



Homocysteine and cardiovascular disease in diabetes mellitus

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Abstract

Background: Patients with diabetes mellitus (DM) have 2- to 6-fold increase in the prevalence of cardiovascular disease (CVD) compared to non-DM subjects. Epidemiological data show that DM is synergic with other conventional risk factors. Total plasma homocysteine (tHcy) is an emerging CVD risk factor. We reviewed the literature to explore the relation between tHcy and CVD in patients with DM. **Methods:** We searched the MEDLINE database for articles on homocysteine, DM and CVD published from January 1991 to October 2000. **Results:** The mean plasma tHcy level is usually low or normal in DM patients, except when nephropathy is present. Levels in that case tend to be higher than in non-DM patients. An independent association with tHcy and CVD was shown in retrospective studies, for DM patients. Prospective studies showed an association between elevated tHcy and all cause mortality in DM patients. In general, the association between elevated levels of tHcy and the outcome was stronger than in non-DM individuals, for all types of study. **Discussion:** To date, there are no prospective work that specifically examined the relationship between levels of tHcy and the presence of CVD in the DM population. Nor are there studies to show that treating elevated tHcy results in a reduction of CVD events. Such studies are ongoing. Nevertheless, since hyperhomocysteinemia is potentially reversible with vitamin therapy, interaction of DM with high levels tHcy on the risk of CVD may have consequences with regard to management of primary and secondary prevention in DM patients who are at particularly high risk of CVD events. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Diabetes mellitus (DM) is a major risk factor for cardiovascular disease (CVD) [1–3]. Epidemiological data especially from the Framingham Heart Study and the Multiple Risk Factor Intervention Trial clearly show the independent contribution of diabetes to cardiovascular risk, and the further incremental risk when other conventional risk factors such as lipids, smoking and hypertension are present [4–7]. Diabetic patients have 2- to 6-fold higher prevalence of atherosclerosis compared to non-diabetic patients [8–10]. For diabetic patients without previous myocardial infarction (MI),

the 7-year incidence of MI was equivalent to that observed in non-diabetic patients with previous MI, in a recent study from Finland [11]. Patients with diabetes also have a higher mortality rate after their first MI than non-diabetics [12]. After a first cardiac event, 50% of patients with diabetes may die in the first year, and half of those who die do so before reaching the hospital. This suggests that primary prevention can play an important role in reducing the incidence of fatal and non-fatal coronary heart disease in diabetic subjects.

Based on this data, the Prevention Conference Writing Group recently made the recommendation that type II diabetes be described as ‘CHD risk equivalent’ in terms of risk factor management, meaning that the current AHA guidelines for risk factor management of patients with established coronary disease should probably be extended to patients with type II diabetes without coronary disease [13].

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Over the last decade, multiple other factors have been identified as potential contributors for CVD. As shown by observational studies, an increase in total plasma homocysteine (tHcy) is one of these new factors [14–57], along with systemic inflammation, coagulation factors, oxidative stress, ventricular hypertrophy and various dyslipidemia subtypes [58,59].

These conventional or new risk factors may work synergistically with diabetes in promoting CVD. The relationship between plasma homocysteine and the excess of CVD in diabetic patients remains to be clarified. A recent study on 1550 subjects revealed that an elevated tHcy concentration was not only an independent risk of vascular disease but also had a multiplicative effect on risk among cigarette smokers and patients with hypertension [38]. This study, however, excluded patients with diabetes. It is also possible that high levels of plasma tHcy and diabetes together confer a multiplicative risk for CVD. The purpose of this review is therefore to explore the relation between homocysteine and CVD in patients with DM.

2. Methods

2.1. Data sources and study selection

We reviewed the scientific literature for all epidemiological studies on CVDs (also using the terms *coronary artery disease*, *heart disease* and *atherosclerosis*) and homocysteine in DM. All types of diabetes were considered according to the relatively small number of studies

expected on the topic. We searched the MEDLINE database for articles published from January 1991 to October 2000 and identified additional studies by examining bibliographies of original articles, review articles, and textbooks. All prospective cohort, case-control and cross-sectional studies were considered, but case reports and series were not included.

A formal meta-analysis was not performed given the lack of homogeneity of the different studies in terms of patient selection, types of diabetes, number of patients and controls, study designs, definition of hyperhomocysteinemia, measuring techniques of plasma homocysteine levels, outcome definition and measurements, and statistical analysis.

3. Results

3.1. Metabolism of homocysteine

Homocysteine, a sulfur-containing amino acid, is a product of methionine metabolism. It lies at the intersection of two major pathways: remethylation and *trans*-sulfuration (Fig. 1) [60–62]. During the first pathway, homocysteine is remethylated in a process that requires methyltetrahydrofolate as a cosubstrate (methyl donor). This chain of reactions requires an adequate supply of folate and vitamin B₁₂ and functional integrity of the enzymes methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MS). In the reaction of *trans*-sulfuration, homocysteine is irreversibly transformed to cystathionine in a

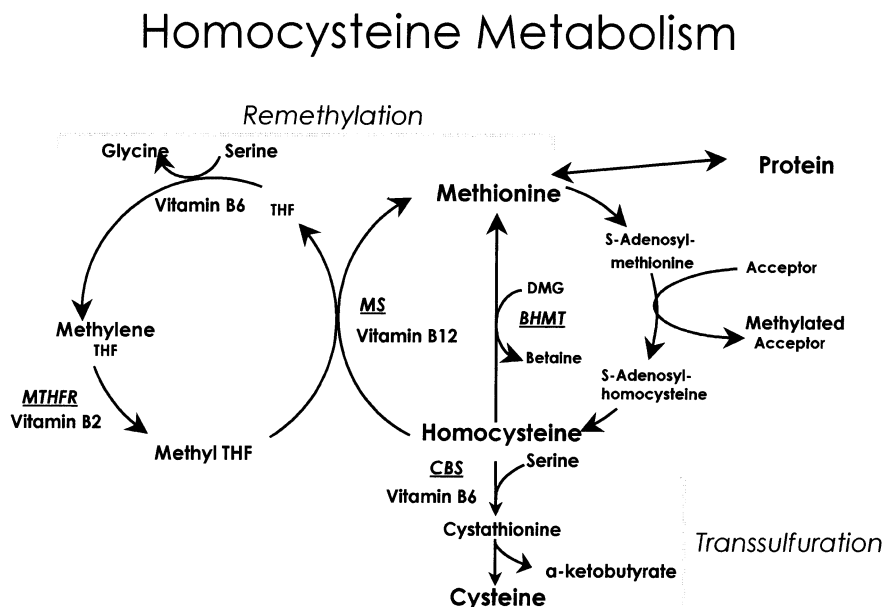


Fig. 1. Homocysteine metabolism: the major routes of homocysteine conversion (remethylation and *trans*-sulfuration) are shown. MTHFR: methylenetetrahydrofolate reductase; MS: methionine synthase; CBS: cystathionine β-synthase; BHMT: betaine-homocysteine methyltransferase; THF: tetrahydrofolate and DMG: dimethylglycine.

process requiring the vitamin B₆ derivative (pyridoxal 5-phosphate) as a co-factor. This reaction is catabolized by cystathionine β-synthase (CBS).

3.2. Determinants of hyperhomocysteinemia

The plasma concentration of tHcy is determined by several factors, both genetic and acquired [47,57,61–68]. The most important include: (1) genetic defects in the genes encoding for enzymes of homocysteine metabolism; CBS, MTHFR, or any of the enzymes participating in the synthesis of methylated vitamin B₁₂. Of these disorders, defects in the gene encoding for CBS, lead to the classic form of congenital homocystinuria, which leads to extreme plasma tHcy level and homocystinuria in homozygous patients or mild-moderate hyperhomocysteinemia in heterozygous subjects. Less severe hyperhomocysteinemia may also be due to a genetic variant of an enzyme, such the thermolabile variant, a mutation in the gene encoding MTHFR that has recently been described. This mutation (C677T) results in the substitution of an alanine residue by valine, rendering the enzyme both thermolabile and less active, which leads to an increase in tHcy levels especially in low folate conditions. (2) Aging, which could lead to an increase in levels of plasma tHcy, perhaps as a result of an age-dependant reduction of enzymatic metabolism or alteration of the renal function. (3) Gender; men have higher tHcy levels than women, probably due to greater muscle mass or different hormone patterns, levels in post-menopausal women approximate those in men. (4) Deficiency of nutritional factors such as folate, vitamin B₁₂ and vitamin B₆, which appear to have a close negative correlation with tHcy levels. (5) Finally, renal dysfunction, which is intimately related to hyperhomocysteinemia, as discussed later in this article.

These factors influencing plasma homocysteine levels must be kept in mind when analyzing results from various studies involving homocysteine. They may be important confounding factors if they vary between the different groups studied and if they are also potentially related to the outcome under the scope [69]. In practice, however, studies rarely control for genetic factors.

3.3. Measurement of homocysteine

Plasma tHcy measurement includes free homocysteine (which accounts for <1% of homocysteine in plasma), the oxidized forms of homocysteine and homocysteine–cysteine (20–30%), and the protein bound form (about 70–80%). Together, these forms consist of tHcy [47]. Several techniques are currently used to assess plasma tHcy levels. Most are based on chromatographic techniques; high-performance liquid chromatography (HPLC) is the most widely used [70,71].

The coefficient of variation of this technique is 1.1% within-assay, 2.1–11.4 between-assay and 3.2% within-pair quality-control specimens [14,18]. Recently, simple and relatively inexpensive immunoassays became also available. They may eventually allow a more widespread measurement of plasma homocysteine levels in clinical laboratories [72].

