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Review

Hyperhomocysteinaemia: a role in the accelerated atherogenesis of chronic renal failure?

M.J.F.M. Janssen [a,*], M. van den Berg [b], C.D.A. Stehouwer [a], G.H.J Boers [c]

[a] *ICaR-VU, Department of Internal Medicine, University Hospital, Free University, De Boelelaan 1117, 1081 HV Amsterdam, Netherlands*
[b] *Department of Surgery, University Hospital, Free University, De Boelelaan 1117, 1081 HV Amsterdam, Netherlands*
[c] *Department of Internal Medicine, University Hospital, Nijmegen, Netherlands*

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Abstract

Moderate hyperhomocysteinaemia has recently been established as an independent risk factor for atherothrombotic disease. It might be caused by heterozygosity for cystathionine β-synthase deficiency, an enzyme involved in the conversion of methionine to cysteine through the transsulphuration pathway or by inherited thermolability of the enzyme which remethylates homocysteine into methionine. In chronic renal failure (CRF) homocysteine levels are significantly elevated at a relatively early stage. The normal kidney possibly plays an important role in homocysteine catabolism, which cannot be performed in CRF. Alternatively, decreased extrarenal catabolism can contribute to the hyperhomocysteinaemia in this disease state. Treatment with folic acid, 5 mg daily, significantly lowers homocysteine levels in chronic renal patients.

Keywords: Hyperhomocysteinaemia; Chronic renal failure; Haemodialysis; Folic acid

1. Introduction

Homocysteine is a sulphydryl amino acid that does not occur in protein structures; it is formed as an intermediate by demethylation of methionine [1]. In many tissues, it can be converted to cystathionine by condensation with serine (Fig. 1). This irreversible reaction, part of the transsulphuration pathway, is catalyzed by the enzyme, cystathionine β-synthase (EC 4.2.1.22; CBS), and requires pyridoxal 5′-phosphate (active form of vitamin B_6) as co-factor. Alternatively, homocysteine can be remethylated to methionine by two different reactions. The first is catalyzed by 5-methyltetrahydrofolate-homocysteine methyltransferase (methionine synthase, EC 2.1.1.13; MH), with 5-methyltetrahydrofolate (5-mTHF, from folic acid) as the methyl-group donor and methylcobalamin (from vitamin B_{12}) as coenzyme. The second pathway is catalyzed by betaine-homocysteine methyltransferase (EC 2.1.1.5; BH), with betaine (Fig. 2) serving as the methyl donor. In normal subjects about 70% of total homocysteine in plasma is protein-bound, for the largest

* Corresponding author. Tel. (20) 5489111; Fax (20) 5484898.

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part to albumin [2–4]. In freshly prepared plasma, the free homocysteine, i.e. the homocysteine that is not protein-bound, exists as a mixture of cysteine-homocysteine mixed disulphide (CHMD, Fig. 2) and trace amounts of homocystine and homocysteine [5].

Normal fasting concentrations of free and total homocysteine are about 3 and about 10 μmol/l, respectively [4–6], premenopausal women having significantly lower values than postmenopausal women and men [7,8]. It has been suggested that efficient homocysteine metabolism is a protective factor against vascular disease in women during their reproductive years [9].

Homocystinuria is an inborn error of metabolism, usually associated with a defect of transsulphuration caused by CBS deficiency, inherited as an autosomal recessive trait. Heritable defects in methyltetrahydrofolate or methylcobalamin synthesis are very rare causes of homocystinuria [1]. Untreated (homozygotic) CBS-deficient patients have high total homocysteine levels in the fasting state up to 200 μmol/l [5]. Clinical characteristics are dislocation of the optic lens, osteoporosis, vertebral anomalies and mental retardation. Arterial and venous thromboembolism, occurring at a rate of 50% by the age of 30 years, constitutes the major cause of death.

The frequency of heterozygosity for homocystinuria is estimated to be 1:70 to 1:290 [1]. Carriers have slightly elevated fasting homocysteine values which overlap with those of normal controls, but they can be detected by l-methionine loading [10,11]. When given an oral dose of methionine of 0.1 g/kg body weight, heterozygotes, when compared with controls, reach higher homocysteine levels 6 h after loading, due to their partially deficient transsulphuration. On the basis of such testing also, up to 35% of patients presenting with symptoms of arterial occlusive dis-

