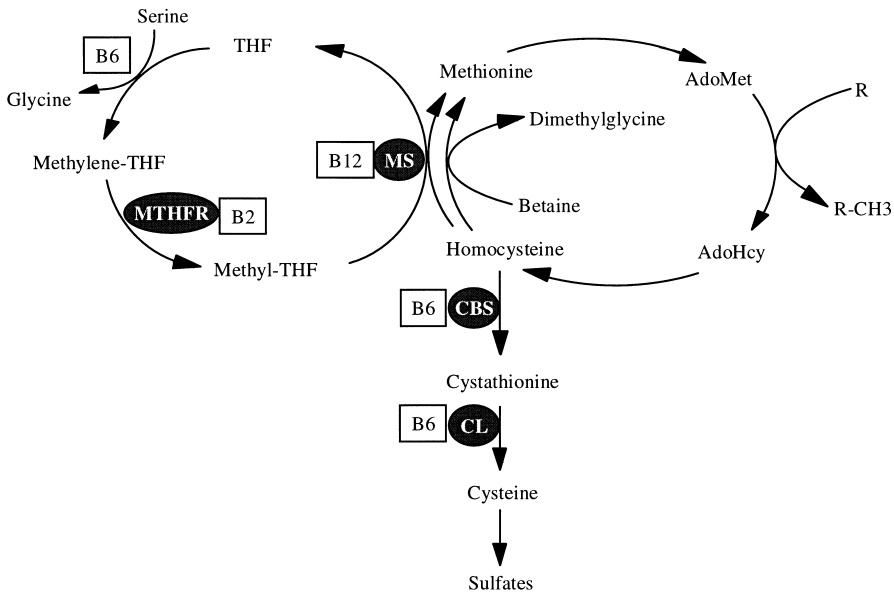

Fig. 1 Homocysteine metabolism: AdoMet; S-adenosyl methionine, AdoHcy; S-adenosyl homocysteine, THF; tetrahydrofolate, CBS; cystathionine β-synthase, CL; cystathionine γ-lyase, MS; methionine synthase, MTHFR; methylenetetrahydrofolate reductase, B6; vitamin B6, B12; vitamin B12, B2; vitamin B2.

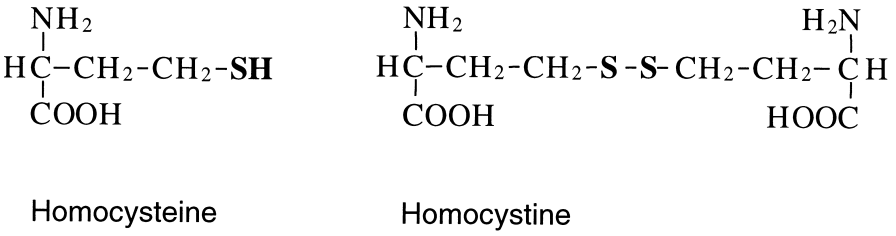

Fig. 2 Structural formulas of homocysteine and homocystine. Homocysteine is an amino acid with a free sulfhydryl (SH) group, whereas homocystine is the symmetric disulphide of two homocysteine molecules.

Homocysteine in blood

The Hcy concentration in plasma or serum is about 10 μmol L^{-1} in adults. However, Hcy exists in various forms (Figs 2 and 3); only trace amounts (~1%) are in the reduced (sulfhydryl) form, the remaining part is oxidized and exists as various disulphides [24]. About 70% is bound to albumin (via a disulphide bond), whereas the remaining 30% exists as free disulphides, mostly as a Hcy-cysteine mixed disulphide [6]. After blood sampling, there is a rapid redistribution between the free and protein-bound fractions of Hcy [25]. This interconversion of the different Hcy species explains the analytical problems that were overcome by the introduction of new methods for total Hcy (tHcy) determination during the 1980s [23].

Terminology

Homocysteine refers to a specific chemical compound; it is an amino acid with a free sulfhydryl group (Fig. 2). The homocysteine symmetric disulphide is termed homocystine, and the term homocystinuria (see below) denotes homocystine in urine and reflects the way in which this disease was originally identified. The other oxidized forms of homocysteine are referred to as homocysteine mixed disulphides (Fig. 3). The abbreviation Hcy is usually used for both homocysteine and its oxidized species. Total homocysteine, abbreviated tHcy, is a methodological term and refers to the *concentration* of homocysteine obtained after plasma/serum has been treated with a reductant which converts the free and bound disulphides into their respective sulfhydryl compounds.

Hyperhomocysteinemia refers to an elevated tHcy concentration in blood, and has been divided into mild (15–30 μmol L^{-1}), intermediate (30–100 μmol L^{-1}) and severe (> 100 μmol L^{-1}) hyperhomocysteinemia [26]. Homocystinuria is now a term exclusively used for the inborn errors of

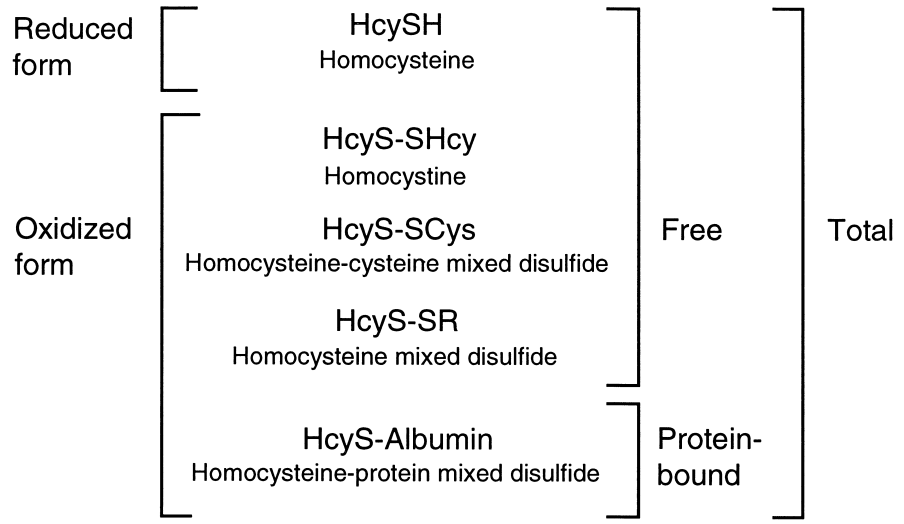

Fig. 3 The various forms of homocysteine (Hcy) in blood.

metabolism leading to severe hyperhomocysteinemia. Because Hcy is always present in blood, the term homocysteinemia is not clinically useful and should be abandoned.

Homocysteine determination

Measurement. A comprehensive review of the history and principles underlying the various methods for Hcy or tHcy determination has been published [23]. The initial methods were based on qualitative tests, such as a positive nitroprusside reaction, or the use of amino acid analyses developed in the late 1950s [27]. In the mid-70 s, these methods were replaced by second-generation amino acid analysers, which for the first time allowed the determination of the Hcy-cysteine level in plasma or serum from healthy subjects. Methods for protein bound Hcy [28] and tHcy [25] determination were introduced some few years later and greatly facilitated clinical research on tHcy. The common principle for all tHcy methods is the use of a reductant in order to cleave the disulphide bonds between Hcy and proteins or other thiols, thereby forming reduced homocysteine, which can be quantified directly or after derivatization. The majority of the assays are based on chromatographic techniques; high performance liquid chromatography (HPLC) with fluorescence detection is the method most commonly used [23]. The development of a rapid and fully automated HPLC method [29, 30] was a prerequisite for performance of the large-scale clinical and epidemiological Hcy studies in Norway [31, 32].

Factors that influence tHcy determination. The procedures for collection and processing of the blood sample are critical steps in the determination of tHcy [30, 33].

It is generally recommended that subjects should be fasting. In healthy subjects, the influence of food intake is, however, limited [33–36]; a protein-rich meal may increase the level by 10–15%, reaching a maximum 6–8 h after food intake [36]. The posture of the subject during blood collection should also be taken into account. Albumin, which binds the major portion of Hcy in plasma, is lower in the recumbent compared to the upright position [37]. The problem might be relevant in case-control studies where the patients may be in bed, whereas the controls are usually called in, and the blood collected in the sitting position.

After blood sampling, there is a time- and temperature-dependent release of Hcy from blood cells [38]. This leads to an artificial increase in plasma/serum tHcy concentration which amounts to 15–20% per hour at room temperature [23]. This artifact can be prevented by keeping the whole blood on ice or by adding a stabilizer such as sodium fluoride [33] or acidic citrate [39]. Once separated from the blood cells, the plasma tHcy concentration is stable for at least 4 days at room temperature, for 2 weeks at 0–2 °C, and for months or years when frozen at –20 °C [23, 40–42].

The within-person or biological variability of tHcy has been tested in several studies, and the tHcy levels show minor variation during repetitive analyses for 24 h [35], weeks [43], months [35, 43–45] or a few years [43]. The intraindividual biological coefficient of variation (CV) has been estimated to be about 9% [44, 45]. In addition, the reliability coefficient, which reflects the ability to correctly classify a subject with respect to its short-term average concentration by a single measurement, is slightly higher for tHcy (0.88) than for total cholesterol (0.85) [45] and most other clinical chemistry analytes [46]. These data therefore suggest that tHcy concentration of an individual is relatively constant, and can be determined by a single measurement when the sample handling is standardized and the method has an acceptable analytical CV.

Methionine-loading test. The concentration of tHcy in plasma or serum is usually between 5 and 15 μmol L^{-1} in adult populations [23]. However, tHcy is increased (2, 4 or 6 h) after the intake of a high dose of methionine (0.1 g kg^{-1} or 3.8 g m^{-2}), hence, stressing the metabolic pathways. This so-called methionine loading test was originally designed to detect heterozygosity for CBS deficiency [47–52], but later studies have demonstrated that an abnormal response to methionine is common in the general population, and probably cannot be fully explained by mutations in the CBS [53]. The fact that up to 40% of subjects with elevated postload tHcy have normal fasting levels [54–56], suggests that this test provides information beyond that obtained by determining fasting tHcy level. The physiological corollary of the methionine loading is

uncertain, but it may mimic the response to dietary methionine intake. The procedure is cumbersome and difficult to apply in large-scale epidemiological studies.

Determinants of total homocysteine concentration

The tHcy level is a function of a complex interaction between multiple genetic and environmental factors [6, 57].

Genetic determinants

Discovery of homocystinuria due to CBS deficiency in 1962 [4, 5] initiated the focus on Hcy as a possible pathogenic factor. These patients have extremely high levels of tHcy in plasma (300–400 μmol L^{-1}) and urine [58]. The clinical condition is characterized by ectopia lentis, osteoporosis, skeletal anomalies, mental retardation and a high incidence of premature vascular episodes [3–7, 9, 59, 60]. CBS deficiency, an autosomal recessive disorder [6, 61], is the most common cause of homocystinuria [6, 62, 63]. However, MTHFR deficiency [64, 65] and methionine synthase deficiency [66] also lead to severe hyperhomocysteinemia (>100 μmol L^{-1}) and thromboembolic vascular disease even in childhood [10].

The genes encoding for CBS [67], MTHFR [68], methionine synthase [66, 69], as well as betaine-homocysteine methyltransferase [70], have now been cloned. In patients with mutations of the CBS gene, more than 50 different pathogenic mutations that have been found [53], and these can partly explain ethnic variations and differences in phenotypic expression amongst patients with CBS deficiency [53, 63, 71, 72].

It was discovered early that obligate heterozygotes for CBS deficiency usually have a normal fasting level of tHcy [49] but frequently respond to the methionine loading test with an abnormal increase in the tHcy concentration [6, 73]. Interestingly, patients with Down syndrome have an additional copy of the CBS gene and generally low tHcy levels, and this condition has been regarded as an atheroma-free model [74, 75].

A common polymorphism in the MTHFR gene causes low activity and thermolability of the enzyme and is associated with elevated tHcy. Thermolability of MTHFR was first described by Kang *et al*. in 1988, who also demonstrated that this enzyme variant was commonly occurring in CVD patients [76, 77]. In 1995 it was recognized that thermolability was due to a C to T substitution at position 677 of this gene. Several studies have demonstrated that the C677T polymorphism is associated with hyperhomocysteinemia in subjects with impaired folate status [77–83]. The C677T allele frequency is high (30–40%) in most populations, and about 10% of the Caucasian population is homozygous, and therefore at particularly high risk of developing hyperhomocysteinemia. However, there is substantial interethnic variation [84, 85]. In populations of African descent [84, 86], Asian Indians (Refsum, unpublished) or Canadian Inuit [87], the prevalence is 0–2%, whereas it may be about 20% in Asians [84] and even higher in Northern Italy [88]. This difference may partly explain variable tHcy levels according to ethnicity [23, 89–91].

Physiological determinants

Blood levels of tHcy are higher in men than in women and increase with age [31, 92–99]. The difference between the genders becomes apparent in puberty [100] and is believed to be related to hormonal factors, but also to lifestyle, diet and vitamin status.

The tHcy concentration is decreased in normal pregnancies [101] but higher levels are observed in pregnancies complicated by recurrent spontaneous abortions or abruptio placentae [102, 103]. The level of tHcy is strongly related to renal function [104]. Two different mechanisms may be involved. First, in healthy subjects, the main source of Hcy is the adenosylmethionine-dependent methylation of guanidoacetate to form creatine and its anhydride creatinine [6, 99]. Creatine/creatinine synthesis is related to muscle-mass, and this may partly explain the higher tHcy (and serum creatinine) levels in men compared to women. Secondly, renal function plays a central role for clearance of both creatinine and Hcy [105–107]. Because the urinary excretion of Hcy is low [25, 108], it has been suggested that an extensive metabolism of Hcy, probably through transsulphuration, takes place in the kidneys [105, 109, 110]. Although this has recently been contested [111], the normal physiological decline in

renal function by age may to some extent explain the increase in tHcy with age [99]. A recent study including the GFR-marker cystatin C supports this assumption, and that the sex difference in tHcy is at least in part due to differences in creatinine synthesis [112].

Nutritional determinants

The postprandial as well as long-term effect of methionine intake from proteins has been investigated, but probably only causes a marginal change in the tHcy level [33–36, 54, 113]. In contrast, dietary intakes [114–117] or plasma levels (Fig. 4) of folate and vitamin B_{12} are inversely related to tHcy concentration. Deficiencies of these vitamins may cause a moderate or even severe hyperhomocysteinemia, and an increased tHcy level is a sensitive marker of disturbed function of both folate and vitamin B_{12} [118]. Intake or plasma levels of vitamin B_6 may be weakly related to basal tHcy [115, 119]. However, most studies find that vitamin B_6 deficiency is associated with normal fasting from tHcy, but with a marked elevation in tHcy after methionine loading [6, 26, 57, 120, 121]. Insufficient dietary intake of the vitamins involved in Hcy metabolism seems to be common in Western countries, especially amongst the elderly [115, 119, 122].

Lifestyle

The influence of various aspects of lifestyle on the tHcy levels has been studied in the Hordaland Homocysteine Study. Data from this large-scale population based study show that smoking and heavy coffee consumption are associated with elevated tHcy levels, whereas physical activity is associated with low tHcy [31, 117, 123]. A moderate alcohol consumption may be associated with reduced tHcy levels [124], whereas a chronic high alcohol consumption is associated with elevated tHcy [124–126], possibly via impaired folate or vitamin B_6 function [126–129].

Drugs

A number of drugs influence tHcy concentration by

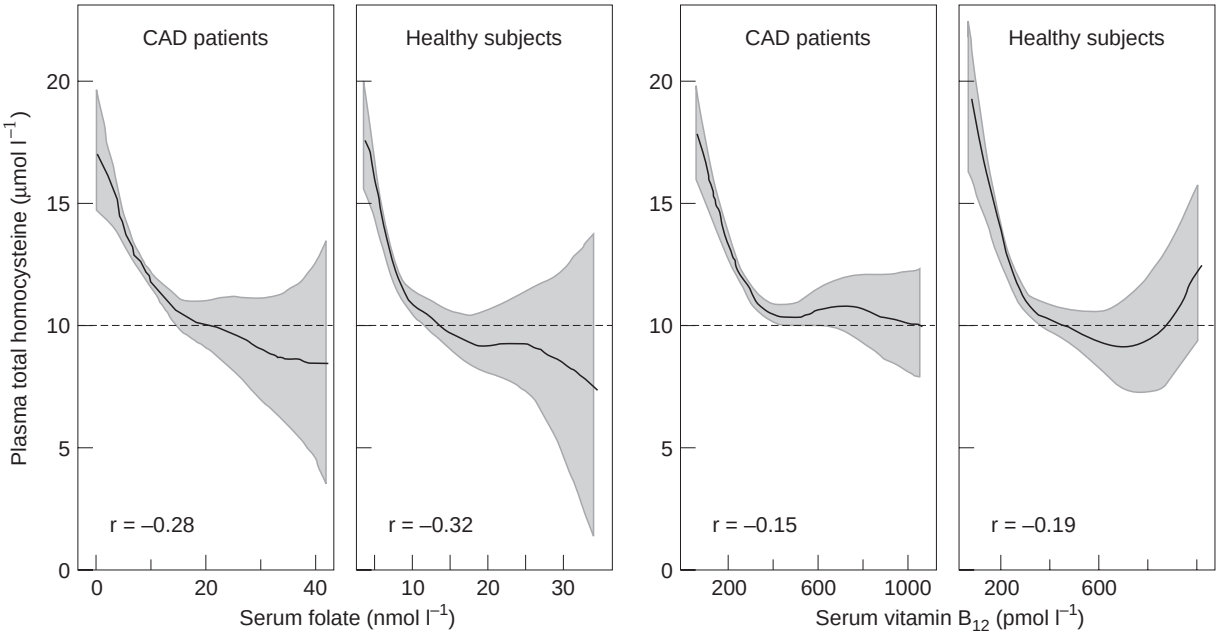

Fig. 4 Serum levels of folate and vitamin B_{12} vs. plasma total homocysteine in 587 patients with coronary artery disease (CAD) [32] and 329 healthy subjects [117, 123]. The relationships between the vitamins and tHcy levels are similar in the two populations, although slightly stronger dose–response relationships are observed amongst the healthy individuals than amongst the patients. In both populations, folate levels show a substantially stronger relationship to tHcy than vitamin $B_{12;}$ whereas folate demonstrates a dose–response relationship to tHcy even at high levels, the relationship to tHcy of vitamin B_{12} is more uncertain at the highest vitamin B_{12} levels. Crude correlations are presented.

interfering with the Hcy metabolism. Folate antagonists such as methotrexate and the vitamin B_{12} antagonist nitrous oxide may lead to a marked tHcy elevation [130]. In renal transplant recipients, treatment with cyclosporin causes a moderate increase in tHcy [131]. The antidiabetic drug metformin may elevate tHcy by affecting folate as well as vitamin B_{12} levels [132, 133], and tHcy is elevated in epileptics treated with anticonvulsant drugs probably because of interference with folate polyglutamation and retention [134].