Plasma homocysteine levels are usually measured in the fasting state but can also be measured after a methionine load, a method that may be more sensitive for detecting mild disturbances. This test may uncover 39% of subjects with otherwise normal basal plasma levels of homocysteine [57,73].

The so-called ‘normal’ plasma homocysteine levels usually range from 5 to 15 μmol/l. Moderate, intermediate and severe hyperhomocysteinemia refer to levels ranging from 16 to 30, 31 to 100 and >100 μmol/l, respectively. However, the definition of hyperhomocysteinemia is not standardized, and differences may exist in the literature for the reference levels [74].

3.4. Hyperhomocysteinemia and cardiovascular disease in the general population

An elevated plasma level of homocysteine was first suspected to be associated with atherogenic and thrombogenic tendencies in patients with classic homocystinuria. This is a rare autosomal recessive disease caused in many cases by CBS deficiency that results in very high plasma tHcy levels (as high as 400 μmol/ml) and urinary homocysteine excretion. This markedly elevated plasma concentration of tHcy is associated with early onset atherosclerosis and life-threatening thrombotic episodes [61,62,75,76]. It has also been shown that milder degrees of hyperhomocysteinemia are associated with an increased risk of developing vascular disease as well.

Experimental studies (both in vivo and in vitro) have demonstrated that high plasma concentrations of tHcy may cause vascular damage and alteration in the coagulation process [77–103] (Table 1). Moreover, observational studies published mainly over the last decade have revealed that an elevated total plasma tHcy concentration is also associated with thrombogenic and cardiovascular disorders in the general population [14–57]. This association seems to be independent from other known risk factors in cross-sectional and case-control studies. The findings are less consistent in some of the prospective studies [104–111]. Nonetheless, in the meta-analysis of Boushey et al., an increase of 5 μmol/l in serum homocysteine enhanced the risk for CVD by 1.6- to 1.8-fold [29]. Furthermore, Omenn et al. [44] calculated a ‘best estimate’ for the increased risk of CAD mortality associated with elevated plasma levels of tHcy. The authors compared RR between homocysteine levels of >15 and <10 μmol/l, after

Table 1
Vascular effects associated with high levels of homocysteine

Atherogenic effects	Thrombogenic effects
Direct endothelium damage, due to homocysteine accumulation in endothelial cells	Reduced level of protein C, a natural anticoagulant involved in the modulation of factor V and VII activity in the coagulation cascade
Decrease bioavailable NO ^a	Inhibition of von Willebrand factor processing and secretion
Generation of free radicals and increase in oxidative stress	Increase in thromboxane synthesis
Increase in the production of Ox-LDL. ^b Enhanced uptake of Ox-LDL by macrophages and formation of foam cells in arterial wall	Platelet activation and aggregation
Dysfunction of the vascular endothelium	Attenuation of the antithrombotic properties of the endothelium
Increase proliferation of vascular smooth-muscle cells	Reduction in serum antithrombin activity and a reduction of TM ^c , which links thrombin
	Thrombin generation

^a Nitric oxide.

^b Oxidized low-density lipoprotein.

^c Thrombomodulin.

adjustment for other cardiovascular risk factors, and suggested that such risk difference was similar to that between total cholesterol levels of 7.1 and 4.9 mmol/l (275 and 189 mg/dl).

Thus, clinical, experimental, and epidemiological evidence all tend to suggest a link between plasma tHcy and CVD. However, the conclusion that an elevated plasma homocysteine level is an independent risk for CVD remains to be proven in controlled clinical trials demonstrating that a reduction in serum tHcy levels will reduce the risk of CVD. There are several ongoing randomized trials in US, Canada, Australia and Europe (≈ 60000 patients currently enrolled) examining the effects of lowering tHcy, by vitamin supplementation, on cardiovascular morbidity and mortality [57]. Before the conclusions of those trials will be published, caution is warranted in extrapolating epidemiological data, as suggested by the Canadian Task Force on Preventive Health Care [104].

In addition, the American Heart Association [112], the International Task Force on the Prevention of Cardiovascular Diseases [113] and the Canadian Cardiovascular Society [114] all agree that results of ongoing clinical studies must be obtained before making change in public health policy for screening and treatment of hyperhomocysteinemia. As declared by the Nutrition Committee of the American Heart Association, routine screening of hyperhomocysteinemia is not recommended for the purpose of risk assessment on a population-wide basis and emphasis should be placed on meeting current recommended daily allowance (RDAs) for folate, vitamin B₆ and vitamin B₁₂, provided by a balanced diet focusing on fruit and vegetables, legumes, meat, fish and poultry as well as grain products [112].

The recent complementary approach instituted by the US Food and Drug Administration and Canadian Government, requiring that all enriched cereal grains

products be fortified with folic acid may eventually help in maintaining lower tHcy concentration in the general population and also the prevalence of hyperhomocysteinemia due to vitamin deficiency. In fact, a study of the Framingham Offspring cohort recently showed substantial increase in folate status, significant reduction in homocysteine levels as well as a reduction in the prevalence of hyperhomocysteinemia of almost 50% by fortified enriched grain products [115].

In contrast to what has been stated for the general population, the Nutrition Committee of the AHA and the Writing Group I of the Prevention Conference V, suggest that screening of hyperhomocysteinemia may be advisable in selected high-risk group of patients, e.g., with personal or family history of premature CVD without other known risk factors [13,112].

Simple, safe, inexpensive and easily available treatment can decrease homocysteine levels. Most patients respond to multivitamin therapy within 2–6 weeks, irrespective of the cause of elevated tHcy levels. In addition, folic acid alone or combined with vitamin B₁₂ or B₆, reduces plasma tHcy levels even in persons who are not frankly vitamin deficient [57].

3.5. Renal function and homocysteine

Impaired renal function is associated with high plasma homocysteine concentrations. In fact, levels of tHcy are 2- to 4-fold higher in patients with chronic renal failure (CRF) than in the general population [116]. Although, there are also possibly extrarenal disturbances (presumably hepatic) in the metabolism of homocysteine due to uremic environment in CRF, impaired renal function is a particularly important determinant of homocysteine levels [117]. In normal kidneys, plasma amino acids are first filtered in glomeruli then reabsorbed almost completely in tubules and finally degraded in renal parenchyma via *trans*-methylation

and *trans*-sulfuration [118]. Bostom showed that degradation in renal tissue following tubular reabsorption of tHcy accounts for a major fraction of total clearance of plasma homocysteine in the rat [119]. It was therefore suggested that the reduced metabolic capacity of the kidney tissue might be rate limiting for renal tHcy clearance and might explain the elevation of plasma tHcy seen in human end stage renal disease [65,120,121]. Wollesen et al. demonstrated that GFR is an independent determinant of plasma tHcy concentration and that it is rate limiting for renal clearance of homocysteine in diabetic patients without overt nephropathy [122]. They found that impaired renal function explains the increase in plasma tHcy (independently of age, serum vitamins, serum creatinine and albuminuria) a result that is consistent with other work [123–140].

The relative hyperfiltration secondary to renal hyperperfusion, which occurs in early diabetes, may lead to increased homocysteine catabolism and may explain the lower than normal mean plasma tHcy in populations of diabetic patients, consistent with the findings of Wollesen et al. [122]. As microalbuminuria is associated with early renal dysfunction, one could expect this marker to be also associated with hyperhomocysteinemia. Hence, it was reported in some studies that microalbuminuric DM patients had higher tHcy values compared with normoalbuminuric patients [141–143]. This positive relationship was independent of diabetes and hypertension according to Hoogeveen et al.'s findings [141]. Other investigators, however, have reported that microalbuminuria alone had no effect on plasma tHcy [122,132].

Finally, a substantial number of retrospective [65,131,133–135,138,144] studies of patients with CRF, with and without dialysis or renal transplant, suggest that hyperhomocysteinemia is strongly associated with CVDs. Same results were found by few prospective studies [145–147]. However, the important topic of hyperhomocysteinemia and CVD in patients with renal dysfunction of different origin is not directly under the scope of the present review.

3.6. Cardiovascular disease in diabetic patients

DM is associated with a sharp increased risk of CVD and mortality [7–10,148,149]. It is estimated that 75% of all non-insulin-dependant DM (NIDDM) patients in the United States die from cardiovascular complications [148]. Additionally, people with type II diabetes develop CVD at a younger age, have a higher rate of diffuse multivessel disease with impaired coronary vasodilatory reserve, and are more likely to develop congestive heart failure (CHF). They also have poorer outcomes after their first and subsequent MI,

with higher rates of both CHF and death in the post-infarct period [12,150,151].

Mechanisms underlying the accelerated atherosclerosis in DM patients are not fully understood. Increased body weight (particularly central adiposity), altered lipid metabolism (hypertriglyceridemia, decreased HDL cholesterol level and presence of small dense LDL cholesterol), hypertension and nephropathy may all contribute, but these adverse cardiovascular risk factors cannot fully explain the excess in cardiovascular morbidity and mortality [152,153]. Diabetes doubles the risk of CVD even when controlled for major known risk factors [7,149]. Gerstein et al. has shown that a moderately elevated glucose level is a continuous risk factor for MI even in non-diabetic patients with either normal or impaired glucose tolerance [154].

It has been demonstrated that extended exposure to hyperglycemia results in the glycosylation of extracellular matrix proteins, which lead to the formation of advanced glycosylated end products that induce cross-linking of collagen and extracellular matrix proteins, in many tissues, including the arterial vessel wall. This also accelerates generation of reactive oxygen species, which increase oxidative stress [155,156]. Consequently, diabetic patients present a decreased synthesis and an increased inactivation of endothelial derived NO. In addition, vascular endothelial cells and lipoproteins in the arterial wall are subject to oxidative modifications. This leads to increased vessel stiffness, Ox-LDL binding by scavenger receptors on macrophages, foam-cell uptake of Ox-LDL, secretion of platelet-derived growth factor and proliferation of vascular smooth-muscle cells. Moreover, these modifications induce endothelial dysfunction and alteration in haemorrhological characteristics, favoring a prothrombotic state (increased platelet adhesion/aggregation, increased fibrinogen and plasminogen-activator-inhibitor-1 (PAI-1) concentrations). All of these mechanisms may contribute to accelerated atherogenesis and increased CAD events seen in patients with diabetes [157–170].