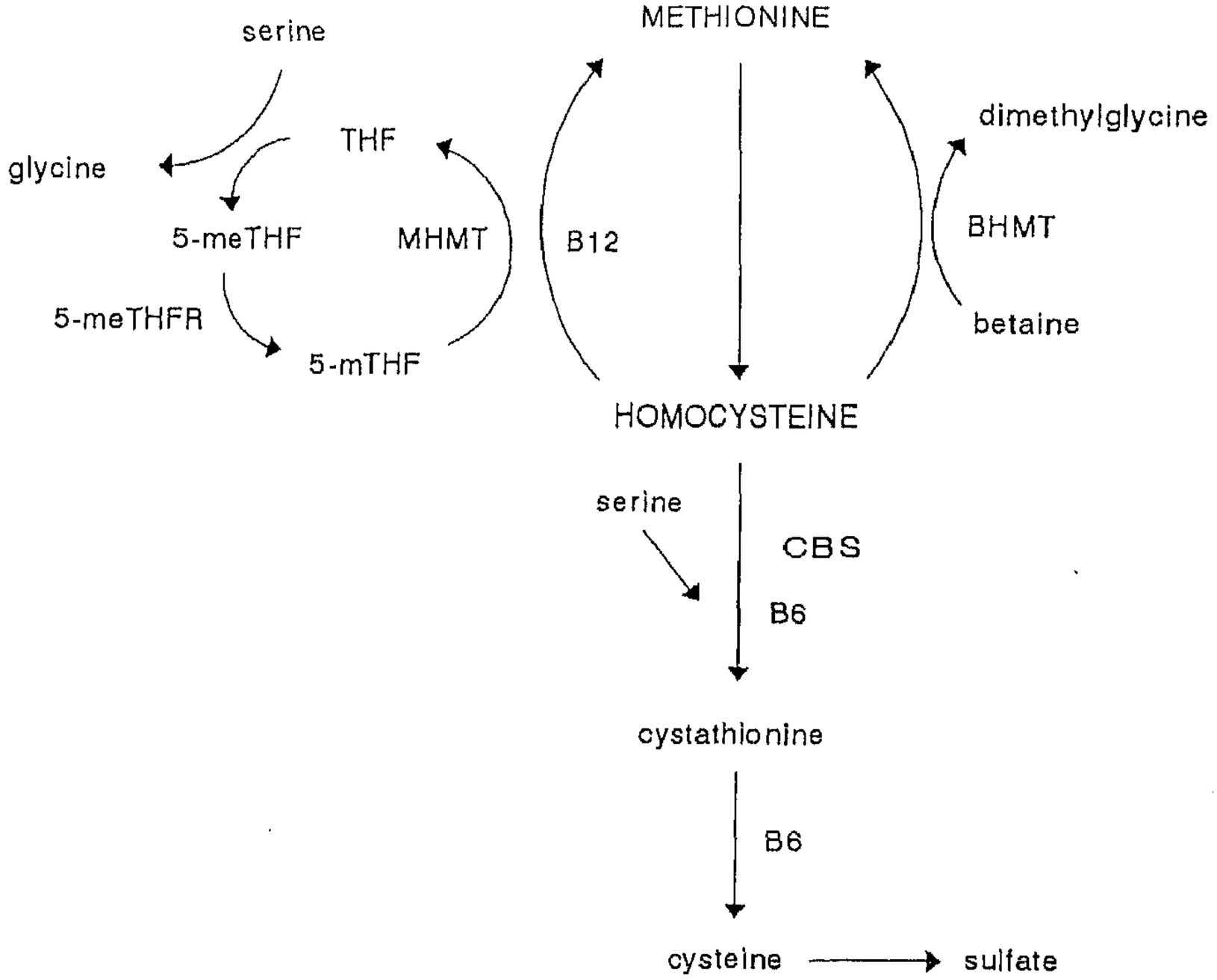

Fig. 1. The main reactions in methionine and homocysteine metabolism by transsulphuration and remethylation. MH = 5,10-methyltetrahydrofolate-homocysteine methyltransferase (methionine synthase); BH = betaine-homocysteine methyltransferase; 5-meTHFR = 5-methylenetetrahydrofolate reductase; CBS = cystathionine β-synthase; 5-mTHF = 5-methyltetrahydrofolate; 5-meTHF = 5-methylenetetrahydrofolate; B_6 = pyridoxal 5′-phosphate (from vitamin B_6).

ease before the age of 50 years could be shown to be hyperhomocysteinaemic, what identified mild hyperhomocysteinaemia as a risk factor for premature occlusive arterial disease [12–15]. In only a few of the reports has the cause of this hyperhomocysteinaemia in vascular patients been investigated. In the majority of these cases an intermediately deficient activity of CBS has been demonstrated in their cultered fibroblasts [12,16]. About 50% of adult patients with the homozygous enzyme defect respond to oral doses of pyridoxine of 750–1000 mg/day by normalization of the biochemical abnomalities [17]. There is evidence of the effectiveness of pyridoxine in preventing further thromboembolic events in these patients [17].

In the case of non-responsiveness to pyridoxine, folic acid and betaine can be given in order to stimulate the remethylation of homocysteine [18,19].

Patients with premature arterial disease and mild hyperhomocysteinaemia comparable with that in carriers of CBS deficiency are currently being treated with pyridoxine 250 mg/day and folic acid 5 mg/day. The effectiveness of this medication in the prevention of further vascular damage is a matter of investigation.

Apart from heterozygosity for CBS deficiency, other enzyme defects may be the cause of moderate hyperhomocysteinaemia. A thermolabile form of 5,10-methylenetetrahydrofolate reductase (EC 1.1.1.68; 5-meTHFR, Fig. 1) has been demonstrated in 17% of patients with premature coronary artery disease, possibly causing a lack of 5-methyltetrahydrofolate (5-mTHF) and hampering homocysteine remethylation [20].