Amongst hypolipidemic drugs, it has been known for several years that a combination of colestipol and niacin may elevate tHcy [135], and the association was primarily related to the folate-antagonistic effect of cholestyramine [135]. This was later confirmed in a study on hyperlipidemic children [136]. The C677T mutation in the MTHFR gene seems to predispose to this effect [137]. Niacin may also induce hyperhomocysteinemia, but by affecting vitamin B_6 status [138].

Theofyllin [139] and azaribine [140] increase tHcy by inhibiting vitamin B_6 function. Notably, azaribine, a drug previously used in the treatment of psoriasis, was related to an increased incidence of thromboembolism, and was therefore prohibited by the Food and Drug Administration in 1976 [140–142].

L-dopa becomes methylated by adenosylmethionine and may therefore elevate tHcy by enhancement of Hcy production [143, 144]. Several hormone related drugs may influence the tHcy level, but the mechanisms have not been elucidated. In women, tHcy levels are lowered by tamoxifen [145, 146], possibly by hormone replacement therapy [147–149], and some oral contraceptives [150–152], and increased by androgen administration [153]. In men, oestrogen plus antiandrogen administration has a substantial tHcy lowering effect [153].

Aminothiols such as penicillamin [154, 155], acetylcysteine [156] and ifosfamide/mesna [157] reduce the plasma tHcy level, probably by increasing renal clearance or by displacing Hcy from the protein binding sites [154, 155]. Such drugs have been suggested for the treatment of homocystinuria [154, 155]. In patients with moderately elevated tHcy levels, aminothiols are not, however, the drugs of choice since long-term therapy with these agents may have side-effects related to disulphide exchange and redox reactions. Vitamin therapy seems to be a more effective, safer and less expensive alternative.

Various diseases

Several clinical conditions are associated with elevated tHcy levels. This can usually be explained by low vitamin status, impaired enzyme function or renal failure.

Hyperproliferative disorders, such as acute lymphoblastic leukaemia [158] and severe psoriasis [24, 159–161] are associated with elevated tHcy levels. This is probably explained by an increased Hcy export from the proliferating cells.

Recently, an increased prevalence of hyperhomocysteinemia has been reported amongst patients with hypothyroidism [162]. Acute hyperinsulinemia is associated with a decrease in tHcy concentration in normal subjects [163]. In diabetes mellitus, elevated tHcy is observed concomitant with the onset of nephropathy [164], which occurs more frequently amongst those with early onset of the disease and with a poor metabolic control [165]. Nephropathy is predicted by microalbuminuria [166], which may be a reliable index of vascular damage both in diabetic and other patient groups [167]. Recent studies show that microalbuminuria is strongly related to plasma tHcy in diabetic patients [168–170], although the relation may be independent of the diabetes per se [170].

Vitamin therapy

Hyperhomocysteinemia, as a risk factor for CVD, represents a strong incentive for investigating folic acid, vitamin B_{12} and B_6, and possibly also betaine, as tHcy lowering therapy. Based on the role of these vitamins in Hcy metabolism (Fig. 1), a differential effect on tHcy levels in relation to fasting and methionine intake is expected. Supplementation with folic acid lowers high fasting as well as postmethionine load tHcy in most subjects, whereas vitamin B_{12} only has an effect in subjects with vitamin B_{12} deficiency [77, 171–173]. Vitamin B_6, even in very high doses, does not affect fasting tHcy levels, but usually reduces abnormal elevation in tHcy after a methionine load. The effect of betaine is less known, but may be effective in some patients with elevated postload tHcy levels [174, 175].

The marked tHcy lowering effect of high doses of

folic acid (5 mg day^{-1}) in the absence of folate deficiency was first reported by Brattström *et al.* in 1988 [176]. This and later studies [172, 174, 177–179] have demonstrated that 0.5–10 mg folic acid, alone or in combinations, reduces fasting as well as postload tHcy levels, usually by about 25–35%. In contrast to vitamin B_6, vitamin B_{12} has a small additional effect, observed in vascular patients as well as in healthy subjects, and it is most pronounced in subjects with hyperhomocysteinemia [179].

In subjects with markedly elevated tHcy ($\geq$40 μmol L^{-1}) and without overt vitamin B_{12} deficiency, a daily supplement of 200 μg folic acid led to a normal tHcy level in 70% of the subjects whereas 5 mg day^{-1} was effective amongst the remainder in this study. Most hyperhomocysteinemic individuals were homozygous for the C677T mutation of the MTHFR gene [82]. The question of the folic acid responsiveness of subjects with the C677T genotype was elaborated further in a recent study demonstrating that these subjects were more responsive than the CC subjects to the tHcy-lowering effects of 1–2 mg folic acid [180].

The effect of low doses of folic acid has been investigated in healthy subjects with normal tHcy levels. Doses of 0.3–0.4 mg day^{-1} seem sufficient to maintain low or normal tHcy in most subjects [181, 182]. The minimal dose required probably depends on the individual's uptake, utilization and stores of folate. Thus, amongst the subjects in the lowest tertile for tHcy, a daily folic acid dose up to 400 μg induced a nonsignificant reduction in the tHcy level (from 7.1 to 6.4 μmol L^{-1}). Individuals with a tHcy level in the two upper tertiles responded to a 100-μg folic acid per day with some reduction in the tHcy level, and a further reduction was observed when the dose was increased to 200 μg and 400 μg [183].

The possible tHcy lowering effects of other vitamins have been tested in subjects with mildly elevated tHcy, but treatment with the antioxidant vitamins ascorbic acid, α-tocopherol or β-carotene did not lower the tHcy levels [184].

Cardiovascular disease

Homocystinuria

Biological plausibility of Hcy as an atherothrombotic agent is derived from the clinical presentation of patients with homocystinuria characterized by severely elevated tHcy. Untreated, 50% of these patients suffer a thromboembolic event (half of which are venous thromboembolic events), and 20% die before the age of 30 years [6, 9]. On autopsy, findings include arteriosclerotic lesions in large and medium-sized arteries, arterial and venous thrombosis and multiple infarctions in different organs. The microscopic findings differ from the atheromatous changes in patients with hyperlipidemia, and include loosening and fragmentation of the internal elastic lamina, intimal hyperplasia and narrowing of the arterial lumen [3, 6, 57]. This difference from hyperlipidemia is also demonstrated *in vivo*; ultrasound assessed carotid intima-media thickness and blood flow velocity of middle cerebral arteries are normal in young patients with homocystinuria but clearly pathologic in familial hypercholesterolemia [185].

Arterial disease

Since the first study by Wilcken and Wilcken in 1976 [12], the results from about 100 clinical and epidemiological studies have shown that even a mild or moderate elevation of tHcy is associated with an increased risk of CVD [13].

Several pooled analyses or meta-analyses based primarily on retrospective studies have been performed. Ueland *et al.* summarized in 1992 the results of 17 studies including approximately 1500 CVD patients and 1400 controls [57]. The findings were consistent across the various forms of CVD, and showed that the fasting tHcy concentration was 32% higher amongst patients. A methionine load test had been performed in some of the studies, and an abnormal postload tHcy was detected in 24% of the patients compared to only 2% of the controls.

In a report by Boers 2 years later, the analysis was restricted to studies with postload Hcy determinations [186]. Amongst the 750 CVD patients, mild hyperhomocysteinemia was detected in 21% with CAD, 24% with cerebrovascular disease and 32% with peripheral vascular disease, compared to 2% amongst 200 controls.

The meta-analysis by Boushey *et al.* in 1995 [187] was based on 24 retrospective and 3 prospective studies and included approximately

4100 patients and a similar number of controls. In this analysis, it was estimated that the relative risk associated with a 5-μmol L^{-1} increment in the tHcy level, was 1.7 (95% CI, 1.5–1.9) for coronary heart disease, 1.9 (95% CI, 1.6–2.3) for cerebrovascular disease and even higher for peripheral artery disease. It was further calculated that at least 10% of the population's risk of CAD might be attributable to tHcy.

Recently, Wald *et al.* summarized the results of eight retrospective studies on tHcy and myocardial infarction [188]. The combined odds for a 5-μmol L^{-1} increase in the tHcy level was 1.84 (95% CI, 152–2.23), which was reduced to 1.65 if one study with an atypically high estimate was excluded.

The relationship between CVD and tHcy was studied in a large European case-control study of 800 cases and 750 controls [55]. Fasting as well as postload tHcy concentrations were about 20% higher in cases compared to controls, and similar increases in risk was observed with elevation in any of the two types of tHcy determinations. A particularly high risk was found in subjects with increased levels of both fasting and postload tHcy concentrations. After adjustment for conventional risk factors, the relative risk per 5-μmol L^{-1} increment in fasting tHcy level was 1.35 (95% CI, 1.2–1.6) for men and 1.42 (95% CI, 0.99–2.05) for women. Notably, users of supplements containing folic acid, vitamin B_6 or vitamin B_{12} had a relative risk of 0.38 (95% CI, 0.2–0.7) compared with nonusers.

Cross-sectional studies using angiography or ultrasound imaging have shown that tHcy is related to the extent of atherosclerosis in the carotid [189–192], peripheral [193] and coronary [32, 194–200] arteries, and tHcy is related to graft vasculopathy in transplant recipients [201].

A recent prospective study also shows that tHcy is related to the progression of coronary atherosclerosis [202]. This may indicate that tHcy promotes the atherosclerotic process. In addition, some prospective studies have shown that tHcy is related to the acute thromboembolic event such as myocardial infarction [203–206], stroke [207] or arterial thrombosis [208]. However, plasma tHcy is low in the acute phase of myocardial infarction [113, 209] and stroke [210]. Because the major part of tHcy is probably bound to albumin [6], this may be related to lower albumin levels in bedfast patients [37, 211] or to other haemodynamic changes due to the acute stress.

Mortality

Whereas most previous prospective investigations have studied presumably healthy individuals, we evaluated mortality in a cohort of 587 patients with angiographically verified CAD in 1991–92 [32]. This distinction may explain the particularly strong effect of tHcy amongst the patients. Five years follow up revealed that, together with left ventricular ejection fraction and creatinine levels, tHcy was the strongest determinant of overall and cardiovascular mortality. The difference in tHcy between those who died and those who survived was 35% in women and 20% in men (Table 1). Notably, plasma tHcy was the strongest modifiable determinant of mortality, and the mortality rate amongst individuals with tHcy <9.0 μmol L^{-1} was less than 1% per year compared with 6% per year in those with tHcy ≥ 15 μmol L^{-1}. In addition, the relation of tHcy with mortality was present in subgroups (Fig. 5).

We also found a strong relationship between tHcy and previous myocardial infarction, which probably explains the strong relationship between tHcy and ejection fraction. Plasma tHcy was only weakly related to the number of stenosed coronary arteries. These observations suggest that elevated tHcy is more strongly related to the acute event than to atherosclerosis.

Two recent studies confirm our finding that tHcy is an important risk factor for mortality in vascular patients [212, 213]. In one of these studies of patients with acute coronary syndromes, tHcy was not related to short-term mortality within 1 month but was related to mortality after a median follow up time of 3.5 years [213]. Three recent community

Table 1 Plasma total homocysteine (tHcy) levels according to mortality amongst 587 patients with angiographically verified coronary artery disease diagnosed at Haukeland University Hospital 1991–92. Mean follow up was 4.6 years

Gender	Number	Mortality (%)	Survivors tHcy (μm L^{-1})	Deceased tHcy (μm L^{-1})
Women	109	10.1	10.9	14.7
Men	478	12.1	11.7	14.0

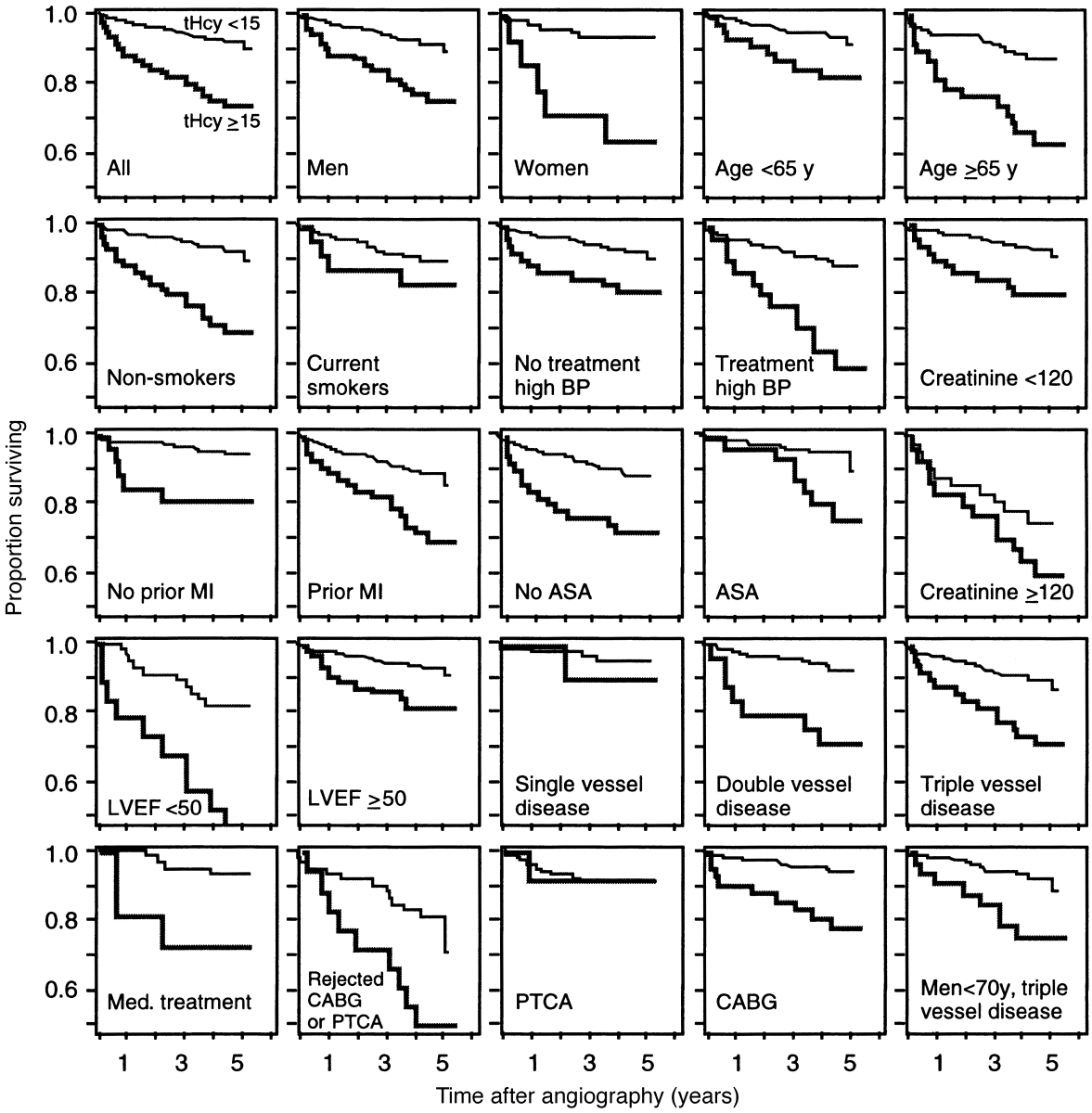

Fig. 5 Kaplan–Meier survival plots comparing patients with plasma total homocysteine (tHcy) above (bold line) and below (thin line) 15 μmol L^{-1} in various subgroups of patients with coronary artery disease [32]. BP denotes blood pressure, MI myocardial infarction, ASA acetylsalicylic acid, LVEF left ventricular ejection fraction, CABG coronary artery bypass grafting, and PTCA percutaneous transluminal coronary angioplasty.

studies now show that plasma tHcy is a risk factor for mortality, also in the general population [188, 214, 215].

Venous thromboembolic disease

Besides its relation with arterial occlusive disease, there is recent evidence that hyperhomocysteinemia is related to venous thrombosis as well [15, 216–227]. This is perhaps the strongest evidence of a thrombotic effect of elevated tHcy.

Cardiovascular risk factors

Like the overall risk for CVD, plasma tHcy is higher in men than in women and increases with age. In women, menopause may confer an increase in tHcy beyond the effect of age [228]. There is an

association between tHcy and several established risk factors, including serum cholesterol [31, 229, 230], blood pressure [31, 92, 230], cigarette smoking [31, 204, 231], diabetes mellitus [164] and renal function [6, 25, 99, 105, 108, 232]. Notably, amongst patients with renal failure, hyperhomocysteinemia is more prevalent than other cardiovascular risk factors [104] and is associated with increased risk of CVD [233]. Elevated tHcy may confer a particularly high risk of CVD in diabetic patients [170]. tHcy is also elevated in patients with hypothyroidism [162], who have a high incidence of CVD [234]. Furthermore, tHcy is related to fibrinogen [195], von Willebrand factor (vWF) [235–237] and the intercellular adhesion molecule ICAM-1 [238]. The effect of high concentrations of tHcy on a number of recognized risk factors related to haemostasis, endothelial function and vascular smooth muscle cells have been investigated in mechanistic studies, and will be discussed below.

Recent studies indicate that elevated tHcy may interact with some hereditary atherothrombotic disorders [239, 240]. The factor V Leiden mutation, which is the most frequent cause of familial venous thrombosis, produces an increased risk of combined venous and arterial thrombosis amongst patients with homocystinuria [241]. Moderate hyperhomocysteinemia, in some but not all studies, seems to interact with the factor V Leiden mutation to increase the risk of idiopathic venous thrombosis beyond that already conferred by the Leiden mutation [220, 222, 240, 242, 243].

Familial hyperhomocysteinemia

The plasma level of tHcy is frequently elevated in subjects with a family history of CVD. This has been documented through various studies on twins, siblings, parents, children, grandchildren and relatives of patients with CVD [93, 100, 244–248]. Even in young children aged 8–12 years, a higher tHcy level is observed in those who reported premature vascular death in a male relative [100]. Hence, the available data strongly suggest that the plasma tHcy is a genetic trait, which may contribute to a family history of CVD.

Experimental evidence and mechanisms

Elevated tHcy is related to numerous processes involved in atherosclerosis or thrombosis. This evidence relates both to *in vitro* and *in vivo* studies, and elevated tHcy is associated with the development of atherosclerosis in animals. There is no unifying hypothesis on the molecular and cellular mechanisms whereby Hcy might influence the pathogenesis of CVD. An overview of the possible mechanisms is presented.

Endothelium. Increasing evidence points to a relationship between elevated Hcy and endothelial dysfunction, which reflects an imbalance between factors involved in vasomotor function, cellular growth, coagulation or thrombolysis [249, 250]. A dysfunctional endothelium is an early marker of atherosclerosis and thrombotic risk [251, 252], and the vasomotor function improves during regression of diet-induced atherosclerosis in monkeys [252, 253]. Umbilical endothelial cells from patients with homocystinuria have normal endothelial function markers [254]. However, a large series of investigations link elevated Hcy levels with injury and dysfunction of the endothelium, and a direct pathophysiological response to Hcy is therefore implied [254].