It also has been shown that in DM the development of CVD as a consequence of macroangiopathy is often associated with preceding nephropathy [171–173]. Plasma homocysteine is increased in renal insufficiency of different origin [123–140]. This has led to the suggestion that hyperhomocysteinemia, resulting from an impairment of renal function, may contribute to the marked increase in cardiovascular morbidity and mortality observed in DM patients. The hypothesis that homocysteine may produce endothelial cell damage in vessels exposed to advanced glycation end products *in vivo* has also been suggested by Hofmann et al. [174]. It is possible that in diabetic patients with macrovascular disease, these abnormalities may in part be mediated or exacerbated by hyperhomocysteinemia.

3.7. Homocysteine levels in diabetic patients

In spite of their higher prevalence of CVD compared with non-diabetic people, the mean plasma tHcy concentration is usually low or normal in insulin-dependant DM (IDDM) and NIDDM patients, except when nephropathy or impaired renal clearance is present [100–102,107,122,128,132,142,175,176,186,187]. Hofmann et al. [174] and Chico et al. [143] in contrast found elevated tHcy in NIDDM patients, but in both studies, the vast majority ($\approx 80\%$) of diabetic patients with fasting elevated tHcy presented nephropathy. As discussed previously, the impairment of renal function seen in diabetic patients appears to be the principal modulator of the homocysteine levels.

Abnormally low levels of plasma tHcy might also be an effect of abnormal homocysteine metabolism in diabetics due to endogenous or exogenous factors. Since insulin has profound effects on amino acid metabolism, it has been suggested that an acquired defect in homocysteine metabolism may occur in diabetic patients [177]. Fonseca et al. [178] studied the effect of acute hyperinsulinemia on plasma tHcy concentrations and demonstrated that plasma tHcy concentrations were not regulated by acute hyperinsulinemia. Additionally, in the study of Araki et al. [179], there was no indication that insulin or sulfonylureas alter tHcy metabolism. In contrast, metformin may induce vitamin B₁₂ malabsorption and thereby increase the serum tHcy level [180,181]. Nevertheless, Hoogeveen et al. has not found an important effect of metformin on serum tHcy levels in subjects with NIDDM [182].

3.8. Hyperhomocysteinemia and cardiovascular disease in diabetes mellitus

3.8.1. Cross-sectional and case-control studies

A recent Japanese study of 145 men and woman, without overt renal dysfunction (serum creatinine $\leq 114.9 \mu\text{mol/l}$ or 1.3 mg/dl), admitted for coronary angiogram to evaluate suspected CAD [183], reported that homocysteine correlated strongly with the CAD score only in the group of DM patients (approximately one third of patients). Other variables such as age, BMI, creatinine and lipid profile did not correlate with the CAD score in either group. Also, the CAD score for patients with elevated tHcy was higher than in patients with normal homocysteine levels. Highest results were found in DM patients with hyperhomocysteinemia. Age-adjusted OR of coronary artery stenosis in patients with elevated tHcy relative to patients with normal tHcy was 2.0. In the DM group, this ratio was increased up to 6.6. In addition, logistic regression analysis adjusting for age, CV risk factors and creatinine, showed that plasma tHcy level was indepen-

dently associated with the CAD score in the DM group, but not in the non-DM group. These results suggest there might exist an independent relationship between hyperhomocysteinemia and increased risk of coronary artery disease in patients with DM. Furthermore, it also suggests that DM may interact with homocysteine conferring a multiplicative risk of CAD. Nevertheless, this cross-sectional study did not control for vitamin status, an important determinant of homocysteine level and a possible independent determinant of cardiovascular status [24,32,46,107,184–189]. It may be a particularly important confounding factor when a group of DM patients are compared to non-DM subjects, since the nutritional composition of their diet may have noticeable differences. This study was also made with a relatively small number of diabetic patients; which also lessens the strengths of the conclusions (Table 2).

Another recent study, with a comparable number of subjects but all stable DM patients without major renal dysfunction (serum $< 130 \mu\text{mol/l}$ or 1.5 mg/dl), showed significantly higher fasting tHcy levels in subjects with history of CAD vs those without CAD [190]. Additionally, the prevalence of CAD was 19% in the lower quartile of fasting tHcy vs 55% in the top quartile, and there was a significant increase in the prevalence of CAD with increasing quartile of fasting Hcy. The calculated OR for CAD in the highest quartile vs the lower quartile was 5. Moreover, after adjustment for age and family history, fasting tHcy was independently associated with coronary artery disease in regression analysis. However, important variables were not included in the statistical model, such as sex, vitamin status (folate, vitamin B₁₂ and pyridoxine), as well as clearance of creatinine. Those variables were correlated with homocysteine levels, and could have been important confounding factors of the outcomes. Finally, the absence of a group of non-diabetic patients limits our interpretation regarding the extent to which determinants of the interaction between hyperhomocysteinemia and CVD may behave in a manner that is typical for DM patients.

An additional study from the Netherlands [191], examined 631 adults of the city of Hoorn (of which 173 had DM and 170 were glucose intolerant) who were randomly sampled. It was shown that a $5\text{-}\mu\text{mol/l}$ increment of serum tHcy is associated with an increased risk of CVD. The adjusted OR (for age, sex, CV risk factors and creatinine) was 1.4, which was of similar magnitude in each of the vascular beds examined (coronary, peripheral and cerebral). After stratification by glucose tolerance category, the adjusted ORs per $5\text{-}\mu\text{mol/l}$ increment in serum tHcy of CVD were 1.4 in normal glucose tolerance group, 1.6 in impaired glucose tolerance group and 2.3 in DM patients. Although this was a cross-sectional study, which cannot resolve the tem-

poral and causal relationship between homocysteine concentration and CVD and that vitamin status was once more not included in the statistical model, these results also tend to suggest that high serum tHcy is a stronger (1.6-fold) predictor of risk for CVD in DM patients than in patients with normal or impaired glucose tolerance.

Munshi et al. [192] reported a case-control study of 28 DM men under 60 years old, without renal dysfunction (serum creatinine ≤ 125 $\mu\text{mol/l}$ or 1.4 mg/dl) or vitamin deficiency compared to 18 healthy volunteers. It was shown that post-methionine load (PML) hyperhomocysteinemia occurred with significantly greater frequency in DM patients than in controls. Peak plasma homocysteine after a methionine load was also more often elevated in DM patients with CVD than without

CVD, although not significantly. The calculated OR for CVD (coronary, peripheral and cerebral) was 1.5 for DM patients with elevated tHcy vs DM without elevated tHcy. Peak and 24-h plasma homocysteine following a methionine load were also significantly higher in DM patients with CVD than in controls but no significant difference was found between the groups of diabetics with CVD vs those without CVD (data not shown). This study was conducted with a very small number of subjects, however, which lessens the power. It is possible that the difference in homocysteine levels in DM patients with macrovascular disease vs those without could have been statistically significant with a greater number of patients or if basal levels of tHcy have been used instead of PML. Some DM patients without overt vascular disease and normal basal tHcy

Table 2
Cross-sectional and case-control studies of homocysteine and CVD in DM

Reference	Participants (N)	Age (year) ^a	tHcy ($\mu\text{mol/l}$) ^b	Main results
[183]	145 (46 DM, all ♂)	64.1 \pm 10.3	13.1 \pm 4.0	OR (age-adjusted) = 2.013 ($P = 0.042$) for ≥ 1 stenotic (75%) artery in all patients with elevated ^c tHcy vs normal tHcy. OR = 6.632 ($P = 0.065$) for the DM group and OR = 1.566 ($P = 0.191$) in the non-DM group In stepwise logistic regression analysis ^d , tHcy was independently associated with CAD score in DM group, $P = 0.026$ (not in non-DM group)
[190]	150 (all DM)	56.6 \pm 10.2	No CAD 10.0 (4–32) CAD 13.0 (7–26) ($P = 0.02$)	Prevalence of CAD was 19.4% in lowest quartile of fasting Hcy vs 55% in highest quartile, with stepwise increase in prevalence of CAD with increasing quartile ^e ($P < 0.01$ for trend) OR (calculated) = 5.078, for CAD in the highest quartile vs lower
[191]	631 (NGT ^g : 288; IGT ^h : 170; DM: 173)	64.3 \pm 7.2	11.4 (9.4–14.1)	In multiple regression analysis ^f , fasting Hcy was independently associated with presence of CAD ($P = 0.02$) Adjusted ⁱ OR = 1.39 (1.15, 1.68) for CVD, per 5- $\mu\text{mol/l}$ increment of tHcy. OR = 1.38 (1.03–1.85) in NGT, OR = 1.55 (1.01–2.38) in IGT, and OR = 2.33 (1.11–4.90) in DM ($P = 0.07$ for interaction)
[192]	28 DM (all ♂)	47.9 \pm 7	DM+CVD: 9.5 \pm 2.5 DM No CVD: 9.3 \pm 3.2	PML ^j tHcy was elevated ^k in 32% of DM patients (27% without CVD and 35% with CVD) vs 5.5% of healthy controls ($P < 0.02$) OR (calculated) = 1.455, of CVD for DM with elevated PML tHcy vs DM without elevated PML tHcy
[179]	18 CTRL (all ♂) 136 DM	42.8 \pm 4 64.4 \pm 10.5	CTRL 9.2 \pm 3.7 DM+CVD: 10.8 \pm 3.8 DM No CVD: 8.3 \pm 3.1 ^m	In DM patients, multiple logistic regression analysis ^l showed that log-transformed tHcy was associated with CVD ($P = 0.010$)
	90 CTRL	64.6 \pm 9.8	CTRL 7.5 \pm 2.1 ^m	

^a Mean \pm S.D.

^b Mean \pm S.D. or Median (range).

^c > 14 $\mu\text{mol/l}$.

^d Adjusted for age, HTN, current smoking, BMI, total cholesterol, HDL, LDL, Lp(a), fibrinogen, creatinine.

^e < 9 , 9–11, 11–14, > 14 .

^f Adjusted for age and family history of CAD.

^g Normal glucose tolerance.

^h Impaired glucose tolerance.

ⁱ Adjusted for age, sex, HTN, hypercholesterolemia, smoking and creatinine.

^j Post-methionine load.

^k $>$ control + 2 S.D.

^l Adjusted for age, sex, systolic blood pressure and serum creatinine.

^m $P = 0.001$ vs DM with CVD.

levels can have high plasma Hcy concentration following a methionine load and it is possible that these patients have not had time to express early clinical signs of the vascular disease.

Conversely, a somewhat larger case-control study from Japan [179] showed that diabetic patients with CVD (coronary, peripheral and cerebral) had significantly higher plasma levels of tHcy than DM patients without macroangiopathy or non-DM patients. After excluding patients with nephropathy (proteinuria ≥ 30 mg/dl and/or creatinine ≥ 1.2 mg/dl or $106 \mu\text{mol/l}$), the plasma levels of tHcy in patients with CVD remained significantly increased compared to those without CVD (10.6 ± 3.6 vs $8.1 \pm 3.0 \mu\text{mol/l}$, $P < 0.001$). Also, following adjustment for the effect of age and sex, log-transformed tHcy levels were significantly associated with CVD in a logistic regression analysis. When adjusting for additional confounders (blood pressure and creatinine) the multivariate analysis showed that log-transformed tHcy was still a significant independent predictor for the presence of CVD in DM patients. According to the authors, these results support the hypothesis that hyperhomocysteinemia is associated with CVD and it is independent of other risk factors of atherosclerosis in DM. However, here we cannot compare the risk of DM patients with hyperhomocysteinemia, to the risk of non-DM patients with elevated tHcy. In addition, as in other retrospective studies, the design does not resolve the causal and temporal relationship between elevated tHcy and CVD in DM patients. Furthermore, since we do not have the baseline characteristics of the subjects and controls according to their vitamin status, it should have been included in the regression analysis as a potential confounder.

3.8.2. Prospective studies

The Hoorn population-based study used a prospective case-control design [193] to examine blood samples of 171 patients who died, within a 5-year follow-up period, from the initial cohort of 2484 citizens, and 640 controls who survived in a sample matched for age, sex and glucose tolerance. In the overall group, there were 184 patients with DM, and 63% of them had newly diagnosed diabetes at the beginning of the study. The adjusted OR (for age, sex, CV risk factor serum albumin and HbA1c) for 5-year mortality associated with elevated tHcy was 1.6. It was 1.3 in non-DM patients compared with 2.5 in DM patients. The investigators thus concluded that hyperhomocysteinemia was a stronger predictor of mortality in diabetic than in non-diabetic subjects (1.9-fold), independent of other conventional risk factors. Additionally, adjusted OR for 5-year mortality was also higher in cases of known diabetes than in cases of newly diagnosed DM. Vitamin status and renal function were, however, not part of the multivariate analysis. When looking at the 5-year mor-

tality for each $5\text{-}\mu\text{mol/l}$ increment in serum creatinine, the OR was 1.6 in DM patients, vs 1.2 for non-DM patients. Because, of the limited number of cases, interaction of hyperhomocysteinemia and diabetes with on CVD mortality was not investigated. The other CVD events were not examined neither (Table 3).

Another Scandinavian study, with 211 DM patients followed for 6.4 years [194], showed that the all cause mortality rate per 100 person-years was 3.9 (not shown), and it was significantly higher in the highest tertile (7.7 per 100 person years) of tHcy than in the middle (2.4 per 100 person years) and lowest tertile (2.2 per 100 person years) ($P < 0.001$, high vs middle and high vs low). Cumulative all-cause and CVD mortality were also significantly higher in the highest tertile than in the middle or the lowest tertile of tHcy. Plasma tHcy was significantly and independently associated with all cause and CVD mortality in univariate and age-adjusted regression analysis (not shown). However, in the Cox multiple regression analysis adjusted for age, sex, various factors related to renal function and CVD risk factors (but not for vitamin status) the plasma tHcy appeared to be a significant predictor for all cause mortality only. In fact, adjusted RR for all-cause mortality per $1 \mu\text{mol/l}$ increased level of tHcy was 1.1. It was thus calculated that for each $5\text{-}\mu\text{mol/l}$ tHcy increase (≈ 1 S.D. in most populations), the total mortality increased by $\approx 50\%$ in this DM population. There was only 30 CVD death in the cohort studied. It is well known that small number of cases always give a less precise estimate and a wider confidence interval. Therefore, if a composite end point has been created for CVD mortality and other CVD events in the present and the former studies, the authors would possibly have reached a sufficient number of cases to demonstrate a significant relation between elevated levels of homocysteine and composite CVD events in diabetic patients.

In a large study of 1788 residents (239 with diabetes) of Jerusalem [195], with a follow up of ≈ 10 years, it was demonstrated that despite lower mean tHcy concentration in diabetes, values above the median ($> 10 \mu\text{mol/l}$) were a risk marker for death (data not shown). Furthermore, among diabetic patients there was a graded association of quartiles of tHcy with age and sex-adjusted total mortality with the RR reaching 2.5 in the top quartile. The association was shown to persist, but was somewhat reduced with additional adjustment for CV risk factors, creatinine and albumin. Vitamin status was again not considered here in the multivariate analysis despite it might be a potential confounder. The authors affirmed that the association was similar for CVD mortality as it was for total mortality, but the results were not stated in the article. They did not mention the other CVD events. In addition, despite the presence of a large group of non-DM patients in the present cohort, a comparison between patients with and

Table 3
Prospective studies of homocysteine and mortality in DM

Reference	Follow-up (year)	Participants (N)	Age (year) ^a	tHcy (μmol/l) ^a	Main results
[193]	5.0	Cases ^b 171 (38 DM)	66.6 ± 7.1	12.9 (9.9, 16.2)	Adjusted ^c OR = 1.56 (1.07, 2.3) for 5-year mortality associated with elevated ^d tHcy. OR = 1.34 (0.87–2.06) for non-DM group, and OR = 2.51 (1.07–5.91) in DM patients, <i>P</i> = 0.08 for interaction. Adjusted ^c OR = 3.18 (0.74–13.74) for 5-year mortality of patients with elevated tHcy and known DM (<i>N</i> = 69) vs OR = 2.58 (0.90–7.40) for patients with newly diagnosed DM (<i>N</i> = 115) (<i>P</i> = 0.04).
[194]	6.4	CTRL ^f 640 (146 DM) 211 (all DM)	63.9 ± 7.0 58 ± (23–69)	11.5 (9.4, 14.1) 7.0 (2.4–46.7)	OR = 1.17 (0.92–1.5) for 5-year mortality for each 5-μmol/l increment of serum tHcy in non-DM, and OR = 1.60 (1.02–2.51) in DM subjects. NGT and IGT were pooled. Cumulative all-cause mortality was 44, 14 and 15% in decreasing tertile ^g of tHcy (<i>P</i> < 0.01, high vs middle and high vs low). Cumulative CVD mortality was 24, 9 and 11% in decreasing tertile of tHcy (<i>P</i> < 0.05, high vs middle and high vs low). RR = 1.09 (1.03–1.14) for all cause mortality per 1 μmol/l increment in tHcy, in Cox multiple regression analysis ^h , RR = 1.5 (calculated) per 5 μmol/l increment in tHcy. RR = 1.03 (0.93, 1.14) for CVD mortality per 1 μmol/l increment in tHcy in Cox multiple regression analysis ^h .
[195]	9–11	1788 (239 DM)	65.5 ± ?	DM ⁱ : ♂ 10.9, ♀ 9.8; no DM: ♂ 12.3, ♀ 10.8	For all cause mortality, in Cox proportional hazard analysis ^j , RR = 2.5 (1.17–5.29), 2.22 (1.06–4.64), 1.26 (0.57–2.79), 1.00 for DM patients, for each decreasing quartile ^k of tHcy (<i>P</i> = 0.0056, for trend).

^a Mean ± S.D. or Median (IQR).

^b Subjects who died during 5-year follow-up.

^c Adjusted for age, sex, hypertension, hypercholesterolemia, current smoking, serum albumin and HBA1c.

^d > 14 μmol/l.

^e Adjusted for age and sex.

^f Survivors taken from a random sample (age, sex and glucose stratified) of the initial cohort.

^g ≥ 8.2, 6.2–8.1 and ≤ 6.1 μmol/l.

^h Adjusted for age, sex, BMI, duration of DM, creatinine, Ccr, Log₁₀ albumin excretion rate, total and HDL cholesterol, HBA1c, systolic and diastolic BP, pre-existing CHD and smoking.

ⁱ DM vs no DM, *P* < 0.05, for both sexes.

^j Adjusted for age and sex.

^k ≥ 13.02, 10.01–13.02, 8.2–10.0, < 8.2.

without DM, for their risk of mortality according to their levels of tHcy was not presented.