Endothelium and coagulation, in vitro studies. The vascular endothelium has a vital function in regulating the balance between pro-and anticoagulant factors [255, 256], and several of these factors have been used as markers of endothelium function. Studies with cultured endothelial cells show that elevated Hcy may activate coagulation factor V [257], inhibit the binding of tissue plasminogen activator to endothelial cells [258, 259] by hampering the binding to annexin II [259], reducing the thrombomodulin-dependent activation of protein C [260–262], suppressing the expression of anticoagulant heparan sulphate [263], stimulating the expression of procoagulant tissue factor [264], and decreasing the production of the two important vasorelaxant and antiaggregatory substances, nitric oxide [265] and prostacyclin [266–268]. These results indicate that elevated tHcy may modulate the endothelium in a way that promotes thrombosis. One study indicates an opposite effect by demonstrating in vitro inhibition of vWF production [269],

but this finding has been contested in later *in vivo* studies [235, 236].

Endothelium and coagulation, in vivo studies. Diet-induced hyperhomocysteinemia is associated with altered levels of endothelial markers in animals. Thus, elevated levels of vWF and a concomitant decrease in angiotensin converting enzyme levels have been observed in hyperhomocysteinemic rats [236], whereas a decreased thrombomodulin-dependent protein C activation was detected in the thoracic aorta of monkeys [270].

In humans, elevated levels of tHcy, vWF and thrombomodulin have been observed in patients with peripheral artery disease, and the concentrations decrease after 12 weeks daily treatment with vitamin B_6 plus folic acid [235]. However, a recent study from this group suggests that elevated protein markers are a consequence rather than a cause of endothelial injury [237].

Endothelial-dependent vasodilatation. Endothelial dysfunction also causes an imbalance between relaxing and contracting forces [271], which is ascertained by impaired vasomotor responses to various stimuli [252]. A marked impairment of endothelium-dependent vasodilatation in response to acetylcholine and adenosine diphosphate (ADP) has been demonstrated in hyperhomocysteinemic monkeys [270], but normalization of plasma tHcy by B-vitamins is insufficient to correct the vascular dysfunction when the animals persist on a hypercholesterolemic diet [272].

Impairment of flow-mediated endothelium-dependent vasodilatation was demonstrated in children with homocystinuria, by using high-resolution ultrasound [273]. Similar observations have recently been made in adults with moderately elevated tHcy [274–277], and the endothelial dysfunction is improved by folic acid supplementation [278].

Cytotoxic effect on endothelial cells. Elevated Hcy has been associated with endothelial damage in several *in vivo* [79–282] and *in vitro* studies [283–288]. Although negative studies have been published [289], the data may suggest a cytotoxic effect of Hcy on the endothelial cells. This is supported by a recent finding of a marked increase in circulating endothelial cells (endothelemia) in vascular patients subjected to methionine loading [290]. Other studies indicate that Hcy directly inhibits the growth of such cells [291, 292] as well as other cell types (HeLa cell line) [293].

Inflammation. As an initial step in atherosclerosis, endothelial injury involves inflammation and targeting of monocytes [294]. In the study by de Jong *et al.* [237], tHcy was not associated with signs of inflammation as evaluated by CRP. In contrast, amongst the participants of the Physicians' Health Study, plasma tHcy was significantly related to the concentration of the intercellular adhesion molecule ICAM-1 [238].

Oxidative stress. The underlying mechanisms for the Hcy-associated endothelial injury and dysfunction are not established. *In vivo* studies indicate that hyperhomocysteinemia induces oxidative stress [295, 296]. This is further elaborated by *in vitro* studies showing that oxygen and catalytic activity of copper [284, 285, 287, 288], caeruloplasmin [285] or hypoxanthine/xanthine [287] are required to provoke the Hcy-induced cellular injury. The response may be inhibited by the metal chelator, desferal [288]. Because the concentration of the copper containing protein caeruloplasmin is increased in patients with homocystinuria [297], copper-catalysed oxidation of Hcy with subsequent hydrogen peroxide formation has been proposed to be involved in the pathogenesis of CVD [284, 285].

The Hcy effects are also related to elevated levels of the oxidation products thiobarbituric acid reactive substances [288]. In addition, catalase, which breaks down hydrogen peroxide, inhibits the response [283, 285, 287, 288]. This indicates a central role for hydrogen peroxide in mediating the oxidative stress on endothelial cells [283, 285, 287, 288].

The observation that endothelium-independent vasodilatation is not affected whereas endothelium-dependent response is impaired in hyperhomocysteinemic humans, indicates that high tHcy reduces endothelial nitric oxide activity [273–277]. Hcy may decrease the bioavailability of nitric oxide by forming S-nitroso-Hcy [265]. Another potential mechanism is impaired function of the intracellular antioxidant enzyme glutathione peroxidase, which catalyses the reduction of hydrogen peroxide and lipid peroxides [298, 299]. This effect of Hcy may be specific since it is not provoked by cysteine [299].

Prolonged Hcy administration may also reduce intracellular levels of glutathione [293].

The initial response to elevated Hcy appears, however, to be stimulation of intracellular production of glutathione [299], nitric oxide or S-nitroso-Hcy [300]. The scavenging potential of glutathione and nitric oxide may be saturated after prolonged Hcy elevation. This may lead to toxic effects of Hcy by increased formation of hydrogen peroxide [293, 300–302] or other reactive oxygen species, such as the superoxide radical or hydroxyl radical [265, 301, 303].

Peroxidation of lipids. The oxidation hypothesis of atherosclerosis implies a causal role of oxidized lipoproteins [304, 305]. Signs of increased low-density lipoprotein (LDL) peroxidation by Hcy have been demonstrated in cell-free systems [306, 307] and *in vivo* in animals [308, 309]. However, other *in vivo* and *in vitro* studies [310–313] refute these findings, and one *in vitro* study indicates that elevated Hcy may actually serve as an antioxidant and protect LDL against oxidative modification [314]. In contrast, a recent human study suggests an increased lipid peroxidation by elevated tHcy after methionine load [315].

Smooth muscle cells and collagen. Several investigations have shown that hyperhomocysteinemia promotes the growth of vascular smooth muscle [279, 291, 316–320] and other cells in the aortic wall [236]. Although diverging results have been obtained [292], Hcy may provoke this effect by induction of cyclin A gene expression [316], enhancement of platelet derived growth factor [318], or stimulation of protein kinase C activation [321]. In addition, Hcy promotes the production of collagen [317, 320], which may account for the intimal hyperplasia in patients with homocystinuria [3]. The fragmentation of the internal elastic lamina observed in these patients [3, 322] may be related to serine elastase induction by Hcy [322].

Platelet aggregation. Some *in vitro* [266] and *in vivo* [279, 280, 282, 308, 323] experiments indicate that Hcy enhances platelet aggregation. This is also supported by the finding of an increased thromboxane biosynthesis amongst patients with homocystinuria [324, 325] and in hyperhomocysteinemic rats [308], which is believed to reflect *in vivo* platelet activation. However, results from several other studies argue against enhanced platelet aggregation in hyperhomocysteinemia [265, 325–327].

Miscellaneous mechanisms. Other mechanisms linking elevated tHcy with CVD or thrombosis include activation of coagulation factor XII [328], enhanced binding of lipoprotein(a) to fibrin [329], stimulation of the macrophage tissue factor activity [308, 323], and impaired anticoagulant pathway as indicated by elevated levels of prothrombin fragments [330].

Validity and clinical relevance of the suggested mechanisms. The validity and clinical relevance of the different observations have been questioned. Most experiments were carried out with one particular chemical form of Hcy and do not reflect the complex redox reactions or interconversion of the different Hcy species in blood [24]. In addition, millimolar concentrations were often applied, which is more than 100-fold higher than observed in moderate hyperhomocysteinemia [331]. This may also explain why many *in vitro* observations have not been reproduced *in vivo* [6, 286]. Finally, often the effects provoked by high Hcy are not specific since they can be obtained with other thiols [286, 306, 332].

Is homocysteine causally related to CVD?

A critical question is whether elevated tHcy is a cause of CVD or just an epiphenomenon. The evidence supporting a causal relationship as well as that disputing it, will therefore be critically reviewed.

Observational clinical and epidemiological evidence

The belief that Hcy may be an atherothrombotic agent was originally derived from the clinical presentation of patients with homocystinuria [3, 6, 8]. The assumption is supported by an extensive series of cross-sectional or case-control studies almost consistently demonstrating a higher risk of CVD amongst subjects with moderate tHcy elevations [13, 55, 57, 186, 187, 189–191, 193–200]. In addition, elevated tHcy potentiates the risk conferred by other risk factors such as hypertension [55, 333] and smoking [55].

Prospective studies. The most reliable epidemiological evidence originates from prospective studies because samples for tHcy determination are collected before disease occurrence. Presently, when investigations with mortality as endpoint are included, results from at least 24 prospective studies have been reported (Table 2). Sixteen of the studies are based primarily on healthy subjects, including five from the Physicians' Health Study and two from the British Regional Heart Study. All the community or occupational cohort studies have used a nested case-control design, which rely upon the quality of tHcy determinations in frozen samples. This approach is probably appropriate [23, 40–42]. The tHcy level was significantly related to subsequent CVD or mortality in nine of the studies [188, 203, 204, 206, 207, 214, 215, 222, 334], non-significantly related in three studies [42, 335, 336], whereas no association was observed in the remaining four [194, 337–339].

In eight of the prospective studies, patients with systemic lupus erythematosus, renal disease or various types of CVD were followed, and tHcy significantly predicted the prognosis [32, 208, 212–214, 340, 341] or progression of atherosclerosis [202] in all these investigations. The stronger predictive power of tHcy in clinical cohorts, compared to the community-based studies may be due to a shorter follow up. In the Physicians' Health Study, the tHcy relation with myocardial infarction was attenuated when follow up was extended from 5 [203] to 9 years [336]. The five studies from this cohort of US physicians included men who presumably are better nourished and with better vitamin status than the general population [336]. This may impede the detection of any tHcy-associated risk. Estimates of the reliability constant of tHcy suggest that the magnitude of the risk associations may be underestimated by more than 10% when they are based on a single individual tHcy determination [44, 45, 342].

Dose–response. A strong dose–response relationship between tHcy and mortality was observed in our patient cohort [32] (Fig. 6, left panel). The tHcy-associated risk was slightly strengthened with cardiovascular mortality as endpoint in this study. We noticed a particularly strong mortality relation at tHcy levels above 15 μmol L^{-1}, but the relationship at lower tHcy was similar to that observed in the meta-analyses by Boushey *et al.* [187] and Wald *et al.* [188].

The association between tHcy and CVD or mortality cannot be used to determine the benefits that might be derived from tHcy intervention trials. However, the data can be used in the design of such studies, and we have calculated the hypothetical mortality reduction associated with a decrease in

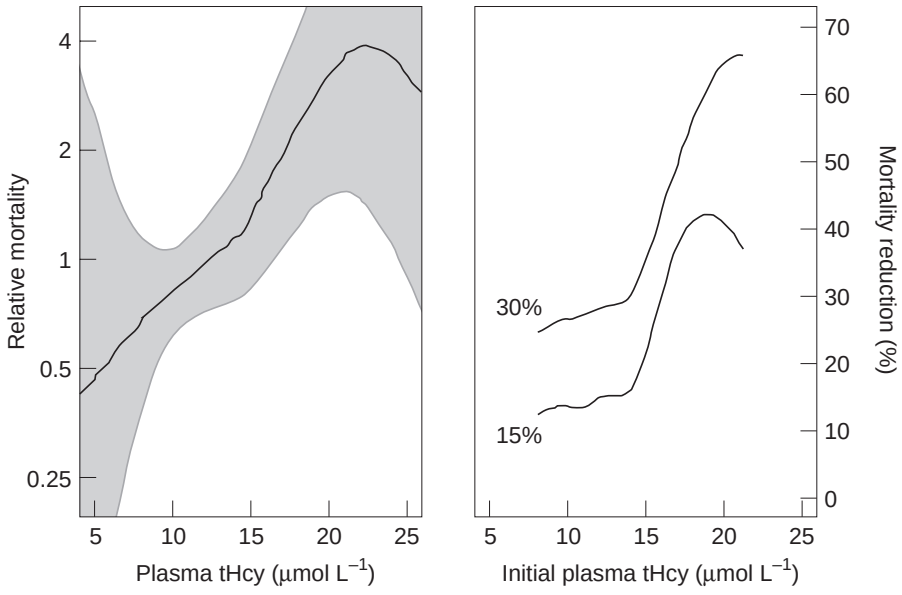

Fig. 6 Dose–response relation between plasma total homocysteine (tHcy) and mortality. The left panel shows the dose–response relationship between plasma tHcy and mortality adjusted for age, sex, left ventricular ejection fraction, creatinine, total cholesterol, and presence of single, double or triple vessel disease, using generalized additive logistic regression. Relative mortality was approximated by the odds ratio. The solid line indicates the estimated dose–response curve and the shaded area the 95% confidence interval. Based on the dose–response relationship of the left panel, the right panel shows the estimated potential reduction in mortality associated with a reduction in tHcy of 15 and 30%. The Initial plasma tHcy refers to the tHcy level before the hypothetical reduction.

Table 2 Prospective studies of the relationship between tHcy and CVD or mortality

Cohort type or population [reference], year	Outcome	Sample size	Cases/events	Controls	Age (years)	Sex	Follow up (years) mean	Follow up (years) max	Result	Significant	Difference in tHcy (μmol L^{-1})[a]
Community/occupational cohorts[b]											
Physicians' Health Study [203], 1992	AMI + CADM		271	271	40–84	M	5	≈7	Pos	Yes	0.6
Malmö Study [42], 1993	Stroke/AMI		13/15	21/27	NA	M	11	16	Pos	No	2.8/1.1
North Karelia Project [337], 1994	AMI + stroke		265	269	40–64	MF	9	14	Neg	No	0.2
Physicians' Health Study [335], 1994	Ischemic stroke		109	427	40–84	M	5	≈7	Pos	No	0.5
British Regional Heart Study [207], 1995	Stroke		107	118	40–59	M	12.8	≈14	Pos	Yes	1.8
Tromsø Study [204], 1995	CHD		122	478	NA-61	MF	4	≈5	Pos	Yes	1.4
Physicians' Health Study [336], 1996	AMI		333	333	40–84	M	7.5	13	Pos	No	NA
Physicians' Health Study [222], 1997	VTE		145	646	40–84	M	10	12	Pos	Yes[c]	0.6
Physicians' Health Study [343], 1997	CAD[d]		149	149	40–84	M	9	11	Neg	No	0.5
MRFIT [338], 1997	AMI + CADM		240	472	35–57	MF	7/15[e]	≈17	Neg	No	–0.5
Framingham [214], 1998 (abstract)	Mortality		653	1280	60–91	MF	10	NA	Pos	Yes	NA
ARIC [339], 1998	CHD		232	537	NA	MF	3.3	NA	Neg	No	NA
BUPA [188], 1998	Mortality		229	1126	35–64	M	8.7	12	Pos	Yes	1.3
The Hoorn Study [215], 1998 abstract	Mortality		188	631	50–75	MF	NA	7	Pos	Yes	1.2
British Regional Heart Study [206], 1998 abstract[f]	AMI		386	454	40–59	M	NA	NA	Pos	Yes	0.7
Scottish Heart & MONICA [334], 1998 abstract	CHD		335	335	35–64	MF	7.6	NA	Pos	Yes	1.5/2.7[g]
Patient cohorts											
SLE pts [208], 1996	AVTE	337	94		mean 35	MF	4.8	8	Pos[h]	Yes	2.9
Hemodialysis pts [233], 1997	CVE	73	16		mean 56	MF	1.4	2.1	Pos	Yes	NA
CAD pts [32], 1998	Mortality	587	64		32–80	MF	4.6	5.2	Pos	Yes	2.5
End stage renal disease pts [340], 1998	CVD + mortality	167	86		mean 56	MF	1.5	NA	Pos	Yes	16
Peripheral artery disease [341], 1998 abstract	New CVD event	162	48		NA	MF	1.7	5.3	Pos	Yes	NA
CAD pts [202], 1998 (abstract)	CAD progression	218			NA	M	2	2	Pos	Yes	
Vascular disease pts [212], 1998 abstract	CVDM	641	27		<60	MF	4.5	NA	Pos	Yes	NA
Acute coronary syndromes [213], 1998 abstract	CVDM + AMI	420	NA		NA	MF	3.5	NA	Pos[I]	Yes	NA

AMI; acute myocardial infarction, CAD; coronary artery disease, CADM; mortality due to CAD, NA; not available, CHD; coronary heart disease, VTE; venous thrombotic or embolic event, AVTE; arterial and VTE, SLE; systemic lupus erythematosus, CVE; cardiovascular event (nonfatal and fatal), CVDM; CVD mortality.

[a]Between cases and noncases. [b]The Physicians' Health Study is a cohort study of US physicians. All community cohort studies are nested case-control. [c]tHcy is a risk factor for idiopathic venous thrombosis but not for any venous thrombosis. [d]Patients referred for coronary surgery; none had had a prior AMI. [e]AMI; follow up 7 years, CADM; follow up 15 years. [f]Results on essentially the same patients are reported in [205]. [g]Difference for men/women. [h]tHcy is a risk factor for atherothrombotic events but not venous thrombosis. [i]tHcy is not a risk factor for short-term mortality (28 days).

tHcy if all the associated effects can be reversed by tHcy lowering (Fig. 6, right panel). Although highly speculative, and likely to exaggerate any effect of tHcy lowering, the calculation shows that a moderate reduction in tHcy may be associated with a substantial mortality reduction even amongst patients with tHcy <15 μmol L^{-1}, further suggesting that the entire CAD population may benefit from tHcy lowering therapy.

Vitamin intake and tHcy lowering therapy

Observational studies have shown that high intakes of fruit and vegetables, the major sources of folates [344], are associated with lower rates of stroke and coronary heart disease [345]. A protective effect on CVD from folate or vitamin B_6 is also supported by recent observational prospective studies [339, 346, 347].

A strong evidence of a beneficial effect related to increased vitamin intake is provided by clinical observations amongst patients with homocystinuria due to cystathionine β-synthase deficiency. In these patients, the cardiovascular morbidity is markedly reduced in those responding to Hcy lowering therapy with vitamin B_6 [6]. This effect is achieved even though tHcy is not normalized. Betaine seems to have a similar protective effect in vitamin B_6-nonresponsive patients [58, 348].

Amongst patients with mild to moderate hyperhomocysteinemia, the data on the effect of tHcy lowering therapy are sparse. One study on nonrandomized patients recently showed that a combination regimen of folic acid, vitamins B_6 and B_{12} reduced the progression of carotid atherosclerosis [349]. In another study of patients with premature peripheral arterial occlusive disease, the patients with initial hyperhomocysteinemia were treated with vitamins B_6 and folic acid and tended to have a lower incidence of new cardiovascular events than patients with normal tHcy levels [341].