The median plasma tHcy concentrations in the two previous populations were very low compared to other study populations. Lower levels of tHcy are suspected to occur in early diabetes secondary to hyperfiltration [122]. We may thus hypothesize here that a relatively large number of patients with DM did not have a very advanced disease. It was also the case in the Hooegeven et al. study, since about two third were new cases of diabetes. This may explain, at least in part, why it may have been harder to show a significant effect of elevated homocysteine coupled with the presence of recent diabetes on cardiovascular mortality. Additionally, it may have been more difficult to obtain an adequate number of patients with sufficiently high levels of tHcy to demonstrate a relation between high homocysteine levels and CVD mortality in DM patients.

4. Discussion

The purpose of this review was to explore the relationship between plasma levels of homocysteine and CVD in diabetic patients. An independent association was suggested by cross-sectional and case-control studies. Moreover, this association seemed to be stronger in diabetic subjects than in non-diabetics. This suggests that presence of elevated levels of homocysteine and diabetes at the same time may confer a synergistic risk of CVD. Prospective studies suggested a stronger causal relationship between hyperhomocysteinemia and all cause mortality in DM than in non-DM patients, but the causal relationship was less clear for cardiovascular morbidity and mortality.

To date, there are no prospective study that has examined the relationship between elevated levels of homocysteine and the presence of CVD in the DM population. Large-scale epidemiological studies are underway in patients with type II diabetes. It is hoped that the authors will consider measuring homocysteine levels, especially in light of recent data showing that some lipid modifying medications, especially the fibric acid derivatives, increases plasma homocysteine levels [196–200].

The biological mechanism for the interaction between diabetes and elevated homocysteine with regard to CVD is still not well known. Previous data suggested that it was biologically plausible that elevated plasma levels of homocysteine may enhance atherogenic and/or thrombogenic pathways, but a considerable number of experimental studies were conducted in artificial and non-physiologic conditions. However, it may also accelerate the direct cytotoxic effects of glucose and the oxidative modification of glucose to endothelial cells. Furthermore, the cumulative effect of diabetes and

elevated homocysteine levels might be of more importance in DM patients with impaired renal function, since it has been shown that diabetic patients with reduced glomerular filtration have higher levels of plasma tHcy.

Thus, because of the particularly enhanced risk of CVD in DM patients, interaction with hyperhomocysteinemia may have consequences with regard to management of primary and secondary prevention in these patients. Finally, recognition of hyperhomocysteinemia may be relevant since it is potentially reversible by a number of therapeutic agents, including folic acid, vitamin B₁₂ and pyridoxine. Additional studies are thus necessary to establish prospectively the presence of a multiplicative risk of CVD with elevated homocysteine levels in DM patients as well as studies assessing the effect of vitamin treatment on plasma levels of homocysteine and CVD outcomes in this population.

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References

- [1] Grundy SM, Benjamin IJ, Burke GI, et al. Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. *Circulation* 1999;100(10):1134–46 Review.
- [2] Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation* 1998;97(18):1837–47.
- [3] Wilson PW. Diabetes mellitus and coronary heart disease. *Am J Kidney Dis* 1998;32(5):S89–S100.
- [4] Kannel WB, McGee DL. Diabetes and cardiovascular risk factors: the Framingham study. *Circulation* 1979;59:8–13.
- [5] Kannel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. *Diabetes Care* 1979;2(2):120–6.
- [6] Kannel WB, McGee DL. Diabetes and cardiovascular disease: the Framingham study. *JAMA* 1979;241(19):2035–8.
- [7] Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the multiple risk factor intervention trial. *Diabetes Care* 1993;16(2):434–44.
- [8] Brand FN, Abbott RD, Kannel WB. Diabetes, intermittent claudication, and risk of cardiovascular events. The Framingham study. *Diabetes* 1989;38(4):504–9.
- [9] The World Health Organization Multinational Study of Vascular Disease in Diabetics. Diabetes Drafting Group. Prevalence of small vessel and large vessel disease in diabetic patients from 14 centers. *Diabetologia* 1985; 28 (Suppl):615–40.
- [10] Manson JE, Colditz GA, Stampfer MJ, et al. A prospective study of maturity-onset diabetes mellitus and risk of coronary heart disease and stroke in women. *Arch Intern Med* 1991;151(6):1141–7.

- [11] Haffner SM, Lehto S, Ronnema T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998;339(4):229–34.
- [12] Miettinen H, Lehto S, Salomaa V, et al. Impact of diabetes on mortality after the first myocardial infarction. The FINMONICA myocardial infarction register study group. *Diabetes Care* 1998;21(1):69–75.
- [13] Grundy SM, Bazzarre T, Cleeman J, et al. Prevention conference V: beyond secondary prevention: identifying the high-risk patient for primary prevention: medical office assessment: writing group I. *Circulation* 2000;101(1):E3–E11.
- [14] Malinow MR, Kang SS, Taylor LM, et al. Prevalence of hyperhomocyst(e)inemia in patients with peripheral arterial occlusive disease. *Circulation* 1989;79(6):1180–8.
- [15] Genest J Jr, McNamara JR, Salem DN, Wilson PF, Schaefer EJ, Malinow MR. Plasma homocyst(e)ine levels in men with premature coronary artery disease. *JACC* 1990;16:1114–9.
- [16] Malinow MR. Hyperhomocyst(e)inemia. A common and easily reversible risk factor for occlusive atherosclerosis. *Circulation* 1990;81(6):2004–6. Published erratum appears in *Circulation* 1990;82(4):1547.
- [17] Coull BM, Malinow MR, Beamer N, Sexton G, Nordt F, de Garmo P. Elevated plasma homocyst(e)ine concentration as a possible independent risk factor for stroke. *Stroke* 1990;21(4):572–6.
- [18] 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(7):877–81.
- [19] Brattstrom L, Lindgren A, Israelsson B, et al. Hyperhomocysteinemia in stroke: prevalence, cause, and relationships to type of stroke and stroke risk factors. *Eur J Clin Invest* 1992;22(3):214–21.
- [20] Rees MM, Rodgers GM. Homocysteinemia: association of a metabolic disorder with vascular disease and thrombosis. *Thromb Res* 1993;71(5):337–59 Review.
- [21] 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(4):1107–13.
- [22] von Eckardstein A, Malinow MR, Upson B, Heinrich J, Schulte H, Schönfeld G, 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(3):464.
- [23] Malinow MR, Stampfer MJ. Role of plasma homocyst(e)ine in arterial occlusive diseases. *Clin Chem* 1994;40(6):857–8 Editorial.
- [24] Selhub J, Jacques PF, Bostom AG, et al. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *N Engl J Med* 1995;332(5):286–91.
- [25] 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(8987):1395–8.
- [26] Nygard O, Vollset SE, Refsum H, et al. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. *JAMA* 1995;274(19):1526–33.
- [27] Dalery K, Lussier-Cacan S, Selhub J, Davignon J, Latour Y, Genest J Jr. Homocysteine and coronary artery disease in French Canadian subjects: relation with vitamins B₁₂, B₆, pyridoxal phosphate, and folate. *Am J Cardiol* 1995;75(16):1107–11.
- [28] Arnesen E, Refsum H, Bonna KH, Ueland PM, Forde OH, Nordrehaug JE. Serum total homocysteine and coronary heart disease. *Int J Epidemiol* 1995;24(4):704–9.
- [29] Boushey CJ, Beresford SA, 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(13):1049–57.
- [30] Hopkins PN, Wu LL, Wu J, Hunt SC, James BC, Vincent GM, et al. Higher plasma homocyst(e)ine and increased susceptibility to adverse effects of low folate in early familial coronary artery disease. *Arterioscler Thromb Vasc Biol* 1995;15(9):1314–20.
- [31] 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(10):2825–30.
- [32] Selhub J, Jacques PF, Bostom AG, et al. Relationship between plasma homocysteine, vitamin status and extracranial carotid-artery stenosis in the Framingham study population. *J Nutr* 1996;126:1258S–65S.
- [33] Malinow MR, Ducimetiere P, Luc G, et al. Plasma homocyst(e)ine levels and graded risk for myocardial infarction: findings in two populations at contrasting risk for coronary heart disease. *Atherosclerosis* 1996;126(1):27–34.
- [34] Malinow MR. Plasma homocyst(e)ine: a risk factor for arterial occlusive diseases. *J Nutr* 1996;126(Suppl 4):1238S–43S Review.
- [35] van den Berg M, Stehouwer CD, 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.
- [36] 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(9035):1120–4.
- [37] Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 1997;337(4):230–6.
- [38] 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(22):1775–81.
- [39] Aronow WS, Ahn C, Schoenfeld MR. Association between plasma homocysteine and extracranial carotid arterial disease in older persons. *Am J Cardiol* 1997;79(10):1432–3.
- [40] Aronow WS, Ahn C. Association between plasma homocysteine and coronary artery disease in older persons. *Am J Cardiol* 1997;80(9):1216–8.
- [41] Konecky N, Malinow MR, Tunick PA, et al. Correlation between plasma homocyst(e)ine and aortic atherosclerosis. *Am Heart J* 1997;133(5):534–40.
- [42] Alfthan G, Aro A, Gey KF. Plasma homocysteine and cardiovascular disease mortality. *Lancet* 1997;349(9049):39 Letter.
- [43] Joubran R, Asmi M, Busjahn A, Vergopoulos A, Luft FC, Jouma M. Homocysteine levels and coronary heart disease in Syria. *J Cardiovasc Risk* 1998;5(4):257–61.
- [44] Omenn GS, Beresford SA, Motulsky AG. Preventing coronary heart disease: B vitamins and homocysteine. *Circulation* 1998;97:421–4.
- [45] 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(8):862–7.
- [46] Robinson K, Arheart K, Refsum H, et al. Low circulating folate and vitamin B₆ concentrations: risk factors for stroke, peripheral vascular disease, and coronary artery disease. European COMAC group. *Circulation* 1998;97(5):437–43.
- [47] Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. *Annu Rev Med* 1998;49:31–62.
- [48] Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998;338(15):1042–50 Review.