CVD and genetic causes of mildly elevated tHcy

Recent studies [338, 339] have added to the ongoing debate of whether Hcy itself is a cause of occlusive vascular disease or just an epiphenomenon [89, 350]. In this paragraph we discuss how genetic causes of hyperhomocysteinemia may complement the discussion of whether tHcy is a causal factor for CVD, just a marker of risk, or a combination of the two.

If mild hyperhomocysteinemia itself is responsible for vascular injury, genetic causes of mildly elevated (fasting or postload) tHcy are candidate CVD risk factors. There is no increased risk of CVD in obligatory heterozygote homocystinuria due to CBS deficiency [351], and the prevalence of common mutations in the CBS gene in CVD patients with an abnormal response to methionine is not higher than expected [352–355]. However, heterozygosity for the CBS mutations is rare, and a majority of these subjects have a normal fasting tHcy level, and often normal postload tHcy [6].

The CVD risk associated with the common C677T MTHFR polymorphism has, since 1995, been extensively studied. In this review, we have extended a published metaanalysis [356] on the C677T MTHFR polymorphism, plasma tHcy concentration and CVD risk, by including the most recent articles.

Based on 17 studies of European and North American populations, we found that subjects with the TT genotype ($n = 614$) have on average 3.5 μmol L^{-1} (32%) higher mean plasma tHcy concentration than those with the CC genotype ($n = 2491$; Table 3). This difference in tHcy concentration between the TT and CC genotypes is larger than the difference usually found between CVD patients and control subjects (see Table 2 for comparison). In 28 studies, comprising 6944 cardiovascular patient and 7764 control subjects, the TT genotype is found in 12.5% of the patients and 11.9% of the control subjects (Table 4), giving an odds ratio for CVD of 1.15 (95% CI 0.97–1.38), which is not significant. Interestingly, subgroup analysis of three Japanese studies [372, 379, 386], including 826 CVD patients and 1289 control subjects, shows a significantly increased risk for CVD in those with the TT genotype (OR 2.04, 95% CI 1.55–2.68).

The available data on MTHFR and CVD risk, summarized above, do not suggest a link between mild fasting hyperhomocysteinemia, mediated through the TT genotype, and CVD, at least not in European, North American, and Australian populations. However, there are several reasons why high tHcy may still confer CVD risk whilst MTHFR is not a major risk factor. First, the TT genotype may protect against CVD by mechanisms independent of Hcy, as

Table 3 Plasma total homocysteine (tHcy) concentration in 17 different studies in relation to the C677T/MTHFR genotypes (TT genotype = mutant homozygotes, CT genotype = mutant heterozygotes, CC genotype = normal homozygotes)

	Mean plasma tHcy concentration in μmol L^{-1}		
Author [reference], year	TT genotype (*n*)	CT genotype (*n*)	CC genotype (*n*)
Frosst *et al.* [357], 1995	22.4 (12)	13.8 (9)	12.6 (19)
van der Put *et al.* [358], 1995	17.1 (34)	13.2 (164)	13.4 (194)
Jacques *et al.* [80], 1996	9.9 (45)	8.4 (170)	8.7 (150)
Harmon *et al.* [359], 1996	9.5 (72)	7.1 (273)	6.8 (280)
Kluijtmans *et al.* [352], 1996	16.3 (15)	13.4 (61)	12.3 (93)
Schmitz *et al.* [360], 1996	9.1 (14)	10.6 (46)	9.9 (67)
Ma *et al.* [361], 1996	12.6 (72)	10.9 (240)	10.6 (271)
Deloughery *et al.* [362], 1996	17.2 (22)	13.6 (111)	13.0 (114)
Verhoef *et al.* [363], 1997	15.5 (30)	12.3 (150)	11.4 (138)
Kluijtmans *et al.* [364], 1997	15.4 (51)	13.4 (233)	12.6 (231)
Christensen *et al.* [365], 1997	12.8 (22)	11.0 (98)	10.3 (89)
Schwartz *et al.* [366], 1997	13.5 (43)	10.8 (141)	10.9 (154)
Kluijtmans *et al.* [243], 1998	17.7 (51)	13.3 (229)	12.6 (258)
Legnani *et al.* [367], 1997	13.0 (12)	7.8 (31)	7.4 (20)
Arai *et al.* [368], 1997	19.8 (22)	15.5 (22)	15.4 (22)
Gudnason *et al.* [85], 1998	16.5 (88)	10.4 (338)	9.9 (359)
Lalouschek *et al.* [369], 1998	10.4 (9)	9.8 (35)	9.4 (32)
Approximate means of all studies	14.3 (614)	11.1 (2351)	10.8 (2491)
Elevation in tHcy compared with the CC genotype	3.5 (32%)	0.3 (3%)	

suggested by lower blood pressure in TT subjects [388]. Secondly, high tHcy may cause vascular lesion only in subjects with coexisting risk factors, and such interactive effects have actually been demonstrated in the COMAC study [55]. Interactions of the MTHFR C677T polymorphism with nutrition [389] and genetic traits seem likely and may explain the association found in the Japanese.

The interactions between the MTHFR genotype and conventional risk factors have been addressed in a recent study from the European Concerted Action Project based on genotyping and tHcy measurement in 711 cases and 747 controls. The TT genotype was associated with 3.5 μmol L^{-1} higher tHcy and a 26% higher CVD risk than the CC genotype. The unexpected finding was made in the hyperhomocysteinemic subgroup (tHcy >15 μmol L^{-1}); TT subjects had a lower prevalence of CVD risk factors than CC subjects. After adjustment for these risk factors, the TT genotype was significantly associated with a 50% increased risk for overall CVD and an even higher risk for CAD, compared with the CC genotype. The increase in risk was markedly attenuated after adjustment for tHcy (Meleady *et al.* 1998, submitted). This suggests that the TT associated risk is mediated through elevated tHcy. Furthermore, the unadjusted relative risk associated with the TT genotype agrees with what has been calculated (20–40%) for a tHcy increment of 3.5 μmol L^{-1} [187], and the adjusted relative risk observed in the COMAC study actually exceeds this expected relative risk. Statistical sample size calculations show that at least 2500 patients and an equal number of controls are required to detect an unadjusted 25% risk increase associated with TT genotype, if the prevalence of the genotype is about 10%.

Conclusions

Observational studies show that plasma tHcy is a strong risk factor for CVD and mortality, and genetic causes of mildly elevated tHcy may be associated with increased risk of CVD when other risk factors are accounted for. However, mechanisms are uncertain and results from adequately sized trials with tHcy lowering therapy and clinical endpoints

Table 4 Numbers and frequencies of the three different C677T/MTHFR genotypes (TT genotype = mutant homozygotes, CT genotype = mutant heterozygotes, and CC genotype = normal homozygotes) in 28 studies of patients with cardiovascular disease and controls

		Cases [Genotype (numbers (%))]				Controls [Genotype (numbers (%))]			
Author [reference], year	Type[a]	TT	CT	CC	All	TT	CT	CC	All
Kluijtmans *et al.* [352], 1996	Mixed	9 (15.0)	21 (35.0)	30 (50.0)	60	6 (5.4)	42 (37.8)	63 (56.8)	111
Schmitz *et al.* [360], 1996	CHD	29 (15.3)	66 (34.7)	95 (50.0)	190	27 (14.4)	90 (47.8)	71 (37.8)	188
Ma *et al.* [361], 1996	CHD	33 (11.3)	124 (42.3)	136 (46.4)	293	39 (13.4)	116 (40.0)	135 (46.6)	290
Deloughery *et al.* [362], 1996[b]	Mixed	42 (9.9)	199 (46.8)	184 (43.3)	425	94 (15.8)	262 (44.0)	240 (40.2)	596
Verhoef *et al.* [363], 1997	CHD	13 (10.0)	59 (45.0)	59 (45.0)	131	7 (7.0)	48 (48.0)	45 (45.0)	100
Kluijtmans *et al.* [364], 1997	CHD	70 (9.5)	328 (44.6)	337 (45.9)	735	106 (8.5)	527 (42.2)	617 (49.3)	1250
Christensen *et al.* [365], 1997	CHD	22 (14.5)	68 (44.7)	62 (40.8)	152	13 (10.7)	61 (50.4)	47 (38.8)	121
Schwartz *et al.* [366], 1997	CHD	7 (10.1)	34 (49.3)	28 (40.6)	69	43 (12.7)	141 (41.7)	154 (45.6)	338
Kluijtmans *et al.* [243], 1998	VTE	47 (10.0)	213 (45.2)	211 (44.8)	471	47 (9.9)	203 (42.8)	224 (47.3)	474
Gallagher *et al.* [370], 1996	CHD	19 (17.1)	48 (43.2)	44 (39.7)	111	7 (6.7)	45 (42.9)	53 (50.4)	105
Adams *et al.* [371], 1996	CHD	32 (10.3)	145 (46.8)	133 (42.9)	310	29 (13.1)	97 (43.7)	96 (43.2)	222
Izumi *et al.* [372], 1996	CHD	50 (20.0)	110 (44.0)	90 (36.0)	250	25 (12.4)	102 (50.8)	74 (36.8)	201
Wilcken *et al.* [373], 1996	CHD	53 (11.6)	217 (47.6)	186 (40.8)	456	24 (10.7)	113 (50.2)	88 (39.1)	225
de Franchis *et al.* [374], 1996	Mixed	19 (29.7)	32 (50.0)	13 (20.3)	64	39 (15.1)	129 (50.0)	90 (34.9)	258
Narang *et al.* [375], 1996	CHD	6 (6.5)	31 (34.0)	55 (59.5)	92	5 (10.0)	19 (38.0)	26 (52.0)	50
Brulhart *et al.* [376],1997[c]	CHD	23 (11.9)	84 (43.5)	86 (44.6)	193	73 (16.0)	195 (42.7)	188 (41.3)	456
van Bockxmeer *et al.* [377], 1997	CHD	56 (10.1)	234 (42.2)	265 (47.7)	555	15 (10.5)	58 (40.5)	70 (49.0)	143
Brugada *et al.* [378], 1997	CHD	10 (6.5)	69 (44.5)	76 (49.0)	155	12 (7.7)	73 (47.1)	70 (45.2)	155
Morita *et al.* [379], 1997	CHD	57 (15.8)	188 (51.9)	117 (32.3)	362	79 (10.2)	361 (46.4)	338 (43.4)	778
Verhoef *et al.* [380], 1997	CHD	61 (12.2)	209 (41.8)	230 (46.0)	500	72 (14.4)	200 (40.0)	228 (45.6)	500
Tosetto *et al.* [381], 1997	VTE	8 (12.3)	36 (55.4)	21 (32.3)	65	17 (13.1)	71 (54.6)	42 (32.3)	130
Malinow *et al.* [180], 1997	CHD	17 (12.1)	83 (59.3)	40 (28.6)	140	8 (7.8)	45 (44.1)	49 (48.0)	102
Markus *et al.* [382], 1997	CBV	37 (10.7)	146 (42.3)	162 (47.0)	345	22 (13.7)	63 (39.1)	76 (47.2)	161
Salden *et al.* [383], 1997	VTE	26 (12.0)	102 (47.2)	88 (40.8)	216	18 (11.0)	75 (45.7)	71 (43.3)	164
Reinhardt *et al.* [384], 1998	CHD	23 (13.0)	66 (36.5)	91 (50.5)	180	9 (8.7)	46 (44.2)	49 (47.1)	104
Abbate *et al.* [385], 1998	CHD	24 (28.6)	41 (48.8)	19 (22.6)	84	32 (30.2)	48 (45.3)	26 (24.5)	106
Ou *et al.* [386], 1998	CHD	61 (28.5)	84 (39.3)	69 (32.2)	214	42 (13.5)	158 (51.0)	110 (35.5)	310
Kostulas *et al.* [387], 1998	CBV	13 (10.3)	50 (39.7)	63 (50.0)	126	13 (10.3)	50 (39.7)	63 (50.0)	126
Total numbers of genotypes		867	3087	2990	6944	923	3438	3403	7764
Genotype frequencies (%)		12.5	44.5	43.0	11.9	44.3	43.8		
Allele frequency (%)			34.7			34.0			

[a]CHD = coronary heart disease, CBV = cerebrovascular diseas, VTE = venous thromboembolism. Mixed: different types of atherothromboembolic cardiovascular disease. [b]Patients with venous thrombosis are included in cases. [c]Diabetic cases and controls.

do not exist. Because elevated tHcy may be just a marker of individuals at high risk, a preventive action with B-vitamin supplementation in the general population or in the general CVD population is not justifiable until the results of intervention trials with tHcy lowering therapy are available.

References

1 Butz LW, du Vigneaud V. The formation of a homologue of cystine by the decomposition of methionine with sulfuric acid. *J Biol Chem* 1932; **99**: 135–42.

2 Case records of the Massachusets General Hospital. Marked cerebral symptoms following a limp of three months' duration. *New Engl J Med* 1933; **209**: 1063–6.

3 McCully KS. Vascular pathology of homocysteinemia: Implications for the pathogenesis of arteriosclerosis. *Am J Pathol* 1969; **56**: 111–28.

4 Gerritsen T, Vaughn JG, Weisman HA. The identification of homocysteine in the urine. *Biochem Biophys Res Commun* 1962; **9**: 493–6.

5 Carson NAJ, Neill DW. Metabolic abnormalities detected in a survey of mentally backward individuals in Northern Ireland. *Arch Dis Child* 1962; **37**: 505–13.

6 Mudd SH, Levy HL, *et al.* Disorders of transsulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D. eds. *The Metabolic and Molecular Bases of Inherited Disease,* 1st edn, Vol 1. New York: McGraw-Hill, 1995: 1279–327.

7 Schimke RN, McKusick VA, Huang T, Pollack AD. Homocystinuria. Studies of 20 families with 38 affected members.

JAMA 1965; **193**: 87–95.
8 McCully KS. Homocysteine theory of arteriosclerosis: Development and current status. *Atherosclerosis Rev* 1983; **11**: 157–246.
9 Mudd SH, Skovby F, Levy HL, *et al.* The natural history of homocystinuria due to cystathionine β-synthase deficiency. *Am J Hum Genet* 1985; **37**: 1–31.
10 McCully KS. Homocysteine, folate, vitamin B6, and cardiovascular disease. *JAMA* 1998; **279**: 392–3.
11 McCully KS, Wilson RB. Homocysteine theory of arteriosclerosis. *Atherosclerosis* 1975; **22**: 215–27.
12 Wilcken DEL, Wilcken B. The pathogenesis of coronary artery disease. A possible role for methionine metabolism. *J Clin Invest* 1976; **57**: 1079–82.
13 Refsum H, Ueland PM, Nygård O, Vollset SE. Homocysteine and cardiovascular disease. *Annu Rev Med* 1998; **49**: 31–62.
14 Boers GHJ. Hyperhomocysteinemia as a risk factor for arterial and venous disease. A review of evidence and relevance. *Thromb Haemost* 1997; **78**: 520–2.
15 D'Angelo A, Mazzola G, Crippa L, Fermo I, D'Angelo SV. Hyperhomocysteinemia and venous thromboembolic disease. *Haematologica* 1997; **82**: 211–9.
16 Selhub J. Mild hyperhomocysteinemia and arterial occlusive disease. *Haematologica* 1997; **82**: 129–32.
17 Ueland PM. Pharmacological and biochemical aspects of S-adenosylhomocysteine and S-adenosylhomocysteine hydrolase. *Pharmacol Rev* 1982; **34**: 223–53.
18 Wendel U, Bremer HJ. Betaine in the treatment of homocystinuria due to 5,10-methylenetetrahydrofolate reductase deficiency. *Eur J Pediatr* 1984; **142**: 147–50.
19 Fowler B. Disorders of homocysteine metabolism. *J Inher Metab Dis* 1997; **20**: 270–85.
20 Mato JM, Alvarez L, Ortiz P, Pajares MA. S-Adenosylmethionine synthesis: molecular mechanisms and clinical implications. *Pharmacol Ther* 1997; **73**: 265–80.
21 Sunden SLF, Renduchintala MS, Park EI, Miklasz SD, Garrow TA. Betaine-homocysteine methyltransferase expression in porcine and human tissues and chromosomal localization of the human gene. *Arch Biochem Biophys* 1997; **345**: 171–4.
22 Finkelstein JD. Methionine metabolism in mammals. *J Nutr Biochem* 1990; **1**: 228–37.
23 Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH. Total homocysteine in plasma or serum. Methods and clinical applications. *Clin Chem* 1993; **39**: 1764–79.
24 Ueland PM. Homocysteine species as components of plasma redox thiol status. *Clin Chem* 1995; **41**: 340–2.
25 Refsum H, Helland S, Ueland PM. Radioenzymic determination of homocysteine in plasma and urine. *Clin Chem* 1985; **31**: 624–8.
26 Kang S-S, Wong PWK, Malinow MR. Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. *Ann Rev Nutr* 1992; **12**: 279–98.
27 Stein WH, Moore S. The free amino acids of human blood plasma. *J Biol Chem* 1954; **211**: 915–26.
28 Kang S-S, Wong PWK, Becker N. Protein-bound homocyst(e)ine in normal subjects and in patients with homocystinuria. *Pediatr Res* 1979; **13**: 1141–3.
29 Refsum H, Ueland PM, Svardal AM. Fully automated fluorescence assay for determining total homocysteine in plasma. *Clin Chem* 1989; **35**: 1921–7.
30 Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM. Homocysteine and other thiols in plasma and urine: automated determination and sample stability. *Clin Chem* 1993; **39**: 263–71.
31 Nygård O, Vollset SE, Refsum H, *et al.* Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. *JAMA* 1995; **274**: 1526–33.
32 Nygård O, Nordrehaug JE, Refsum H, Farstad M, Ueland PM, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 1997; **337**: 230–6.
33 Ubbink JB, Vermaak WJH, van der Merwe A, Becker PJ. The effect of blood sample aging and food consumption on plasma total homocysteine levels. *Clin Chim Acta* 1992; **207**: 119–28.
34 Candito M, Aubin-Brunet V, Beaulieu F, Chevalier T, Pringuey D, Darcourt G. Increased postprandial homocysteinemia in a group of depressed patients. *Amino Acids* 1997; **12**: 315–21.
35 Fermo I, De Vecchi E, D'Angelo SV, D'Angelo A, Paroni R. Total plasma homocysteine: influence of some common physiological variables. *Amino Acids* 1993; **5**: 17–21.
36 Guttormsen AB, Schneede J, Fiskerstrand T, Ueland PM, Refsum H. Plasma concentrations of homocysteine and other aminothiol compounds are related to food intake in healthy subjects. *J Nutr* 1994; **124**: 1934–41.
37 Leppänen EA, Grasbäck R. Experimental basis of standardized specimen collection: effect of posture on blood picture. *Eur J Haematol* 1988; **40**: 222–6.
38 Andersson A, Isaksson A, Hultberg B. Homocysteine export from erythrocytes and its implication for plasma sampling. *Clin Chem* 1992; **38**: 1311–5.
39 Willems HP, Bos GM, Gerrits WB, den Heijer M, Vloet S, Blom HJ. Acidic citrate stabilizes blood samples for assay of total homocysteine. *Clin Chem* 1998; **44**: 342–5.
40 Daskalakis I, Lucock MD, Anderson A, Wild J, Schorah CJ, Levene MI. Determination of plasma total homocysteine and cysteine using HPLC with fluorescence detection and an ammonium 7-fluoro-2,1,3-benzoxadiazole-4-sulphonate (SBD-F) derivatization protocol optimized for antioxidant concentration, derivatization reagent concentration, temperature and matrix pH. *Biomed Chromatogr* 1996; **10**: 205–12.
41 Kittner SJ, Malinow MR, Seipp MJ, Upson B, Hebel JR. Stability of blood homocyst(e)ine under epidemiological field conditions. *J Clin Lab Anal* 1995; **9**: 75–6.
42 Israelsson B, Brattström L, Refsum H. Homocysteine in frozen plasma samples – a short cut to establish hyperhomocysteinaemia as a risk factor for arteriosclerosis. *Scand J Clin Lab Invest* 1993; **53**: 465–9.
43 Garg UC, Zheng Z-J, Folsom AR, *et al.* Short-term and long-term variability of plasma homocysteine measurement. *Clin Chem* 1997; **43**: 141–5.
44 Cobbaert C, Arentsen JC, Mulder P, Hoogerbrugge N, Lindemans J. Significance of various parameters derived from biological variability of lipoprotein (a), homocysteine, cysteine, and total antioxidant status. *Clin Chem* 1997; **43**: 1958–64.
45 Clarke R, Khaw KT, Woodhouse P, *et al.* Variability and determinants of plasma total homocysteine in an elderly population. *Clin Chem* 1998; **44**: 102–7.
46 Eckfeldt JH, Chambless LE, Shen YL. Short-term, within-

person variability in clinical chemistry test results. Experience from the Atherosclerosis Risk in Communities Study. *Arch Pathol Lab Med* 1994; **118**: 496–500.
47 Brenton DP, Cusworth DC, Gaull GE. Homocystinuria: metabolic studies on 3 patients. *J Pediatr* 1965; **67**: 58–68.
48 Fowler B, Sardharwalla IB, Robins AJ. The detection of heterozygotes for homocystinuria by oral loading with L-methionine. *Biochem J* 1971; **122**: 23–4.
49 Sardharwalla IB, Fowler B, Robins AJ, Komrower GM. Detection of heterozygotes for homocystinuria. Study of sulphur-containing amino acids in plasma and urine after L-methionine loading. *Arch Dis Child* 1974; **49**: 553–9.
50 Boers GHJ, Smals AGH, Trijbels FJM, *et al.* Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. *N Engl J Med* 1985; **313**: 709–15.
51 Boers GHJ, Fowler B, Smals AGH, *et al.* Improved identification of heterozygotes for homocystinuria due to cystathionine synthase deficiency by the combination of methionine loading and enzyme determination in cultured fibroblasts. *Hum Genet* 1985; **69**: 164–9.
52 Clarke R, Daly L, Robinson K, *et al.* Hyperhomocysteinemia: an independent risk factor for vascular disease. *N Engl J Med* 1991; **324**: 1149–55.
53 Kozich V, Janosik M, Sokolova J, *et al.* Analysis of CBS alleles in Czech and Slovak patients with homocystinuria: report on three novel mutations E176K, W409X and 1223+37 del99. *J Inherit Metab Dis* 1997; **20**: 363–6.
54 Bostom AG, Jacques PF, Nadeau MR, Williams RR, Ellison RC, Selhub J. Post-methionine load hyperhomocysteinemia in persons with normal fasting total plasma homocysteine: initial results from the NHLBI family heart study. *Atherosclerosis* 1995; **116**: 147–51.
55 Graham IM, Daly LE, Refsum HM, *et al.* Plasma homocysteine as a risk factor for vascular disease: The European Concerted Action Project. *JAMA* 1997; **277**: 1775–81.
56 van der Griend R, Haas FJLM, Duran M, Biesma DH, Meuwissen OJAT, Banga JD. Methionine loading test is necessary for detection of hyperhomocysteinemia. *J Lab Clin Med* 1998; **132**: 67–72.
57 Ueland PM, Refsum H, Brattström L. Plasma homocysteine and cardiovascular disease. In: Francis RBJ, ed. *Atherosclerotic Cardiovascular Disease, Hemostasis, and Endothelial Function.* New York: Marcel Dekker, Inc., 1992: 183–236.
58 Wilcken DEL. Homocysteine and vascular disease. *Med L Aust* 1998; **168**: 431–2.
59 Carson NAJ, Dent CE, Field CMB, Gall GE. Homocystinuria: Clinical and pathological review of ten cases. *J Pediatr* 1965; **66**: 565–83.
60 Gibson JB, Carson NAJ, Neill DW. Pathological findings in homocystinuria. *J Clin Pathol* 1964; **17**: 427–37.
61 Schimke RN, McKusick VA, Huang T, Pollack AD. Homocystinuria. *JAMA* 1965; **193**: 711–9.
62 Mudd SH, Finkelstein JD, Irreverre F, Laster L. Homocystinuria: an enzymatic defect. *Science* 1964; **143**: 1443–5.
63 Dawson PA, Cox AJ, Emmerson BT, Dudman NPB, Kraus JP, Gordon RB. Characterisation of five missense mutations in the cystathionine beta-synthase gene from three patients with B-6-nonresponsive homocystinuria. *Eur J Hum Genet* 1997; **5**: 15–21.
64 Mudd SH, Uhlendorf BW, Freeman JM, Finkelstein JD, Shih VE. Homocystinuria associated with decreased methylenetetrahydrofolate reductase activity. *Biochem Biophys Res Commun* 1972; **46**: 905–12.
65 Rozen R. Molecular genetics of methylenetetrahydrofolate reductase deficiency. *J Inherit Metab Dis* 1996; **19**: 589–94.
66 Leclerc D, Campeau E, Goyette P, *et al.* Human methionine synthase: cDNA cloning and identification of mutations in patients of the *cbl*G complementation group of folate/cobalamin disorders. *Hum Mol Genet* 1996; **5**: 1867–74.
67 Kraus JP, Le K, Swaroop M, *et al.* Human cystathionine beta-Synthase cDNA – sequence, alternative splicing and expression in cultured cells. *Hum Mol Genet* 1993; **2**: 1633–8.
68 Goyette P, Sumner JS, Milos R, *et al.* Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. *Nat Genet* 1994; **7**: 195–200.
69 Chen LH, Liu ML, Hwang HY, Chen LS, Korenberg J, Shane B. Human methionine synthase: cDNA cloning, gene localization, and expression. *J Biol Chem* 1997; **272**: 3628–34.
70 Garrow TA. Purification, kinetic properties, and cDNA cloning of mammalian betaine-homocysteine methyltransferase. *J Biol Chem* 1996; **271**: 22 831–8.
71 Giusti B, Comeglio P, Attanasio M, *et al.* Different distribution of the double mutant 'T833C/68 bp insertion' in cystathionine beta-synthase gene in Northern and Southern Italian populations. *Thromb Haemost* 1997; **78**: 1293.
72 Kim CE, Gallagher PM, Guttormsen AB, *et al.* Functional modeling of vitamin responsiveness in yeast: a common pyridoxine-responsive cystathionine beta-synthase mutation in homocystinuria. *Hum Mol Genet* 1997; **6**: 2213–21.
73 Sperandeo MP, Candito M, Sebastio G, *et al.* Homocysteine response to methionine challenge in four obligate heterozygotes for homocystinuria and relationship with cystathionine beta-synthase mutations. *J Inherit Metab Dis* 1996; **19**: 351–6.
74 Scott J, Weir D. Homocysteine and cardiovascular disease. *QJM* 1996; **89**: 561–3.
75 Murdoch JC, Rodger JC, Rao SS, Fletcher CD, Dunnigan MG. Down's syndrome: an atheroma-free model? *Br Med J* 1977; **2**: 226–8.
76 Kang SS, Wong PWK, Zhou J, *et al.* Thermolabile methylenetetrahydrofolate reductase in patients with coronary artery disease. *Metabolism* 1988; **37**: 611–3.
77 Kang S-S, Zhou J, Wong PWK, Kowalisyn J, Strokosch G. Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. *Am J Hum Genet* 1988; **43**: 414–21.
78 Frosst P, Blom HJ, Milos R, *et al.* Identification of a candidate genetic risk factor for vascular disease: a common mutation at the methylenetetrahydrofolate reductase locus. *Nat Genet* 1995; **10**: 111–3.
79 Engbersen AMT, Franken DG, Boers GHJ, Stevens EMB, Trijbels FJM, Blom HJ. Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. *Am J Hum Genet* 1995; **56**: 142–50.
80 Jacques PF, Bostom AG, Williams RR, *et al.* Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 1996; **93**: 7–9.
81 Kang S-S. Critical points for determining moderate hyperhomocyst(e)inemia. *Eur J Clin Invest* 1995; **25**: 806–8.
82 Guttormsen AB, Ueland PM, Nesthus I, *et al.* Determinants and vitamin responsiveness of intermediate hyperhomocys-

teinemia (≥40 μmol/L). The Hordaland Homocysteine Study. *J Clin Invest* 1996; **98**: 2174–83.
83 Woodside JV, Yarnell JWG, Mcmaster D, *et al.* Vitamin B6 status, MTHFR and hyperhomocysteinaemia. *QJM* 1997; **90**: 551–2.
84 Franco RF, Araujo AG, Guerreiro JF, Elion J, Zago MA. Analysis of the 677 C→T mutation of the methylenetetrahydrofolate reductase gene in different ethnic groups. *Thromb Haemost* 1998; **79**: 119–21.
85 Gudnason V, Stansbie D, Scott J, Bowron A, Nicaud V, Humphries S. C677T (thermolabile alanine/valine) polymorphism in methylenetetrahydrofolate reductase (MTHFR): its frequency and impact on plasma homocysteine concentration in different European populations. *Atherosclerosis* 1998; **136**: 347–54.
86 Stevenson RE, Schwartz CE, Du YZ, Adams MJJ. Differences in methylenetetrahydrofolate reductase genotype frequencies, between whites and blacks. *Am J Hum Genet* 1997; **60**: 229–30.
87 Hegele RA, Tully C, Young TK, Connelly PW. V677 mutation of methylenetetrahydrofolate reductase and cardiovascular disease in Canadian Inuit. *Lancet* 1997; **349**: 1221–2.
88 Sacchi E, Tagliabue L, Duca F, Mannucci PM. High frequency of the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene in Northern Italy. *Thromb Haemost* 1997; **78**: 963–4.
89 Motulsky AG. Nutritional ecogenetics: homocysteine-related arteriosclerotic vascular disease, neural tube defects, and folic acid. *Am J Hum Genet* 1996; **58**: 17–20.
90 Vermaak WJ, Ubbink JB, Delport R, Becker PJ, Bissbort SH, Ungerer JP. Ethnic immunity to coronary heart disease? *Atherosclerosis* 1991; **89**: 155–62.
91 Ubbink JB, Delport R, Vermaak WJ. Plasma homocysteine concentrations in a population with a low coronary heart disease prevalence. *J Nutr* 1996; **126** (Suppl. 4): 1254S–7S.
92 Malinow MR, Kang SS, Taylor LM, *et al.* Prevalence of hyperhomocyst(e)inemia in patients with peripheral arterial occlusive disease. *Circ Res* 1989; **79**: 1180–8.
93 Berg K, Malinow MR, Kierulf P, Upson B. Population variation and genetics of plasma homocyst(e)ine level. *Clin Genet* 1992; **41**: 315–21.
94 Kang S-S, Wong PWK, Zhou J, Cook HY. Preliminary report: Total homocyst(e)ine in plasma and amniotic fluid of pregnant women. *Metabolism* 1986; **35**: 889–91.
95 Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 1987; **422**: 43–52.
96 Vester B, Rasmussen K. High performance liquid chromatography method for rapid and accurate determination of homocysteine in plasma and serum. *Eur J Clin Chem Clin Biochem* 1991; **29**: 549–54.
97 Andersson A, Brattström L, Israelsson B, Isaksson A, Hamfelt A, Hultberg B. Plasma homocysteine before and after methionine loading with regard to age, gender, and menopausal status. *Eur J Clin Invest* 1992; **22**: 79–87.
98 Jacobsen DW, Gatautis VJ, Green R, *et al.* Rapid HPLC determination of total homocysteine and other thiols in serum and plasma: Sex differences and correlation with cobalamin and folate levels in normal subjects. *Clin Chem* 1994; **40**: 873–81.
99 Brattström L, Lindgren A, Israelsson B, Andersson A, Hultberg B. Homocysteine and cysteine: determinants of plasma levels in middle-aged and elderly subjects. *J Intern Med* 1994; **236**: 631–41.
100 Tonstad S, Refsum H, Ueland PM. Association between plasma total homocysteine and parental history of cardiovascular disease in children with familial hypercholesterolemia. *Circulation* 1997; **96**: 1803–8.
101 Andersson A, Hultberg B, Brattström L, Isaksson A. Decreased serum homocysteine in pregnancy. *Eur J Clin Chem Clin Biochem* 1992; **30**: 377–9.
102 Steegers-Theunissen RPM, Boers GHJ, Blom H, Trijbels FJM, Eskes TKAB. Hyperhomocysteinemia and recurrent spontaneous abortion or abruptio placenta. *Lancet* 1992; **339**: 1122–3.
103 Wouters MGAJ, Boers GHJ, Blom H, *et al.* Hyperhomocysteinemia: a risk factor in women with unexplained recurrent early pregnancy loss. *Fertil Steril* 1993; **60**: 820–5.
104 Bostom AG, Lathrop L. Hyperhomocysteinemia in end-stage renal disease: prevalence, etiology, and potential relationship to arteriosclerotic outcomes. *Kidney Int* 1997; **52**: 10–20.
105 Bostom A, Brosnan JT, Hall B, Nadeau MR, Selhub J. Net uptake of plasma homocysteine by the rat kidney in vivo. *Atherosclerosis* 1995; **116**: 59–62.
106 Arnadottir M, Hultberg B, Nilsson-Ehle P, Thysell H. The effect of reduced glomerular filtration rate on plasma total homocysteine concentration. *Scand J Clin Lab Invest* 1996; **56**: 41–6.
107 Guttormsen AB, Ueland PM, Svarstad E, Refsum H. Kinetic basis of hyperhomocysteinemia in patients with chronic renal failure. *Kidney Int* 1997; **52**: 495–502.
108 Guttormsen AB, Svarstad E, Ueland PM, Refsum H. Elimination of homocysteine from plasma in subjects with endstage renal failure. *Irish J Med Sci* 1995; **164** (Suppl. 15): 8.
109 House JD, Brosnan MGEE, *et al.* Renal homocysteine metabolism. In: Odonovan DJ, Endou H, Schoolwerth AC, Tizianello A, Walls J, eds. *Nutritional and Acid-Base Aspects of Amino Acid Metabolism.* Basel: Karger, 1997: 79–84.
110 House JD, Brosnan ME, Brosnan JT. Characterization of homocysteine metabolism in the rat kidney. *Biochem J* 1997; **328**: 287–92.
111 van Guldener C, Donker AJM, Jakobs C, Teerlink T, de Meer K, Stehouwer CDA. No net renal extraction of homocysteine in fasting humans. *Kidney Int* 1998; **54**: 166–9.
112 Norlund L, Grubb A, Fex G, *et al.* The increase of plasma homocysteine concentrations with age is partly due to the deterioration of renal function as determined by plasma cystatin C. *Clin Chem Lab Med* 1998; **36**: 175–8.
113 Verhoef P, Stampfer MJ, Buring JE, *et al.* Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B-6, B-12, and folate. *Am J Epidemiol* 1996; **143**: 845–59.
114 Jacques PF, Sulsky SI, Sadowski JA, Phillips JCC, Rush D, Willett WC. Comparison of micronutrient intake measured by a dietary questionnaire and biochemical indicators of micronutrient status. *Am J Clin Nutr* 1993; **57**: 182–9.
115 Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* 1993; **270**: 2693–8.
116 Koehler KM, Pareotubbeh SL, Liang HC, Romero LJ,

Baumgartner RN, Garry PJ. Some vitamin sources relating to plasma homocysteine provide not only folate but also vitamins B-12 and B-6. *J Nutr* 1997; **127**: 1534–5.

117 Nygård O, Refsum H, Ueland PM, Vollset SE. Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study. *Am J Clin Nutr* 1998; **67**: 263–70.

118 Allen RH, Stabler SP, Savage DG, Lindenbaum J. Metabolic abnormalities in cobalamin (vitamin B12) and folate deficiency. *FASEB J* 1993; **7**: 1344–53.

119 Joosten E, van den Berg A, Riezler R, *et al.* Metabolic evidence that deficiencies of vitamin B12, folate and vitamin B6 occur commonly in the elderly. *Am J Clin Nutr* 1993; **58**: 468–76.

120 Miller JW, Ribaya-Mercado JD, Russell RM, *et al.* Effect of vitamin-B-6 deficiency on fasting plasma homocysteine concentrations. *Am J Clin Nutr* 1992; **55**: 1154–60.

121 Ubbink JB, Vermaak WJH, van der Merwe A, Becker PJ. Vitamin B-12, vitamin B-6, and folate nutritional status in men with hyperhomocysteinemia. *Am J Clin Nutr* 1993; **57**: 47–53.

122 Tucker KL, Selhub J, Wilson PWF, Rosenberg IH. Dietary intake pattern relates to plasma folate and homocysteine concentrations in the Framingham Heart Study. *J Nutr* 1996; **126**: 3025–31.

123 Nygård O, Refsum H, Ueland PM, *et al.* Coffee consumption and total plasma homocysteine: The Hordaland Homocysteine Study. *Am J Clin Nutr* 1997; **65**: 136–43.

124 Vollset SE, Nygård O, Kvåle G, *et al.* The Hordaland Homocysteine Study: lifestyle and total plasma homocysteine in Western Norway. In: Graham I, Refsum H, Rosenberg IH, Ueland P,eds. *Homocysteine Metabolism: from Basic Science to Clinical Medicine.* Boston: Kluwer Academic Publishers, 1997: 177–82.

125 Hultberg B, Berglund M, Andersson A, Frank A. Elevated plasma homocysteine in alcoholics. *Alcohol Clin Exp Res* 1993; **17**: 687–9.

126 Cravo ML, Glória LM, Selhub J, *et al.* Hyperhomocysteinemia in chronic alcoholism: correlation with folate, vitamin B-12, and vitamin B-6 status. *Am J Clin Nutr* 1996; **63**: 220–4.