- [49] Stehouwer CD, Weijnenberg MP, van den Berg M, Jakobs C, Feskens EJ, Kromhout D. Serum homocysteine and risk of coronary heart disease and cerebrovascular disease in elderly men: a 10-year follow-up. *Arterioscler Thromb Vasc Biol* 1998;18(12):1895–901.
- [50] Bostom AG, Silbershatz H, Rosenberg ICH, et al. Nonfasting plasma total homocysteine levels and all-cause and cardiovascular disease mortality in elderly Framingham men and women. *Arch Int Med* 1999;159:1077–80.
- [51] Yoo J-H, Park J-E, Hong K-P, et al. Moderate hyperhomocyst(e)inemia is associated with the presence of coronary artery disease and the severity of coronary arteriosclerosis in Koreans. *Thromb Res* 1999;94:45–52.
- [52] Taylor LM, Moneta GL, Sexton GJ, Schuff RA, Porter JM, et al. Progressive blinded study of the relationship between plasma homocysteine and progression of symptomatic peripheral arterial disease. *J Vasc Surg* 1999;28:8–21.
- [53] Whincup PH, Refsum H, Perry IJ, et al. Serum total homocysteine and coronary heart disease: prospective study in middle aged men. *Heart* 1999;82(4):448–54.
- [54] Kark JD, Selhub J, Adler B, et al. Nonfasting plasma total homocysteine level and mortality in middle-aged and elderly men and women in Jerusalem. *Ann Intern Med* 1999;131(5):321–30.
- [55] Bostom AG, Rosenberg IH, Silbershatz H, et al. Nonfasting plasma total homocysteine levels and stroke incidence in the elderly persons: the Framingham study. *Ann Intern Med* 1999;131(5):352–5.
- [56] Bots ML, Launer LJ, Lindemans J, et al. Homocysteine and short-term risk of myocardial infarction and stroke in the elderly: the Rotterdam study. *Arch Intern Med* 1999;159(1):38–44.
- [57] Eikelboom JW, Lonn E, Genest J Jr, Hankey G, Yusuf S. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. *Ann Intern Med* 1999;131(5):363–75.
- [58] Grundy SM, Pasternak R, Greenland P, Smith S Jr, Fuster V. AHA/ACC scientific statement: assessment of cardiovascular risk by use of multiple-risk-factor assessment equations: a statement for healthcare professionals from the American Heart Association and the American College of Cardiology. *J Am Coll Cardiol* 1999;34(4):1348–59 Review.
- [59] Harjai KJ. Potential new cardiovascular risk factors: left ventricular hypertrophy, homocysteine, lipoprotein(a), triglycerides, oxidative stress, and fibrinogen. *Ann Intern Med* 1999;131(5):376–86 Review.
- [60] Finkelstein JD. The metabolism of homocysteine: pathways and regulation. *Eur J Pediatr* 1998;157(Suppl 2):S40–4.
- [61] Sebastio G, Sperandio MP, Panico M, de Franchis R, Kraus JP, Andria G. The molecular basis of homocystinuria due to cystathionine beta-synthase deficiency in Italian families, and report of four novel mutations. *Am J Hum Genet* 1995;56(6):1324–33.
- [62] Selhub J. Homocysteine metabolism. *Annu Rev Nutr* 1999;19:217–46.
- [63] Andersson A, Brattstrom 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(2):79–87.
- [64] Mudd S, Levy H, Skoby F. Disorders of transsulfuration. In: Scriver C, Beaudet A, Sly W, Valle D, editors. *The metabolic and molecular bases of inherited disease*. New York: McGraw-Hill, 1995:1279–327.
- [65] Bostom AG, Shemin D, Lapane KL, et al. Hyperhomocysteinemia and traditional cardiovascular disease risk factors in end-stage renal disease patients on dialysis: a case-control study. *Atherosclerosis* 1995;114(1):93–103.
- [66] Kang SS, Wong PW. Genetic and nongenetic factors for moderate hyperhomocyst(e)inemia. *Atherosclerosis* 1996;119(2):135–8.
- [67] Kang SS, Wong PW, Susmano A, Sora J, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. *Am J Hum Genet* 1991;48(3):536–45.
- [68] 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(1):111–3 Letter.
- [69] Hennekens CH, Buring JE, Mayrent SL, editors. *Epidemiology in medicine*, 1st ed. Philadelphia, PA: Lippincott, 1987.
- [70] 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(9):549–54.
- [71] te Poele-Pothoff MT, van den Berg M, Franken DG, et al. Three different methods for the determination of total homocysteine in plasma. *Ann Clin Biochem* 1995;32(Pt 2):218–20.
- [72] Frantzen F, Faaren AL, Alheim I, Nordhei AK. Enzyme conversion immunoassay for determining total homocysteine in plasma or serum. *Clin Chem* 1998;44(2):311–6.
- [73] 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(1):147–51.
- [74] Kang SS, Wong PWK, Malinow MR. Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. *Ann Rev Nutr* 1992;12:279–98.
- [75] 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):1–31.
- [76] McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. *Am J Pathol* 1969;56(1):111–28.
- [77] McDonald L, Bray C, Field C, Love F, Davies B. Homocystinuria, thrombosis and the blood platelets. *Lancet* 1964;1:745–6.
- [78] Harker LA, Slichter SJ, Scott CR, Ross R. Homocysteinemia. Vascular injury and arterial thrombosis. *N Engl J Med* 1974;291(11):537–43.
- [79] Harker LA, Ross R, Slichter SJ, Scott CR. Homocystine-induced arteriosclerosis. The role of endothelial cell injury and platelet response in its genesis. *J Clin Invest* 1976;58(3):731–41.
- [80] Wall RT, Harlan JM, Harker LA, Striker GE. Homocystine-induced endothelial cell injury in vitro: a model for the study of vascular injury. *Thromb Res* 1980;18(1–2):113–21.
- [81] Graeber JE, Slott JH, Ulane RE, Schulman JD, Stuart MJ. Effect of homocysteine and homocysteine on platelet and vascular arachidonic acid metabolism. *Pediatr Res* 1982;16(6):490–3.
- [82] Harker LA, Harlan JM, Ross R. Effect of sulfapyrazone on homocysteine-induced endothelial injury and arteriosclerosis in baboons. *Circ Res* 1983;53(6):731–9.
- [83] Harman LS, Mottley C, Mason RP. Free radical metabolites of L-cysteine oxidation. *J Biol Chem* 1984;259(9):5606–11.
- [84] Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. *J Clin Invest* 1986;77(4):1370–6.
- [85] 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(21):10098–103.
- [86] Parthasarathy S. Oxidation of low-density lipoprotein by thiol compounds leads to its recognition by the acetyl LDL receptor. *Biochim Biophys Acta* 1987;917(2):337–40.