127 Barak AJ, Beckenhauer HC, Tuma DJ. Betaine, ethanol, and the liver: a review. *Alcohol* 1996; **13**: 395–8.

128 Hidiroglou N, Camilo ME, Beckenhauer HC, *et al.* Effect of chronic alcohol ingestion on hepatic folate distribution in the rat. *Biochem Pharmacol* 1994; **47**: 1561–6.

129 Gloria L, Cravo M, Camilo ME, *et al.* Nutritional deficiencies in chronic alcoholics: relation to dietary intake and alcohol consumption. *Am J Gastroenterol* 1997; **92**: 485–9.

130 Refsum H, Ueland PM. Clinical significance of pharmacological modulation of homocysteine metabolism. *Trends Pharmacol Sci* 1990; **11**: 411–6.

131 Arnadottir M, Hultberg B, Vladov V, Nilsson-Ehle P, Thysell H. Hyperhomocysteinemia in cyclosporin-treated renal transplant recipients. *Transplantation* 1996; **61**: 509.

132 Carlsen SM, Følling I, Grill V, Bjerve KS, Schneede J, Refsum H. Metformin increases total serum homocysteine levels in non-diabetic male patients with coronary heart disease. *Scand J Clin Lab Invest* 1997; **57**: 521–7.

133 Hoogeveen EK, Kostense PJ, Jakobs C, Bouter LM, Heine RJ, Stehouwer CDA. Does metformin increase the serum total homocysteine level in non-insulin-dependent diabetes mellitus? *J Intern Med* 1997; **242**: 389–94.

134 Carl GF, Hudson FZ, Mcguire BS. Phenytoin-induced depletion of folate in rats originates in liver and involves a mechanism that does not discriminate folate form. *J Nutr* 1997; **127**: 2231–8.

135 Blankenhorn DH, Malinow MR, Mack WJ. Colestipol plus niacin therapy elevates plasma homocyst(e)ine levels. *Coron Art Dis* 1991; **2**: 357–60.

136 Tonstad S, Knudtzon J, Sivertsen M, Refsum H, Ose L. Efficacy and safety of cholestyramine therapy in peripubertal and prepubertal children with familial hypercholesterolemia. *J Pediatr* 1996; **129**: 42–9.

137 Tonstad S, Refsum H, Ose L, Ueland PM. The C677T mutation in the methylenetetrahydrofolate reductase gene predisposes to hyperhomocysteinemia in children with familial hypercholesterolemia treated with cholestyramine. *J Pediatr* 1998; **132**: 365–8.

138 Basu TK, Mann S. Vitamin B-6 normalizes the altered sulfur amino acid status of rats fed diets containing pharmacological levels of niacin without reducing niacin's hypolipidemic effects. *J Nutr* 1997; **127**: 117–21.

139 Ubbink JB, van der Merwe A, Delport R, *et al.* The effect of a subnormal vitamin B6 status on homocysteine metabolism. *J Clin Invest* 1996; **98**: 177–84.

140 Drell W, Welch AD. Azaribine-homocystinemia-thrombosis in historical perspectives. *Pharmac Ther* 1989; **41**: 195–206.

141 Slavik M, Hyanek J, Elis J, Homolka J. Typical hyperaminoaciduria after high doses of 6-azauridine triacetate. *Biochem Pharmacol* 1969; **18**: 1782–4.

142 Shupack JL, Grieco AJ, Epstein AM, Sansaricq C, Snyderman SE. Azaribine, homocystinemia, and thrombosis. *Arch Dermatol* 1977; **113**: 1301–2.

143 Daly D, Miller JW, Nadeau MR, Selhub J. The effect of L-dopa administration and folate deficiency on plasma homocysteine concentrations in rats. *J Nutr Biochem* 1997; **8**: 634–40.

144 Miller JW, Shukitt-Hale B, Villalobos-Molina R, Nadeau MR, Selhub J, Joseph JA. Effect of L-Dopa and the catechol-O-methyltransferase inhibitor Ro 41–0960 on sulfur amino acid metabolites in rats. *Clin Neuropharmacol* 1997; **20**: 55–66.

145 Anker G, Lønning PE, Ueland PM, Refsum H, Lien EA. Plasma levels of the atherogenic amino acid homocysteine in postmenopausal women with breast cancer treated with tamoxifen. *Int J Cancer* 1995; **60**: 365–8.

146 Lien EA, Anker G, Lønning PE, Refsum H, Ueland PM. Effects of hormones on the plasma levels of the atherogenic amino acid homocysteine. *Biochem Soc Trans* 1997; **46**: 247–9.

147 van der Mooren MJ, Wouters MGAJ, Blom HJ, Schellekens LA, Eskes TKAB, Rolland R. Hormone replacement therapy may reduce high serum homocysteine in postmenopausal women. *Eur J Clin Invest* 1994; **24**: 733–6.

148 van der Mooren MJ, Demacker PN, Blom HJ, de Rijke YB, Rolland R. The effect of sequential three-monthly hormone replacement therapy on several cardiovascular risk estimators in postmenopausal women. *Fertil Steril* 1997; **67**: 67–73.

149 Mijatovic V, Kenemans P, Jakobs C, van Baal WM, Peters-Muller ER, van der Mooren MJ. A randomized controlled study of the effects of 17beta-estradiol-dydrogesterone on plasma homocysteine in postmenopausal women. *Obstet Gynecol* 1998; **91**: 432–6.

150 Steegers-Theunissen RPM, Boers GHJ, Steegers EAP, Trijbels

FJM, Thomas CMG, Eskes TKAB. Effects of sub-50 oral contraceptives on homocysteine metabolism: a preliminary study. *Contraception* 1992; **45**: 129–39.
151 Wong PWK, Kang SS. Accelerated atherosclerosis. *Am J Med* 1988; **84**: 1093–4.
152 Brattström L, Israelsson B, Olsson A, Andersson A, Hultberg B. Plasma homocysteine in women on oral oestrogen-containing contraceptives and in men with oestrogen-treated prostatic carcinoma. *Scand J Clin Lab Invest* 1992; **52**: 283–7.
153 Giltay EJ, Hoogeveen EK, Elbers JM, Gooren LJ, Asscheman H, Stehouwer CD. Effects of sex steroids on plasma total homocysteine levels: a study in transsexual males and females. *J Clin Endocrinol Metab* 1998; **83**: 550–3.
154 Kang S-S, Wong PWK, Curley K. The effect of D-penicillamine on protein-bound homocyst(e)ine in homocystinurics. *Pediatr Res* 1982; **16**: 370–2.
155 Kang S-S, Wong PWK, Glickman PB, MacLeod CM, Jaffe IA. Protein-bound homocyst(e)ine in patients with rheumatoid arthritis undergoing D-penicillamine treatment. *J Clin Pharmacol* 1986; **26**: 712–5.
156 Wiklund O, Fager G, Andersson A, Lundstam U, Masson P, Hultberg B. N-Acetylcysteine treatment lowers plasma homocysteine but not serum lipoprotein (a) levels. *Atherosclerosis* 1996; **119**: 99–106.
157 Lauterburg BH, Nguyen T, Hartmann B, Junker E, Küpfer A, Cerny T. Depletion of total cysteine, glutathione, and homocysteine in plasma by ifosfamide/mesna therapy. *Cancer Chemother Pharmacol* 1994; **35**: 132–6.
158 Refsum H, Wesenberg F, Ueland PM. Plasma homocysteine in children with acute lymphoblastic leukemia. Changes during a chemotherapeutic regimen including methotrexate. *Cancer Res* 1991; **51**: 828–35.
159 Weinstein GD. Biochemical and pathophysiological rationale for amethopterin in psoriasis. *NY Acad Sci* 1971; **186**: 452–66.
160 McDonald CJ, Calabresi P. Psoriasis and occlusive vascular disease. *Br J Dermatol* 1978; **99**: 469–75.
161 Refsum H, Helland S, Ueland PM. Fasting plasma homocysteine as a sensitive parameter to antifolate effect. A study on psoriasis patients receiving low-dose methotrexate treatment. *Clin Pharmacol Ther* 1989; **46**: 510–20.
162 Nedrebø BG, Ericsson UB, Nygård O, *et al.* Plasma total homocysteine in hyperthyroid and hypothyroid patients. *Metabolism* 1998; **47**: 89–93.
163 Fonseca VA, Mudaliar S, Schmidt B, Fink LM, Kern PA, Henry RR. Plasma homocysteine concentrations are regulated by acute hyperinsulinemia in nondiabetic but not type 2 diabetic subjects. *Metabolism* 1998; **47**: 686–9.
164 Hultberg B, Agardh E, Andersson A, *et al.* Increased levels of plasma homocysteine are associated with nephropathy but not severe retinopathy in type 1 diabetes mellitus. *Scand J Lab Clin Invest* 1991; **51**: 277–82.
165 Hultberg B, Agardh CD, Agardh E, Lovestam-Adrian M. Poor metabolic control, early age at onset, and marginal folate deficiency are associated with increasing levels of plasma homocysteine in insulin-dependent diabetes mellitus. A five-year follow-up study. *Scand J Clin Lab Invest* 1997; **57**: 595–600.
166 Robinson K, Dennis VW. From microalbuminuria to hyperhomocysteinemia. *Kidney Int* 1998; **54**: 281–2.
167 Lydakis C, Lip GYH. Microalbuminuria and cardiovascular risk. *QJM* 1998; **91**: 381–91.
168 Chico A, Perez A, Cordoba A, *et al.* Plasma homocysteine is related to albumin excretion rate in patients with diabetes mellitus: a new link between diabetic nephropathy and cardiovascular disease? *Diabetologia* 1998; **41**: 684–93.
169 Lanfredini M, Fiorina P, Peca MG, *et al.* Fasting and post-methionine load homocyst(e)ine values are correlated with microalbuminuria and could contribute to worsening vascular damage in non-insulin-dependent diabetes mellitus. *Metabolism* 1998; **47**: 915–21.
170 Hoogeveen EK, Kostense PJ, Beks PJ, *et al.* Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non-insulin-dependent diabetes mellitus: a population-based study. *Arterioscler Thromb Vasc Biol* 1998; **18**: 133–8.
171 Naurath HJ, Joosten E, Riezler R, Stabler SP, Allen RH, Lindenbaum J. Effects of vitamin B12, folate, and vitamin B6 supplements in elderly people with normal serum vitamin concentrations. *Lancet* 1995; **346**: 85–9.
172 Brattström L. Vitamins as homocysteine-lowering agents. *J Nutr* 1996; **126**: 1276S–80S.
173 Ubbink JB. The role of vitamins in the pathogenesis and treatment of hyperhomocyst(e)inaemia. *J Inherited Metab Dis* 1997; **20**: 316–25.
174 Dudman NPB, Wilcken DEL, Wang J, Lynch JF, Macey D, Lundberg P. Disordered methionine/homocysteine metabolism in premature vascular disease. Its occurrence, cofactor therapy, and enzymology. *Arterioscler Thromb* 1993; **13**: 1253–60.
175 van den Berg M, Franken DG, Boers GHJ, *et al.* Combined vitamin B-6 plus folic acid therapy in young patients with arteriosclerosis and hyperhomocysteinemia. *J Vasc Surg* 1994; **20**: 933–40.
176 Brattström LE, Israelsson B, Jeppsson J-O, Hultberg BL. Folic acid – an innocuous means to reduce plasma homocysteine. *Scand J Clin Lab Invest* 1988; **48**: 215–21.
177 Ubbink JB, Vermaak WJH, van der Merwe A, Becker PJ, Delport R, Potgieter HC. Vitamin requirements for the treatment of hyperhomocysteinemia in humans. *J Nutr* 1994; **124**: 1927–33.
178 Franken DG, Boers GH, Blom HJ, Trijbels JM. Effect of various regimens of vitamin B6 and folic acid on mild hyperhomocysteinaemia in vascular patients. *J Inherit Metab Dis* 1994; **17**: 159–62.
179 Homocysteine Lowering Trialist' Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. *Br Med J* 1998; **316**: 894–8.
180 Malinow MR, Nieto FJ, Kruger WD, *et al.* The effects of folic acid supplementation on plasma total homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes. *Arterioscler Thromb Vasc Biol* 1997; **17**: 1157–62.
181 Jacob RA, Mei-Miau W, Henning S, Swendseid ME. Homocysteine increases as folate decreases in plasma of healthy men during short-term dietary folate and methyl group restriction. *J Nutr* 1994; **124**: 1072–80.
182 O'Keefe CA, Bailey LB, Thomas EA, *et al.* Controlled dietary folate affects folate status in nonpregnant women. *J Nutr* 1995; **125**: 2717–25.
183 Ward M, McNulty H, McPartlin J, Strain JJ, Weir DG, Scott JM. Plasma homocysteine, a risk factor for cardiovascular disease, is lowered by physiological doses of folic acid. *QJM*

1997; **90**: 519–24.
184 Woodside JV, Yarnell JWG, Mcmaster D, *et al.* Effect of B-group vitamins and antioxidant vitamins on hyperhomocysteinemia: a double-blind, randomized, factorial-design, controlled trial. *Am J Clin Nutr* 1998; **67**: 858–66.
185 Rubba P, Mercuri M, Faccenda F, *et al.* Premature carotid atherosclerosis: does it occur in both familial hypercholesterolemia and homocystinuria? *Stroke* 1994; **25**: 943–50.
186 Boers GHJ. Hyperhomnocysteinemia: a newly recognized risk factor for vascular disease. *Neth J Med* 1994; **45**: 34–41.
187 Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: Probable benefits of increasing folic acid intakes. *JAMA* 1995; **274**: 1049–57.
188 Wald NJ, Watt HC, Law MR, Weir DG, McPartlin J, Scott JM. Homocysteine and ischemic heart disease: results of a prospective study with implications regarding prevention. *Arch Intern Med* 1998; **158**: 862–7.
189 Malinow MR, Nieto FJ, Szklo M, Chambless LE, Bond G. Carotid artery intimal-medial wall thickening and plasma homocyst(e)ine in asymptomatic adults. The Atherosclerosis Risk in Communities Study. *Circulation* 1993; **87**: 1107–13.
190 Selhub J, Jacques PF, Bostom AG, *et al.* Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *N Engl J Med* 1995; **332**: 286–91.
191 Tonstad S, Joakimsen O, Stenslandbugge E, *et al.* Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. *Arterioscler Thromb Vasc Dis* 1996; **16**: 984–91.
192 Voutilainen S, Alfthan G, Nyyssonen K, Salonen R, Salonen JT. Association between elevated plasma total homocysteine and increased common carotid artery wall thickness. *Ann Med* 1998; **30**: 300–6.
193 van den Berg M, Stehouwer CDA, Bierdrager E, Rauwerda JA. Plasma homocysteine and severity of atherosclerosis in young patients with lower-limb atherosclerotic disease. *Arterioscler Thromb Vasc Biol* 1996; **16**: 165–71.
194 Verhoef P, Kok FJ, Kruyssen DACM, *et al.* Plasma total homocysteine, B vitamins and risk of coronary atherosclerosis. *Arterioscler Thromb Vasc Biol* 1997; **17**: 989–95.
195 von Eckardstein A, Malinow MR, Upson B, *et al.* Effects of age, lipoproteins, and hemostatic parameters on the role of homocyst(e)inemia as a cardiovascular risk factor in men. *Arterioscler Thromb* 1994; **14**: 460–4.
196 Ubbink JB, Vermaak WJH, Bennett JM, Becker PJ, van Staden DA, Bissbort S. The prevalence of homocysteinemia and hypercholesterolemia in angiographically defined coronary heart disease. *Klin Wochenschr* 1991; **69**: 527–34.
197 Malinow MR. Plasma homocyst(e)ine: a risk factor for arterial occlusive diseases. *J Nutr* 1996; **126**: 1238S–43S.
198 Pancharuniti N, Lewis CA, Sauberlich HE, *et al.* Plasma homocyst(e)ine, folate, and vitamin B-12 concentrations and risk for early-onset coronary artery disease. *Am J Clin Nutr* 1994; **59**: 940–8.
199 Robinson K, Mayer EL, Miller DP, *et al.* Hyperhomocysteinemia and low pyridoxal phosphate: common and independent reversible risk factors for coronary artery disease. *Circulation* 1995; **92**: 2825–30.
200 Dalery K, Lussier-Cacan S, Selhub J, Davignon J, Latour Y, Genest JJ. Homocysteine and coronary artery disease in French Canadian subjects: Relation with vitamins B12, B6, pyridoxal phosphate, and folate. *Am J Cardiol* 1995; **75**: 1107–11.
201 Ambrosi P, Garcon D, Riberi A, *et al.* Association of mild hyperhomocysteinemia with cardiac graft vascular disease. *Atherosclerosis* 1998; **138**: 347–50.
202 Willems FF, Aengevaeren WRM, Jukema JW, *et al.* Hyperhomocysteinemia and progression of coronary atherosclerosis [abstract]. *Neth J Med* 1998; **52**: S36–S37.
203 Stampfer MJ, Malinow MR, Willett WC, *et al.* A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. *JAMA* 1992; **268**: 877–81.
204 Arnesen E, Refsum H, Bønaa KH, Ueland PM, Førde OH, Nordrehaug JE. Serum total homocysteine and coronary heart disease. *Int J Epidemiol* 1995; **24**: 704–9.
205 Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG. Serum total homocysteine and coronary heart disease in middle-aged British men [abstract]. *Heart* 1996; **75**: P53.
206 Whincup PH, Refsum H, Perry IJ, *et al.* Serum total homocysteine and coronary heart disease: prospective study in middle aged men [abstract]. *Neth J Med* 1998; **52**: S36.
207 Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. *Lancet* 1995; **346**: 1395–8.
208 Petri M, Roubenoff R, Dallal GE, Nadeau MR, Selhub J, Rosenberg IH. Plasma homocysteine as a risk factor for atherothrombotic events in systemic lupus erythematosus. *Lancet* 1996; **348**: 1120–4.
209 Egerton W, Silberberg J, Crooks R, Ray C, Xie L, Dudman N. Serial measures of plasma homocyst(e)ine after acute myocardial infartion. *Am J Cardiol* 1996; **77**: 759–61.
210 Lindgren A, Brattström L, Norrving B, Hultberg B, Andersson A, Johansson BB. Plasma homocysteine in the acute and convalescent phases after stroke. *Stroke* 1995; **26**: 795–800.
211 Nygård O. The Hordaland Homocysteine Studies. Determinants and prognostic value of plasma total homocysteine [dissertation]. Bergen; 1998.
212 Meleady R, Lindgren A, Boers GHJ, *et al.* Plasma homocysteine as a prognostic risk factor for vascular disease [abstract]. *Eur Heart J* 1998; **19**: 214.
213 Al-Obaidi M, Noble M, Conroy R, Graham I, Stubbs P. Admission plasma homocysteine predicts long-term mortality in patients with acute coronary ischemia [abstract]. *Eur Heart J* 1998; **19**: P1040.
214 Bostom AG, Silbershatz H, Jacques PF, *et al.* Serum total homocysteine levels predict all cause and cardiovascular disease mortality in elderly Framingham men and women [abstract]. *Circulation* 1998; **97**: 818.
215 Hoogeveen EK, Kostense PJ, de Vegt F, *et al.* Hyperhomocysteinemia is a risk factor for mortality: The Hoorn Study [abstract]. *Neth J Med* 1998; **52**: S8.
216 Brattström L, Tengborn L, Israelsson B, Hultberg B. Plasma homocysteine in venous thromboembolism. *Haemostasis* 1991; **21**: 51–7.
217 Simioni P, Prandoni P, Burlina A, *et al.* Hyperhomocysteinemia and deep-vein thrombosis – a case-control study. *Thromb Haemost* 1996; **76**: 883–6.
218 den Heijer M, Blom HJ, Gerrits WBJ, *et al.* Is hyperhomocysteinemia a risk factor for recurrent venous thrombosis? *Lancet* 1995; **345**: 882–5.
219 Amundsen T, Ueland PM, Waage A. Plasma homocysteine levels in patients with deep venous thrombosis. *Arterioscler*

Thromb Vasc Biol 1995; **15**: 1321–3.
220 den Heijer M, Koster T, Blom HJ, *et al.* Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med* 1996; **334**: 759–62.
221 den Heijer M. Hyperhomocysteinemia and venous thrombosis [dissertation]. University of Leiden, The Netherlands, 1997; 120.
222 Ridker PM, Hennekens CH, Selhub J, Miletich JP, Malinow RM, Stampfer MJ. Interrelation of hyperhomocyst(e)inemia, factor V Leiden, and risk of future venous thromboembolism. *Circulation* 1997; **95**: 1777–82.
223 Bienvenu T, Ankri A, Chadefaux B, Montalescot G, Kamoun P. Elevated total plasma homocysteine, a risk factor for thrombosis. Relation to coagulation and fibrinolytic parameters. *Thromb Res* 1993; **70**: 123–9.
224 Falcon CR, Cattaneo M, Panzeri D, Martinelli I, Mannucci PM. High prevalence of hyperhomocyst(e)inemia in patients with juvenile venous thrombosis. *Arterioscler Thromb* 1994; **14**: 1080–3.
225 Fermo I, D'Angelo SV, Paroni R, Mazzola G, Calori G, D'Angelo A. Prevalence of moderate hyperhomocysteinemia in patients with early-onset venous and arterial occlusive disease. *Ann Intern Med* 1995; **123**: 747.
226 Cattaneo M, Martinelli I, Mannucci PM. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med* 1996; **335**: 974–5.
227 Tazi Z, Cacoub P, Koskas F, *et al.* Value of an extensive biological study in venous or arterial thrombosis. *Presse Med* 1996; **25**: 531–6.
228 Wouters MGAJ, Moorrees MTEC, van der Mooren MJ, *et al.* Plasma homocysteine and menopausal status. *Eur J Clin Invest* 1995; **25**: 801–5.
229 Kang S-S, Wong PWK, Cook HY, Norusis M, Messer JV. Protein-bound homocyst(e)ine: a possible risk factor for coronary artery disease. *J Clin Invest* 1986; **77**: 1482–6.
230 Araki A, Sako Y, Fukushima Y, Matsumoto M, Asada T, Kita T. Plasma sulfhydryl-containing amino acids in patients with cerebral infarction and in hypertensive subjects. *Atherosclerosis* 1989; **79**: 139–46.
231 Bergmark C, Mansoor MA, Swedenborg J, de Faire U, Svardal AM, Ueland PM. Hyperhomocysteinemia in patients operated for lower extremity ischaemia below the age of 50. Effect of smoking and extent of disease. *Eur J Vasc Surg* 1993; **7**: 391–6.
232 Chauveau P, Chadefaux B, Coudé M, *et al.* Hyperhomocysteinemia, a risk factor for atherosclerosis in chronic uremic patients. *Kidney Int* 1993; **43** (Suppl. 41): S72–S77.
233 Bostom AG, Shemin D, Verhoef P, *et al.* Elevated fasting total plasma homocysteine levels and cardiovascular disease outcomes in maintenance dialysis patients. A prospective study. *Arterioscler Thromb Vasc Biol* 1997; **17**: 2554–8.
234 Gomberg-Maitland M, Frishman WH. Thyroid hormone and cardiovascular disease. *Am Heart J* 1998; **135** (2 pt 1): 187–96.
235 van den Berg M, Boers GHJ, Franken DG, *et al.* Hyperhomocysteinaemia and endothelial dysfunction: young patients with peripheral arterial occlusive disease. *Eur J Clin Invest* 1995; **25**: 176–81.
236 Lubec B, Labudova O, Hoeger H, *et al.* Homocysteine increases cyclin-dependent kinase in aortic rat tissue. *Circulation* 1996; **94**: 2620–5.
237 de Jong SC, Stehouwer CDA, van den Berg M, Vischer UM, Rauwerda JA, Emeis JJ. Endothelial marker proteins in hyperhomocysteinemia. *Thromb Haemost* 1997; **78**: 1332–7.
238 Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J. Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. *Lancet* 1998; **351**: 88–92.
239 Seligsohn U, Zivelin A. Thrombophilia as a multigenic disorder. *Thromb Haemost* 1997; **78**: 297–301.
240 Gemmati D, Serino ML, Moratelli S, Mari R, Ballerini G, Scapoli GL. Coexistence of antithrombin deficiency, factor V Leiden and hyperhomocysteinemia in a thrombotic family. *Blood Coagul Fibrinol* 1998; **9**: 173–6.
241 Mandel H, Brenner B, Berant M, *et al.* Coexistence of hereditary homocystinuria and factor V Leiden – effect on thrombosis. *N Engl J Med* 1996; **334**: 763–8.
242 Cattaneo M, Tsai MY, Bucciarelli P, *et al.* A common mutation in the methylenetetrahydrofolate reductase gene (C677T) increases the risk for deep-vein thrombosis in patients with mutant factor V (Factor V. Q (506)). *Arterioscler Thromb Vasc Biol* 1997; **17**: 1662–6.
243 Kluijtmans LAJ, den Heijer M, Reitsma PH, Heil SG, Blom HJ, Rosendaal FR. Thermolabile methylenetetrahydrofolate reductase and factor V Leiden in the risk of deep-vein thrombosis. *Thromb Haemost* 1998; **79**: 254–8.
244 Franken DG. Hyperhomocysteinemia: inherited causes and effects of treatment [dissertation]. University Hospital Nijmegen, The Netherlands, 1996; 175.
245 Franken DG, Boers GHJ, Blom HJ, Cruysberg JRM, Trijbels FJM, Hamel BCJ. Prevalence of familial mild hyperhomocysteinemia. *Atherosclerosis* 1996; **125**: 71–80.
246 Genest JJJ, McNamara JR, Upson B, *et al.* Prevalence of familial hyperhomocyst(e)inemia in men with premature coronary artery disease. *Arterioscler Thromb* 1991; **11**: 1129–36.
247 Wu LL, Wu J, Hunt SC, *et al.* Plasma homocyst(e)ine as a risk factor for early familial coronary artery disease. *Clin Chem* 1994; **40**: 552–61.
248 Tonstad S, Refsum H, Sivertsen M, Christophersen B, Ose L, Ueland PM. Relation of total homocysteine and lipid levels in children to premature cardiovascular death in male relatives. *Pediatr Res* 1996; **40**: 47–52.
249 Rubbanyi GM. The role of the endothelium in cardiovascular homeostasis and diseases. *J Cardiovasc Pharmacol* 1993; **22**: S1–S14.
250 De Meyer GR, Herman AG. Vascular endothelial dysfunction. *Prog Cardiovasc Dis* 1997; **39**: 325–42.
251 Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993; **362**: 801–9.
252 Benzuly KH, Padget RC, Kaul S, Piegors DJ, Armstrong ML, Heistad DD. Functional improvement precedes structural regression of atherosclerosis. *Circulation* 1994; **89**: 1810–8.
253 Harrison DG, Armstrong ML, Freiman PC, Heistad DD. Restoration of endothelium-dependent relaxation by dietary treatment of atherosclerosis. *J Clin Invest* 1987; **80**: 1808–11.
254 van der Molen EF, Hiipakka MJ, van Lith-Zanders H, *et al.* Homocysteine metabolism in endothelial cells of a patient homozygous for cystathionine beta-synthase (CS) deficiency. *Thromb Haemost* 1997; **78**: 827–33.
255 Rock G, Wells P. New concepts in coagulation. *Crit Rev Clin Lab Sci* 1997; **34**: 475–501.

256 Bombeli T, Mueller M, Haeberli A. Anticoagulant properties of the vascular endothelium. *Thromb Haemost* 1997; **77**: 408–23.
257 Rodgers GM, Kane WH. Activation of endogenous factor V by a homocysteine-induced vascular endothelial cell activator. *J Clin Invest* 1986; **77**: 1909–16.
258 Hajjar KA. Homocysteine-induced modulation of tissue plasminogen activator binding to its endothelial cell membrane receptor. *J Clin Invest* 1993; **91**: 2873–9.
259 Hajjar KA, Mauri L, Jacovina AT, *et al.* Tissue plasminogen activator binding to the annexin II tail domain – direct modulation by homocysteine. *J Biol Chem* 1998; **273**: 9987–93.
260 Rodgers GM, Conn MT. Homocysteine, an atherogenic stimulus, reduces protein-C activation by arterial and venous endothelial cells. *Blood* 1990; **75**: 895–901.
261 Lentz SR, Sadler JE. Inhibition of thrombomodulin surface expression and protein-C activation by the thrombogenic agent homocysteine. *J Clin Invest* 1991; **88**: 1906–14.
262 Hayashi T, Honda G, Suzuki K. An atherogenic stimulus homocysteine inhibits cofactor activity of thrombomodulin and enhances thrombomodulin expression in human umbilical vein endothelial cells. *Blood* 1992; **79**: 2930–6.
263 Nishinaga M, Ozawa T, Shimada K. Homocysteine, a thrombogenic agent, suppresses anticoagulant heparan sulfate expression in cultured porcine aortic endothelial cells. *J Clin Invest* 1993; **92**: 1381–6.
264 Fryer RH, Wilson BD, Gubler DB, Fitzgerald LA, Rodgers GM. Homocysteine, a risk factor for premature vascular disease and thrombosis, induces tissue factor activity in endothelial cells. *Arterioscler Thromb* 1993; **13**: 1327–33.
265 Stamler JS, Osborne JA, Jaraki O, *et al.* Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest* 1993; **91**: 308–18.
266 Graeber JE, Slott JH, Ulane RE, Schulman JD, Stuart MJ. Effect of homocysteine and homocystine on platelet and vascular arachidonic acid metabolism. *Pediatr Res* 1982; **16**: 490–3.
267 Panganamala RV, Karpen CW, Merola AJ. Peroxide mediated effects of homocysteine on arterial prostacyclin synthesis. *Prostagland Leuk Med* 1986; **22**: 349–56.
268 Quéré I, Habib A, Tobelem G, *et al.* Inhibition of cyclooxygenase activity in human endothelial cells by homocysteine. In: Samuelsson B, Ramwell PW, Paoletti R, Folco G, Granstrom E, Nicosia S,eds. *Prostaglandins and Related Compounds,* Vol 23. New York: Raven Press, 1995: 397–9.
269 Lentz SR, Sadler JE. Homocysteine inhibits von Willebrand Factor processing and secretion by preventing transport from the endoplasmic reticulum. *Blood* 1993; **81**: 683–9.
270 Lentz SR, Sobey CG, Piegors DJ, *et al.* Vascular dysfunction in monkeys with diet-induced hyperhomocyst(e)inemia. *J Clin Invest* 1996; **98**: 24–9.
271 Cines DB, Pollak ES, Buck CA, *et al.* Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* 1998; **91**: 3527–61.
272 Lentz SR, Malinow MR, Piegors DJ, Bhopatkarteredesai M, Faraci FM, Heistad DD. Consequences of hyperhomocyst(e)inemia on vascular function in atherosclerotic monkeys. *Arterioscler Thromb Vasc Biol* 1997; **17**: 2930–4.
273 Celermajer DS, Sorensen K, Ryalls M, *et al.* Impaired endothelial function occurs in the systemic arteries of children with homozygous homocystinuria but not in their heterozygous parents. *J Am Coll Cardiol* 1993; **22**: 854–8.
274 Tawakol A, Omland T, Gerhard M, Wu JT, Creager MA. Hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilation in humans. *Circulation* 1997; **95**: 1119–21.
275 Woo KS, Chook P, Lolin YI, *et al.* Hyperhomocyst(e)inemia is a risk factor for arterial endothelial dysfunction in humans. *Circulation* 1997; **96**: 2542–4.
276 Chambers JC, McGregor A, Jean-Marie J, Kooner JS. Acute hyperhomocysteinemia and endothelial dysfunction. *Lancet* 1998; **351**: 36–7.
277 Bellamy MF, Ramsey MW, Goodfellow J, *et al.* Hyperhomocysteinemia and endothelial dysfunction with low vitamin B-12/folate status [abstract]. *Circulation* 1996; **94** (Suppl. I): I-462.
278 Bellamy MF, McDowell IFW, Ramsey MW, Brownlee M, Lewis MJ. Folic acid supplements lower plasma homocysteine and improve flow-mediated endothelial function in healthy adults. *Neth J Med* 1998; **52** (Suppl.): S14.
279 Harker LA, Slichter SJ, Scott CR, Ross R. Homocystinemia. Vascular injury and arterial thrombosis. *N Engl J Med* 1974; **291**: 537–43.
280 Harker LA, Ross R, Slichter SJ, Scott CR. Homocysteine-induced arteriosclerosis. The role of endothelial cell injury and platelet response in its genesis. *J Clin Invest* 1976; **58**: 731–41.
281 Hladovec J. Experimental homocystinemia, endothelial lesions and thrombosis. *Blood Vessels* 1979; **16**: 202–5.
282 Harker LA, Harlan JM, Ross R. Effect of sulfinpyrazone on homocysteine-induced endothelial injury and arteriosclerosis in baboons. *Circ Res* 1983; **53**: 731–9.
283 Wall RT, Harlan JM, Harker LA, Striker GE. Homocysteine-induced endothelial cell injury in vitro: a model for the study of vascular injury. *Thromb Res* 1980; **18**: 113–21.
284 DeGroot PG, Willems C, Boers GHJ, Gonsalves MD, van Aken WG, van Mourik JA. Endothelial cell dysfunction in homocystinuria. *Eur J Clin Invest* 1983; **13**: 405–10.
285 Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. *J Clin Invest* 1986; **77**: 1370–6.
286 Dudman NPB, Hicks C, Wang J, Wilcken DEL. Human arterial endothelial cell detachment in vitro: its promotion by homocysteine and cysteine. *Atherosclerosis* 1991; **91**: 77–83.
287 Berman RS, Martin W. Arterial endothelial barrier dysfunction – Actions of homocysteine and the hypoxanthine-xanthine oxidase free radical generating system. *Br J Pharmacol* 1993; **108**: 920–6.
288 Jones BG, Rose FA, Tudball N. Lipid peroxidation and homocysteine induced toxicity. *Atherosclerosis* 1994; **105**: 165–70.
289 Reddy GSR, Wilcken DEL. Experimental homocysteinemia in pigs: comparison with studies in sixteen homocystinuric patients. *Metabolism* 1982; **31**: 778–83.
290 Hladovec J, Sommerova Z, Pisarikova A. Homocysteinemia and endothelial damage after methionine load. *Thromb Res* 1997; **88**: 361–4.
291 Tsai JC, Perrella MA, Yoshizumi M, *et al.* Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. *Proc Natl Acad Sci, USA* 1994; **91**: 6369–73.