- [87] Rodgers GM, Conn MT. Homocysteine, an atherogenic stimulus, reduces protein C activation by arterial and venous endothelial cells. *Blood* 1990;75(4):895–901.
- [88] Lentz SR, Sadler JE. Inhibition of thrombomodulin surface expression and protein C activation by the thrombogenic agent homocysteine. *J Clin Invest* 1991;88(6):1906–14.
- [89] Blom HJ, Engelen DP, Boers GH, Stadhouders AM, Sengers RC, de Abreu R, et al. Lipid peroxidation in homocysteinemia. *J Inherited Metab Dis* 1992;15:419–22.
- [90] Ueland PM, Brattstrom L, Refrim H. Plasma homocysteine and cardiovascular disease. In: Francis RB Jr, editor. *Atherosclerotic cardiovascular disease, hemostasis and endothelial function*. New York: Marcel Dekker, 1992:183–235.
- [91] Lentz SR, Sadler JE. Homocysteine inhibits von Willebrand factor processing and secretion by preventing transport from the endoplasmic reticulum. *Blood* 1993;81(3):683–9.
- [92] 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(1):308–18.
- [93] Tsai JC, Perrella MA, Yoshizumi M, Hsieh CM, Haber E, Schlegel R, et al. Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. *Proc Natl Acad Sci USA* 1994;91:6369–73.
- [94] Blom HJ, Kleinveld HA, Boers GHJ, et al. Lipid peroxidation and susceptibility of low-density lipoprotein to in vitro oxidation in hyperhomocysteinemia. *Eur J Clin Invest* 1995;25:149–54.
- [95] Blundell G, Jones BG, Rose FA, Tudball N. Homocysteine mediated endothelial cell toxicity and its amelioration. *Atherosclerosis* 1996;12:163–72.
- [96] Halvorsen B, Brude I, Drevon CA, Nysom J, Ose L, Christiansen EN, et al. Effect of homocysteine on copper ion-catalysed, azo compound-initiated, and mononuclear cell-mediated oxidative modification of low density lipoprotein. *J Lipid Res* 1996;37:1591–600.
- [97] Lentz SR, Sobey CG, Piegors DJ, et al. Vascular dysfunction in monkeys with diet-induced hyperhomocyst(e)inemia. *J Clin Invest* 1996;98(1):24–9.
- [98] Tsai JC, Wang H, Perrella MA, Yoshizumi M, Sibinga NE, Tan LC, et al. Induction of cyclin A gene expression by homocysteine in vascular smooth muscle cells. *J Clin Invest* 1996;97:146–53.
- [99] Woo KS, Chook P, Lolin YI, et al. Hyperhomocyst(e)inemia is a risk factor for arterial endothelial dysfunction in humans. *Circulation* 1997;96(8):2542–4.
- [100] Tawakol A, Omland T, Gerhard M, Wu JT, Creager MA. Hyperhomocyst(e)inemia: is associated with impaired endothelial-dependent vasodilatation in humans. *Circulation* 1997;95:1119–21.
- [101] 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(10):2074–81.
- [102] Chambers JC, Obeid O, McGregor A, Powell-Tuck J, Boustead L, Kooner JS. The relationship between hyperhomocysteinemia and endothelial dysfunction is concentration-dependant, and present even at physiological levels. *Circulation* 1998;98:1–192.
- [103] Al-Obaidi MK, Philippou H, Stubbs PJ, et al. Relationship between homocysteine, Factor VIIa, and thrombin generation in acute coronary syndromes. *Circulation* 2000;101:372–7.
- [104] Booth GL, Wang EE. Preventive health care: 2000 update: screening and management of hyperhomocysteinemia for the prevention of coronary artery events. *CMAJ* 2000;163(1):21–9.
- [105] Verhoef P, Hennekens CH, Malinow MR, Kok FJ, Willet WC, Stampfer MJ. A prospective study of plasma homocyst(e)ine and risk of ischemic stroke. *Stroke* 1994;25:1924–30.
- [106] Alfthan G, Pekkanen J, Jauhiainen M, Pitkaniemi J, Karvonen M, Tuomilehto J, et al. Relation of serum homocysteine and lipoprotein (a) concentrations to atherosclerosis disease in a prospective Finnish population based study. *Atherosclerosis* 1994;106:9–19.
- [107] Chasan-Taber L, Selhub J, Rosenberg IH, Malinow MR, 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.
- [108] Verhoef P, Hennekens CH, Allen RH, Stabler SP, Willet WC, Stampfer MJ. Plasma total homocysteine and risk of angina pectoris with subsequent coronary artery bypass surgery. *Am J Cardiol* 1997;79:799–801.
- [109] Evans RW, Shaten BJ, Hempel JD, Cutler JA, Kuller LH. Homocysteine and risk of cardiovascular disease in the multiple risk factor intervention trial. *Arterioscler Thromb Vasc Biol* 1997;17:1947–53.
- [110] Folsom AR, Nieto FJ, McGovern PG, et al. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related to genetic polymorphisms, and b vitamins. The Atherosclerosis risk in communities (ARIC) study. *Circulation* 1998;98:204–10.
- [111] Ubbink JB, Fehily AM, Pickering J, Elwood PE, Hayward Vermaak WJ. Homocysteine and ischaemic heart disease in the Caerphilly cohort. *Atherosclerosis* 1998;140:349–56.
- [112] Malinow MR, Bostom AG, Krauss RM. Homocyst(e)ine, diet, and cardiovascular diseases: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. *Circulation* 1999;99(1):178–82.
- [113] International task force for the prevention of coronary artery disease. Coronary artery disease: reducing the risk. *Nutr Metab Cardiovasc Dis* 1998; 8:229.
- [114] Genest J Jr. Emerging risk factors associated with cardiovascular diseases. Canadian cardiovascular society 1998 consensus conference on the prevention of cardiovascular diseases. *Can J Cardiol* 1999;15:73G–6G.
- [115] Jacques PF, Selhub J, Bostom AG, Wilson PF, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *NEJM* 1999;340(19):1449–54.
- [116] Manns BJ, Burgess ED, Hyndman ME, et al. Hyperhomocyst(e)inemia and the prevalence of atherosclerotic vascular disease in patients with end-stage renal disease. *AJKD* 1999;34(4):669–77.
- [117] Bostom AG, Culleton BF. Hyperhomocysteinemia in chronic renal disease. *J Am Soc Nephrol* 1999;10:891–900.
- [118] Dudman NP, Guo XW, Gordon RB, Dawson PA, Wilcken DE. Human homocysteine catabolism: three major pathways and their relevance to development of arterial occlusive disease. *J Nutr* 1996;126(Suppl 4):1295S–300S.
- [119] Bostom A, Brosnan JT, Hall B, Nadeau MR, Selhub J. Net uptake of plasma homocysteine by the rat kidney in vivo. *Atherosclerosis* 1995;116(1):59–62.
- [120] Hultberg B, Andersson A, Sterner G. Plasma homocysteine in renal failure. *Clin Nephrol* 1993;40(4):230–5.
- [121] Robinson K, Gupta A, Dennis V, et al. Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations. *Circulation* 1996;94(11):2743–8.
- [122] Wollesen F, Brattstrom L, Refsum H, Ueland PM, Berglund L, Berne C. Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. *Kidney Int* 1999;55(3):1028–35.
- [123] Wilcken DE, Gupta VJ. Sulphur containing amino acids in chronic renal failure with particular reference to homocystine and cysteine–homocystine mixed disulphide. *Eur J Clin Invest* 1979;9(4):301–7.

- [124] Wilcken DE, Gupta VJ, Reddy SG. Accumulation of sulphur-containing amino acids including cysteine–homocysteine in patients on maintenance haemodialysis. *Clin Sci* 1980;58(5):427–30.
- [125] Wilcken DE, Dudman NP, Tyrrell PA, Robertson MR. Folic acid lowers elevated plasma homocysteine in chronic renal insufficiency: possible implications for prevention of vascular disease. *Metabolism* 1988;37(7):697–701.
- [126] Wilcken DEL, Gupta BJ, Betts AK. Homocysteine in the plasma of renal transplant recipients: effects of cofactors for methionine metabolism. *Clin Sci* 1981;61:743–9.
- [127] Kang SS, Wong PW, Bidani A, Milanez S. Plasma protein-bound homocyst(e)ine in patients requiring chronic haemodialysis. *Clin Sci* 1983;65(3):335–6 Letter.
- [128] 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 Clin Lab Invest* 1991;51(3):277–82.
- [129] Chauveau P, Chadeaux B, Coude M, Aupetit J, Hannedouche R, Kamoun P, et al. Increased plasma homocysteine concentration in patients with chronic renal failure. *Miner Electrolyte Metab* 1992;18:196–8.
- [130] Arnadottir M, Brattstrom L, Simonsen O, Thysell H, Hultberg B, Andersson A, et al. The effect of high-dose pyridoxine and folic acid supplementation on serum lipid and plasma homocysteine concentration in dialysis patients. *Clin Nephrol* 1993;40:236–40.
- [131] Chauveau P, Chadeaux B, Coude M, Aupetit J, Hannedouche T, Kamoun P, et al. Hyperhomocysteinemia, a risk factor for atherosclerosis in chronic uremic patients. *Kidney Int* 1993;43(Suppl 41):S72–7.
- [132] Agardh CD, Agardh E, Andersson A, Hultberg B. Lack of association between plasma homocysteine levels and microangiopathy in type 1 diabetes mellitus. *Scand J Clin Lab Invest* 1994;54(8):637–44.
- [133] Friedman JA, Dwyer JT. Hyperhomocysteinemia as a risk factor for cardiovascular disease in patients undergoing hemodialysis. *Nutr Rev* 1994;53:197–200.
- [134] Massy AZ, Chadeaux-Vekemans B, Chevalier A, Bader CA, Druke TB, Legendre D, et al. Hyperhomocysteinemia: a significant risk factor for cardiovascular disease in renal transplant recipients. *Nephrol Dial Transplant* 1994;9:1103–8.
- [135] Bachmann J, Tepel M, Raidt H, Riezler R, Graefe U, Langer K, et al. Hyperhomocysteinemia and the risk for vascular disease in hemodialysis patients. *J Am Soc Nephrol* 1995;6:121–5.
- [136] Janssen MJFM, van Guldener C, de Jong GMT, van den Berg M, Stehouwer CDA, Donker AJM. Folic acid treatment of hyperhomocysteinemia in dialysis patients. *Miner Electrolyte Metab* 1996;22:110–4.
- [137] 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(1):41–6.
- [138] Dennis VW, Robinson K. Homocysteinemia and vascular disease in end-stage renal disease. *Kidney Int* 1996;57(Suppl):S11–7.
- [139] Bostom AG, Shemin D, Lapane KL, et al. Hyperhomocysteinemia, hyperfibrinogenemia, and lipoprotein (a) excess in maintenance dialysis patients: a matched case-control study. *Atherosclerosis* 1996;125:91–101.
- [140] Bostom AG, Lathrop L. Hyperhomocysteinemia in end-stage renal disease: prevalence, etiology, and potential relationship to atherosclerotic outcomes. *Kidney Int* 1997;52(1):10–20.
- [141] Hoogeveen EK, Kostense PJ, Jager A, et al. Serum homocysteine levels and protein intake are related to risk of microalbuminuria: the Hoorn study. *Kidney Int* 1998;54(1):203–9.
- [142] 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-dependant diabetes mellitus patients. *Metabolism* 1998;47(8):915–21.
- [143] 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(6):684–93.
- [144] Robinson K, Gupta A, Dennis V, Arheart K, Chaudhary D, Green R, et al. Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations. *Circulation* 1996;94:2743–8.
- [145] Bostom AG, Shemin D, Verhoef P, Nadeau MR, Jacques PF, Selhub J, et al. Elevated fasting total plasma homocysteine levels and cardiovascular disease outcomes in maintenance dialysis patients. *Arterioscler Thromb Vasc Biol* 1997;17:2554–8.
- [146] Moustapha A, Naso A, Nahlawi M, Gupta A, Arheart K, Jacobsen D, et al. Prospective study of hyperhomocysteinemia as an adverse cardiovascular risk factor in end-stage renal disease. *Circulation* 1998;97:138–41.
- [147] Ducloux D, Mote G, Challier B, Gibey R, Chalopin JM. Serum total homocysteine and cardiovascular disease occurrence in chronic, stable renal transplant recipients: a prospective study. *J Am Soc Nephrol* 2000;11:134–7.
- [148] Geiss LS, Herman WH, Smith PJ. Mortality in non-insulin-dependent diabetes. In: Harris M, editor. *Diabetes in America*, 2nd ed. Bethesda: National Institute of Health, 1995:233–55.
- [149] Kannel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. *Diabetes Care* 1979;2:120–6.
- [150] Singer DE, Moulton AW, Nathan DM. Diabetic myocardial infarction: interaction with other risk factors. *Diabetes* 1989;38:350–7.
- [151] Aronson D, Rayfield EJ, Chesebro JH. Mechanisms determining course and outcome of diabetic patients who have acute myocardial infarction. *Ann Intern Med* 1997;126:296–306.
- [152] Fuller JH, Shipley MJ, Rose G, Jarrett RJ, Keen H. Mortality from coronary heart disease and stroke in relation to degree of glycemia: the Whitehall study. *Br Med J* 1983;287(6396):867–70.
- [153] Jensen T, Borch-Johnsen K, Kofoed-Enevoldsen A, Deckert T. Coronary heart disease in young type 1 (insulin-dependent) diabetic patients with and without diabetic nephropathy: incidence and risk factors. *Diabetologia* 1987;30(3):144–8.
- [154] Gerstein HC, Pais P, Pogue J, Yusuf S. Relationship of glucose and insulin levels to the risk of myocardial infarction: a case-control study. *J Am Coll Cardiol* 1999;33(3):612–9.
- [155] Brownlee M. Glycation products and the pathogenesis of diabetic complications. *Diabetes Care* 1992;15(12):1835–43 Review.
- [156] Vlassara H, Bucala R. Recent progress in advanced glycation and diabetic vascular disease: role of advanced glycation end product receptors. *Diabetes* 1996;45(Suppl 3):S65–6 Review.
- [157] Monnier VM, Glomb M, Elgawish A, Sell DR. The mechanism of collagen cross-linking in diabetes: a puzzle nearing resolution. *Diabetes* 1996;45(Suppl 3):S67–72.
- [158] Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 1988;318:1315–21.
- [159] Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care* 1996;19:257–67.
- [160] Sowers JR, Epstein M. Diabetes mellitus and associated hypertension, vascular disease, and nephropathy. *Hypertension* 1995;26:869–79.