292 Wang H, Yoshizumi M, Lai KH, *et al.* Inhibition of growth and p21 (ras) methylation in vascular endothelial cells by homocysteine but not cysteine. *J Biol Chem* 1997; **272**: 25380–5.
293 Hultberg B, Andersson A, Isaksson A. The cell-damaging effects of low amounts of homocysteine and copper ions in human cell line cultures are caused by oxidative stress. *Toxicology* 1997; **123**: 33–40.
294 Holvoet P, Collen D. Thrombosis and atherosclerosis. *Curr Opin Lipidol* 1997; **8**: 320–8.
295 Mansoor MA, Bergmark C, Svardal AM, Lønning PE, Ueland PM. Redox status and protein binding of plasma homocysteine and other aminothiols in patients with early-onset peripheral vascular disease. *Arterioscler Thromb Vasc Biol* 1995; **15**: 232–40.
296 Andersson A, Lindgren A, Hultberg B. Effect of thiol oxidation and thiol export from erythrocytes on determination of redox status of homocysteine and other thiols in plasma from healthy subjects and patients with cerebral infarction. *Clin Chem* 1995; **41**: 361–6.
297 Dudman NPB, Wilcken DEL. Increased plasma copper in patients with homocystinuria due to cystathionine beta synthase deficiency. *Clin Chim Acta* 1983; **127**: 105–13.
298 Freedman JE, Frei B, Welch GN, Loscalzo J. Glutathione peroxidase potentiates the inhibition of platelet function by s-nitrosthiols. *J Clin Invest* 1995; **96**: 394–400.
299 Upchurch GR, Welch GN, Fabian AJ, *et al.* Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. *J Biol Chem* 1997; **272**: 17012–7.
300 Upchurch GR, Welch GN, Fabian AJ, Pigazzi A, Keaney JF, Loscalzo J. Stimulation of endothelial nitric oxide production by homocyst(e)ine. *Atherosclerosis* 1997; **132**: 177–85.
301 Stamler JS, Slivka A. Biological chemistry of thiols in the vasculature and in vascular-related disease. *Nutr Rev* 1996; **54**: 1–30.
302 Upchurch GR, Welch GN, Loscalzo J. Homocysteine, EDRF and endothelial function. *J Nutr* 1996; **126** (Suppl. 4): S1290–S1294.
303 Loscalzo J. The oxidant stress of hyperhomocyst(e)inemia. *J Clin Invest* 1996; **98**: 5–7.
304 Stocker R. Lipoprotein oxidation: mechanisms, aspects, methodological approaches and clinical relevance. *Curr Opin Lipidol* 1994; **5**: 422–33.
305 Berliner JA, Heinecke JW. The role of oxidized lipoproteins in atherogenesis. *Free Radic Biol Med* 1996; **20**: 707–27.
306 Parthasarathy S. Oxidation of low-density lipoprotein by thiol compounds leads to its recognition by the acetyl LDL receptor. *Biochim Biophys Acta* 1987; **917**: 337–40.
307 Hirano K, Ogihara T, Miki M, *et al.* Homocysteine induces iron-catalyzed lipid peroxidation of low-density lipoprotein that is prevented by alpha-tocopherol. *Free Radic Res* 1994; **21**: 267–76.
308 Durand P, Lussier-Cacan S, Blache D. Acute methionine load-induced hyperhomocysteinemia enhances platelet aggregation thromboxane biosynthesis, and macrophage-derived tissue factor activity in rats. *FASEB J* 1997; **11**: 1157–68.
309 Young PB, Kennedy S, Molloy AM, Scott JM, Weir DG, Kennedy DG. Lipid peroxidation induced in vivo by hyperhomocysteinaemia in pigs. *Atherosclerosis* 1997; **129**: 67–71.
310 Blom HJ, Engelen DPE, Boers GHJ, *et al.* Lipid peroxidation in homocysteinaemia. *J Inherit Metab Dis* 1992; **15**: 419–22.
311 Dudman NPB, Wilcken DEL, Stocker R. Circulating lipid hydroperoxide levels in human hyperhomocysteinemia – relevance to development of arteriosclerosis. *Arterioscler Thromb* 1993; **13**: 512–6.
312 Blom HJ, Kleinveld HA, Boers GHJ, *et al.* Lipid peroxidation and susceptibility of low-density lipoprotein to in vitro oxidation in hyperhomocysteinaemia. *Eur J Clin Invest* 1995; **25**: 149–54.
313 Córdoba-Porras A, Sanchez-Quesada JL, González-Sastre F, Ordónez-Llanos J, Blanco-Vaca F. Susceptibility of plasma low- and high-density lipoproteins to oxidation in patients with severe hyperhomocysteinemia. *J Mol Med* 1996; **74**: 771–6.
314 Halvorsen B, Brude I, Drevon CA, *et al.* Effect of homocysteine on copper ion-catalyzed, azo compound-initiated, and mononuclear cell-mediated oxidative modification of low density lipoprotein. *J Lipid Res* 1996; **37**: 1591–6000.
315 Domagala TB, Libura M, Szczeklik A. Hyperhomocysteinemia following oral methionine load is associated with increased lipid peroxidation. *Thromb Res* 1997; **87**: 411–6.
316 Tsai JC, Wang H, Perella MA, *et al.* Induction of cyclin A gene expression by homocysteine in vascular smooth muscle cells. *J Clin Invest* 1996; **97**: 146–53.
317 Majors A, Ehrhart LA, Pezacka EH. Homocysteine as a risk factor for vascular disease – enhanced collagen production and accumulation by smooth muscle cells. *Arterioscler Thromb Vasc Biol* 1997; **17**: 2074–81.
318 Nishio E, Watanabe Y. Homocysteine as a modulator of platelet-derived growth factor action in vascular smooth muscle cells: a possible role for hydrogen peroxide. *Br J Pharmacol* 1997; **122**: 269–74.
319 Tang LL, Mamotte CDS, Van Bockxmeer FM, Taylor RR. The effect of homocysteine on DNA synthesis in cultured human vascular smooth muscle. *Atherosclerosis* 1998; **136**: 169–73.
320 Tyagi SC. Homocysteine redox receptor and regulation of extracellular matrix components in vascular cells. *Am J Physiol* 1998; **274**: C396–C405.
321 Dalton ML, Gadson PF, Wrenn RW, Rosenquist TH. Homocysteine signal cascade: production of phospholipids, activation of protein kinase C, and the induction of c-fos and c-myb in smooth muscle cells. *FASEB J* 1997; **11**: 703–11.
322 Jourdheuil-Rahmani D, Rolland PH, Rosset E, Branchereau A, Garcon D. Homocysteine induces synthesis of a serine elastase in arterial smooth muscle cells from multi-organ donors. *Cardiovasc Res* 1997; **34**: 597–602.
323 Durand P, Prost M, Blache D. Pro-thrombotic effects of a folic acid deficient diet in rat platelets and macrophages related to elevated homocysteine and decreased n-3 polyunsaturated fatty acids. *Atherosclerosis* 1996; **121**: 231–43.
324 Di Minno G, Davi G, Margaglione M, *et al.* Abnormally high thromboxane biosynthesis in homozygous homocystinuria – evidence for platelet involvement and probucol-sensitive mechanism. *J Clin Invest* 1993; **92**: 1400–6.
325 Coppola A, Albisinni R, Madonna P, Pagano A, Cerbone AM, Di Minno G. Platelet and monocyte variables in homocystinuria due to cystathionine-β-synthase deficiency. *Haematologica* 1997; **82**: 189–90.
326 Uhlemann ER, TenPas JH, Lucky AW, Schulman JD, Mudd SH, Shulman NR. Platelet survival and morphology in

homocystinuria due to cystathionine synthase deficiency. *N Engl J Med* 1976; **295**: 1283–6.
327 Hill-Zobell RL, Pyeritz RE, Scheffel U, *et al.* Kinetics and distribution of 111Indium-labeled platelets in patients with homocystinuria. *N Engl J Med* 1982; **307**: 781–6.
328 Ratnoff OD. Activation of Hageman factor by L-homocystine. *Science* 1968; **162**: 1007–9.
329 Harpel PC, Chang VT, Borth W. 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* 1992; **89**: 10193–7.
330 Kyrle PA, Stümpflen A, Hirschl M, *et al.* Levels of prothrombin fragment F1+2 in patients with hyperhomocysteinemia and a history of venous thromboembolism. *Thromb Haemost* 1997; **78**: 1327–31.
331 Blom HJ, van der Molen EF. Pathobiochemical implications of hyperhomocysteinemia. *Fibrinolysis* 1994; **8**: 86–7.
332 Heinecke JW, Rosen H, Suzuki LA, Chait A. The role of sulfur-containing amino acids in superoxide production and modification of low density lipoprotein by arterial smooth muscle cells. *J Biol Chem* 1987; **262**: 10 098–103.
333 Montalescot G, Ankri A, Chadefaux-Vekemans B, *et al.* Plasma homocysteine and the extent of atherosclerosis in patients with coronary artery disease. *Int J Cardiol* 1997; **60**: 295–300.
334 A, Brook R, Tavendale R, Tunstall-Pedoe. Homocysteine and coronary risk in the general population: analysis from the Scottish Heart Health Study and Scottish Monica surveys [abstract]. *Eur Heart J* 1998; **19**: 213.
335 Verhoef P, Hennekens CH, Malinow MR, Kok FJ, Willett WC, Stampfer MJ. A prospective study of plasma homocyst(e)ine and risk of ischemic stroke. *Stroke* 1994; **25**: 1924–30.
336 Chasan-Taber L, Selhub J, Rosenberg IH, *et al.* A prospective study of folate and vitamin B6 and risk of myocardial infarction in US physicians. *J Am Coll Nutr* 1996; **15**: 136–43.
337 Alfthan G, Pekkanen J, Jauhiainen M, *et al.* Relation of serum homocysteine and lipoprotein (a) concentrations to atherosclerotic disease in a prospective Finnish population based study. *Atherosclerosis* 1994; **106**: 9–19.
338 Evans RW, Shaten BJ, Hempel JD, Cutler JA, Kuller LH. Homocyst(e)ine and risk of cardiovascular disease in the multiple risk factor intervention trial. *Arterioscler Thromb Vasc Biol* 1997; **17**: 1947–53.
339 Folsom AR, Nieto JF, McGovern PG, *et al.* Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphism, and B vitamins: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 1998; **98**: 204–10.
340 Moustapha A, Naso A, Nahlawi M, *et al.* Prospective study of hyperhomocysteinemia as an adverse cardiovascular risk factor in end-stage renal disease. *Circulation* 1998; **97**: 138–41.
341 de Jong SC, Stehouwer CDA, van den Berg M, Geurts TW, Rauwerda JA. Normohomocysteinemia and vitamin-treated hyperhomocysteinemia are associated with similar risks of cardiovascular events in patients with premature peripheral arterial occlusive disease [abstract]. *Neth J Med* 1998; **52**: S41–S42.
342 Law MR, Wald NJ, Wu T, Hackshaw A, Bailey A. Systematic underestimation of association between serum cholesterol concentration and ischaemic heart disease in observational studies: data from the BUPA study. *Br Med J* 1994; **308**: 363–6.
343 Verhoef P, Hennekens CH, Allen RH, Stabler SP, Willett WC, Stampfer MJ. Plasma total homocysteine and risk of angina pectoris with subsequent coronary artery bypass surgery. *Am J Cardiol* 1997; **79**: 799–801.
344 Koehler KM, Pareo-Tubbeh SL, Romero LJ, Baumgartner RN, Garry PJ. Folate nutrition and older adults: challenges and opportunities. *J Am Diet Assoc* 1997; **97**: 167–73.
345 Ness A, Powles JW. Fruit and vegetables, and cardiovascular disease: a review. *Int J Epidemiol* 1997; **26**: 1–13.
346 Morrison HI, Schaubel D, Desmeules M, Wigle DT. Serum folate and risk of fatal coronary heart disease. *JAMA* 1996; **275**: 1893–6.
347 Rimm EB, Willett WC, Hu FB, *et al.* Folate and vitamin B6 from diet and supplements in relation to risk of coronary heart disease among women. *JAMA* 1998; **279**: 359–64.
348 Wilcken DEL, Wang XL. The natural history of vascular disease in homocystinuria and the effects of treatment. *J Inherited Metab Dis* 1997; **20**: 295–300.
349 Peterson JC, Spence JD. Vitamins and progression of atherosclerosis in hyper-homocyst(e)inemia. *Lancet* 1998; **351**: 263.
350 Kuller LH, Evans RW. Homocysteine, vitamins, and cardiovascular disease. *Circulation* 1998; **98**: 196–9.
351 Mudd SH, Havlik R, Levy HL, McKusick VA, Feinleib M. A study of cardiovascular risk in heterozygotes for homocystinuria. *Am J Hum Genet* 1981; **33**: 883–93.
352 Kluijtmans LAJ, van den Heuvel LPWJ, Boers GHJ, *et al.* Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Hum Genet* 1996; **58**: 35–41.
353 Kozich V, Kraus E, de Franchis R, *et al.* Hyperhomocysteinemia in premature arterial disease: examination of cystathionine beta-synthase alleles at the molecular level. *Hum Mol Genet* 1995; **4**: 623–9.
354 Evers S, Koch HG, Grotemeyer KH, Ullrich K, Deufel T, Harms E. Hyperhomocysteinemia in ischemic stroke is not due to mutations in the cystathionine beta-synthase gene. *Cerebrovasc Dis* 1996; **6** (Suppl. 2): 19.
355 Whitehead AS, Ward P, Tan S et al. The molecular genetics of homocystinuria, hyperhomocysteinemia, and premature vascular disease in Ireland. In: Mato JM, Caballero A,eds. *Methionine Metabolism: Molecular Mechanisms and Clinical Implications.* Madrid: Bouncopy, 1994; 79–83.
356 Brattström L, Wilcken DEL, Öhrvik J, Brudin L. The common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease – the result of a meta analysis. *Circulation* 1998: 2520–6.
357 Frosst P, Blom HJ, Milos R et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; **10**: 111–3.
358 van der Put NMJ, Steegers-Theunissen RPM, Frosst P et al. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. *Lancet* 1995; **346**: 1070–1.
359 Harmon DL, Woodside JV, Yarnell JWG et al. The common 'thermolabile' variant of methylene tetrahydrofolate reductase is a major determinant of mild hyperhomocysteinaemia. *QJM* 1996; **89**: 571–7.

360 Schmitz C, Lindpaintner K, Verhoef P, Gaziano JM, Buring J. Genetic polymorphism of methylenetetrahydrofolate reductase and myocardial infarction. A case-control study. *Circulation* 1996; **94**: 1812–4.
361 Ma J, Stampfer MJ, Hennekens CH et al. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. *Circulation* 1996; **94**: 2410–6.
362 Deloughery TG, Evans A, Sadeghi A et al. Common mutation in methylenetetrahydrofolate reductase: correlation with homocysteine metabolism and late-onset vascular disease. *Circulation* 1996; **94**: 3074–8.
363 Verhoef P, Kok FJ, Kluijtmans LJ et al. The 677C→T mutation in the methylenetetrahydrofolate reductase gene: associations with plasma total homocysteine levels and risk of coronary atherosclerotic disease. *Atherosclerosis* 1997; **132**: 105–13.
364 Kluijtmans LAJ, Kastelein JJP, Lindemans J et al. Thermolabile methylenetetrahydrofolate reductase in coronary artery disease. *Circulation* 1997; **96**: 2573–7.
365 Christensen B, Frosst P, Lussier-Cacan S et al. Correlation of a common mutation in the methylenetetrahydrofolate reductase gene with plasma homocysteine in patients with premature coronary artery disease. *Arterioscler Thromb Vasc Biol* 1997; **17**: 569–73.
366 Schwartz SM, Siscovick DS, Malinow MR et al. Myocardial infarction in young women in relation to plasma total homocysteine, folate, and a common variant in the methylenetetrahydrofolate reductase gene. *Circulation* 1997; **96**: 412–7.
367 Legnani C, Palareti G, Grauso F et al. Hyperhomocyst(e)inemia and a common methylenetetrahydrofolate reductase mutation (Ala (223) Val MTHFR) in patients with inherited thrombophilic coagulation defects. *Arterioscler Thromb Vasc Biol* 1998; **17**: 2924–9.
368 Arai K, Yamasaki Y, Kajimoto Y et al. Association of methylenetetrahydrofolate reductase gene polymorphism with carotid arterial wall thickening and myocardial infarction risk in NIDDM. *Diabetes* 1997; **46**: 2102–4.
369 Lalouschek W, Aull S, Korninger L et al. 677C to T mutation in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene and plasma homocyst(e)ine levels in patients with TIA or minor stroke. *J Neurol Sci* 1998; **165**: 156–62.
370 Gallagher PM, Meleady R, Shields DC et al. Homocysteine and risk of premature coronary heart disease. Evidence for a common gene mutation. *Circulation* 1996; **94**: 2154–8.
371 Adams M, Smith PD, Martin D, Thompson JR, Samani NJ. Genetic analysis of thermolabile methylenetetrahydrofolate reductase as a risk factor for myocardial infarction. *QJM* 1996; **89**: 437–44.
372 Izumi M, Iwai N, Ohmichi N, Nakamura Y, Shimoike H, Kinoshita M. Molecular variant of 5,10-methylenetetrahydrofolate reductase is a risk factor of ischemic heart disease in the Japanese population. *Atherosclerosis* 1996; **121**: 293–4.
373 Wilcken DEL, Wang XL, Sim AS, McCredie RM. Distribution in healthy and coronary populations of the methylenetetrahydrofolate reductase (MTHFR) C677T mutation. *Arterioscler Thromb Vasc Biol* 1996; **16**: 878–82.
374 de Franchis R, Mancini FP, D'Angelo A et al. Elevated total plasma homocysteine and 677C→T mutation of the 5,10-methylenetetrahydrofolate reductase gene in thrombotic vascular disease [letter]. *Am J Hum Genet* 1996; **59**: 262–4.
375 Narang R, Callaghan G, Haider AW, Davies GJ, Tuddenham EGD. Methylenetetrahydrofolate reductase mutation and coronary artery disease. *Circulation* 1996; **94**: 2322–3.
376 Brulhart MC, Dussoix P, Ruiz J, Passa P, Froguel P, James RW. The (Ala-Val) mutation of methylenetetrahydrofolate reductase as a genetic risk factor for vascular disease in non-insulin-dependent diabetic patients. *Am J Hum Genet* 1997; **60**: 228–9.
377 van Bockxmeer FM, Mamotte CDS, Vasikaran SD, Taylor RR. Methylenetetrahydrofolate reductase gene and coronary artery disease. *Circulation* 1997; **95**: 21–3.
378 Brugada R, Marian AJ. A common mutation in methylenetetrahydrofolate reductase gene is not a major risk of coronary artery disease or myocardial infarction. *Atherosclerosis* 1997; **128**: 107–12.
379 Morita H, Taguchi J, Kurihara H et al. Genetic polymorphism of 5,10-methylenetetrahydrofolate reductase (MTHFR) as a risk factor for coronary artery disease. *Circulation* 1997; **95**: 2032–6.
380 Verhoef P, Rimm EB, Hunter DJ et al. Methylenetetrahydrofolate reductase polymorphism, diet, and risk of coronary heart disease among men. *16th International Congress of Nutrition*. July 27-August 1, 1997, Montreal, Canada. (Abstract) PW3.12.
381 Tosetto A, Missiaglia E, Frezzato M, Rodeghiero F. The VITA Project: C677T mutation in the methylene-tetrahydrofolate reductase gene and risk of venous thromboembolism. *Br J Haematol* 1997; **97**: 804–6.
382 Markus HS, Ali N, Swaminathan R, Sankaralingam A, Molloy J, Powell J. A common polymorphism in the methylenetetrahydrofolate reductase gene, homocysteine, and ischemic cerebrovascular disease. *Stroke* 1997; **28**: 1739–43.
383 Salden A, Keeney S, Hay CR, Cumming AM. The C677T MTHFR variant and the risk of venous thrombosis. *Br J Haematol* 1997; **99**: 464–72.
384 Reinhardt D, Sigusch HH, Vogt SF, Farker K, Muller S, Hoffmann A. Absence of association between a common mutation in the methylenetetrahydrofolate reductase gene and the risk of coronary artery disease. *Eur J Clin Invest* 1997; **28**: 20–3.
385 Abbate R, Sardi I, Pepe G et al. The high prevalence of thermolabile 5-10 methylenetetrahydrofolate reductase (MTHFR) in Italians is not associated to an increased risk for coronary artery disease (CAD). *Thromb Haemost* 1998; **79**: 727–30.
386 Ou T, Yamakawa-Kobayashi K, Arinami T et al. Methylenetetrahydrofolate reductase and apolipoprotein E polymorphisms are independent risk factors for coronary heart disease in Japanese: a case-control study. *Atherosclerosis* 1998; **137**: 23–8.
387 Kostulas K, Crisby M, Huang WX et al. A methylenetetrahydrofolate reductase gene polymorphism in ischaemic stroke and in carotid artery stenosis. *Eur J Clin Invest* 1998; **28**: 285–9.
388 Nakata Y, Katsuya T, Takami S et al. Methylenetetrahydrofolate reductase gene polymorphism: relation to blood pressure and cerebrovascular disease. *Am J Hypertens* 1998; **11**: 1019–23.
389 Girelli D, Friso S, Trabetti E et al. Methylenetetrahydrofolate

reductase C677T mutation, plasma homocysteine, and folate in subjects from Northern Italy with or without angiographically documented severe coronary atherosclerotic disease: evidence for an important genetic–environmental interaction. *Blood* 1998; **91**: 4158–63.

Received 21 October 1998; accepted 29 December 1998.

Correspondence: Dr Ottar Nygård, Department of Heart Disease, Haukeland University Hospital, 5021 Bergen, Norway (fax: 47 55 975150; e-mail: ottar.nygard@smis.uib.no).