- [161] Daugherty A, Rateri DL, Dumm JN, Heinecke JW. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J Clin Invest* 1980;94:437–44.
- [162] Heinecke JW, Li W, Dachnke HL, Goldstein J. Dityrosine, a specific marker of oxidation, is produced by the myeloperoxidase-hydrogen peroxide chloride system of human neutrophils and macrophages. *J Biol Chem* 1993;268:4069–77.
- [163] King GL, Shiba T, Oliver J, Inoguchi T, Bursell SE. Cellular and molecular abnormalities in the vascular endothelium of diabetes mellitus. *Annu Rev Med* 1994;45:179–88.
- [164] Zimmet PZ, Alberti KGMM. The changing face of macrovascular disease in non-insulin-dependent diabetes mellitus: an epidemic in progress. *Lancet* 1997;350(Suppl 1):1–4.
- [165] Nathan DM, Meigs J, Singer DE. The epidemiology of cardiovascular disease in type 2 diabetes mellitus: how sweet it is or is it? *Lancet* 1997;350(Suppl 1):4–9.
- [166] Tribe RM, Poston L. Oxidative stress and lipids in diabetes: a role in endothelium vasodilator dysfunction? *Vasc Med* 1996;1(3):195–206 Review.
- [167] Bucala RP, Makita Z, Koschinsky T, Cerami A, Vlassara H. Lipid advanced glycosylation: pathway for lipid oxidation in vivo. *Proc Natl Acad Sci USA* 1993;90(14):6434–8.
- [168] Haffner SM. Management of dyslipidemia in adults with diabetes. *Diabetes Care* 1998;21(1):160–78 Review.
- [169] Feener EP, King GL. Vascular dysfunction in diabetes mellitus. *Lancet* 1997;350(Suppl 1):9–13.
- [170] Webster MWI, Scott RS. What cardiologist need to know about diabetes. *Lancet* 1997;350(Suppl 1):23–8.
- [171] Mogensen CE, Christensen CK, Christensen PD, et al. The abnormal albuminuria-syndrome in diabetes. Key to the complications. *Frontiers Diabet* 1993;12:86–121.
- [172] Borch-Johnsen K, Andersen PK, Deckert T. The effect of proteinuria on relative mortality in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1985;28:590–6.
- [173] Niskanen L, Uusitupa M, Sarlund H, et al. Microalbuminuria predicts the development of serum lipoprotein abnormalities favoring atherogenesis in newly diagnosed type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 1990;33(4):237–43.
- [174] Hofmann MA, Kohl B, Zumbach MS, et al. Hyperhomocyst(e)inemia and endothelial dysfunction in IDDM. *Diabetes Care* 1998;21(5):841–8 Corrected and republished article originally printed in *Diabetes Care* 1997; 20(12):1880–6.
- [175] Robillon JF, Canivet B, Candito M, et al. Type 1 diabetes mellitus and homocyst(e)ine. *Diabet Metabolism* 1994;20:494–6.
- [176] Cronin Cc, McPartlin JM, Barry DG, et al. Plasma homocysteine concentrations in patients with type 1 diabetes. *Diabetes Care* 1998;21(11):1843–7.
- [177] Jacobs RL, House JD, Brosnan ME, Brosnan J. Effects of streptozotocin-induced diabetes and of insulin treatment on homocysteine metabolism in the rat. *Diabetes* 1998;47:1967–70.
- [178] Fonseca VA, Mudaliar Sunder, 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(6):686–9.
- [179] Araki A, Sako Y, Ito H. Plasma homocysteine concentrations in Japanese patients with non-insulin-dependent diabetes mellitus: effect of parenteral methylcobalamin treatment. *Atherosclerosis* 1993;103(2):149–57.
- [180] DeFronzo RA, Goodman AM. The multicenter metformin study group. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med* 1995;333:541–9.
- [181] Berchtold P, Bolli P, Arbenz U, Keiser G. Intestinale absorptionsstörung infolge metforminbehandlung. *Diabetologia* 1969;5:405–12.
- [182] Hoogeveen EK, Kostense PJ, Jakobs C, Bouter LM, Heine RJ, Stehouwer CD. Does metformin increase the serum total homocysteine level in non-insulin-dependent diabetes mellitus? *J Intern Med* 1997;242(5):389–94.
- [183] Okada E, Oida K, Tada H, et al. Hyperhomocysteinemia is a risk factor for coronary arteriosclerosis in Japanese patients with type 2 diabetes. *Diabetes Care* 1999;22(3):484–90.
- [184] Morrison HI, Schaubel D, Desmeules M, Wigle DT. Serum folate and risk of fatal coronary heart disease. *JAMA* 1996;275:1893–6.
- [185] 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.
- [186] Rimm EB, Willett WC, Hu FB, Sampson L, Colditz GA, Manson JE, 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.
- [187] Giles WH, Kittner SJ, Croft JB, et al. Serum folate and risk for coronary heart disease: results from a cohort of US adults. *Ann Epidemiol* 1998;8(8):490–6.
- [188] Siri PW, Verhoef P, Kok FJ. Vitamins B₆, B₁₂, and folate: association with plasma total homocysteine and risk of coronary atherosclerosis. *J Am Coll Nutr* 1998;17(5):435–41.
- [189] Bunout D, Petermann M, Hirsh S, et al. Low serum folate but normal homocysteine levels in patients with atherosclerotic vascular disease and matched healthy controls. *Nutrition* 2000;16(6):434–8.
- [190] Smulders YM, Rakic M, Slaats EH, et al. Fasting and post-methionine homocysteine levels in NIDDM. Determinants and correlations with retinopathy, albuminuria, and cardiovascular disease. *Diabetes Care* 1999;22(1):125–32.
- [191] 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(1):133–8.
- [192] Munshi MN, Stone A, Fink L, Fonseca VA. Hyperhomocysteinemia following a methionine load in patients with non-insulin-dependent diabetes mellitus and macrovascular disease. *Metabolism* 1996;45(1):133–5.
- [193] Hoogeveen EK, Kostense PJ, Jakobs C, et al. Hyperhomocysteinemia increases risk of death, especially in type 2 diabetes: 5-year follow-up of the Hoorn study. *Circulation* 2000;101(13):1506–11.
- [194] Stehouwer CD, Gall MA, Hougaard P, Jakobs C, Parving HH. Plasma homocysteine concentration predicts mortality in non-insulin-dependent diabetic patients with and without albuminuria. *Kidney Int* 1999;55(1):308–14.
- [195] Kark JD, Selhub J, Bostom A, Adler B, Rosenberg IH. Plasma homocysteine and all-cause mortality in diabetes. *Lancet* 1999;353(9168):1936–7 Letter.
- [196] De Lorgeril M, Salen P, Paillard F, Lacan P, Richard G. Lipid-lowering drugs and homocysteine. *Lancet* 1999;353:209–10.
- [197] Landray MJ, Townend JN, Martin S, Martin U, Wheeler DC. Lipid-lowering drugs and homocysteine. *Lancet* 1999;353:1714–5.
- [198] Dierkes J, Westphal S, Luley C. Serum homocysteine increases after therapy with fenofibrate or bezafibrate. *Lancet* 1999;354:219–20.
- [199] Jonkers IJAM, De Man FHAF, Onkenhout W, van der Laarse A, Smelt AHM. Implication of fibrate therapy for homocysteine. *Lancet* 1999;354:1208.
- [200] Bissonette R, Treacy E, Rozen R, Boucher B, Cohn JS, Genest Jr J. Fenofibrate raises plasma homocysteine in the fasted and fed states. *Atherosclerosis* 2000;155:455–62.