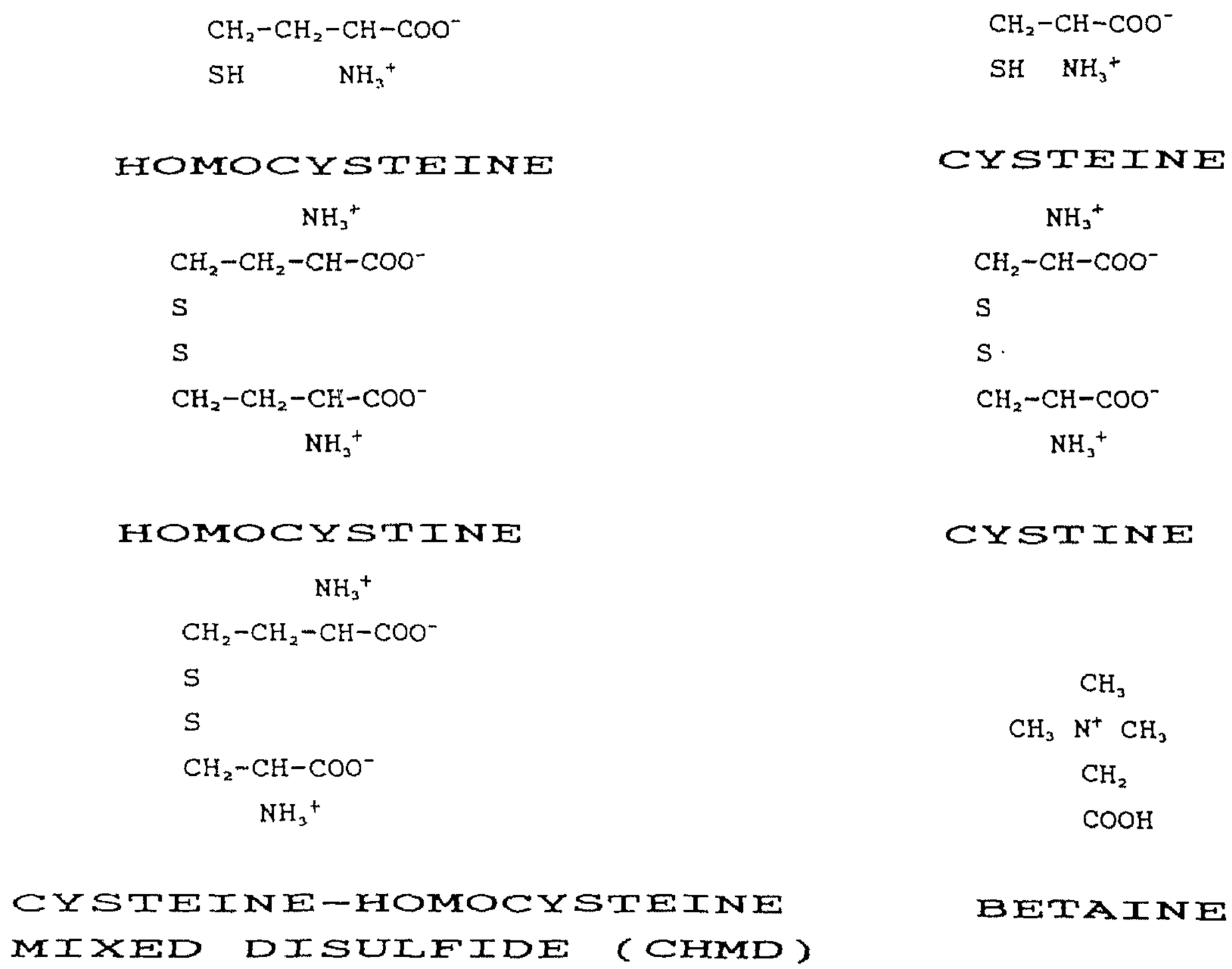

Fig. 2. Structural formulae of some molecules involved in homocysteine metabolism.

Acquired forms of hyperhomocysteinaemia have been found in severe liver disease [21], in vitamin B_{12} [22] and folate deficiency [23], and in chronic renal failure (CRF).

2. The hyperhomocysteinaemia of chronic renal failure

Cardiovascular disease is the major cause of death in patients on maintenance dialysis [24] and is only partially accounted for by the high prevalence of hypertension, smoking, hyperlipidaemia and diabetes mellitus in this population [25].

Plasma homocysteine in CRF patients not requiring maintenance dialysis

Wilcken et al. were the first to demonstrate that plasma homocysteine is increased in patients with chronic renal failure [26]. In 22 patients not requiring dialysis (mean serum creatinine $\pm$SD: 680 ± 300 μmol/l) the fasting plasma cysteine-homocysteine mixed disulphide concentration (CHMD), reflecting unbound homocysteine, was increased significantly compared to the values of an equally large control group: 8.2 ± 3.4 versus 3.1 ± 1.0 μmol/l ($P < 0.001$). The increase was positively correlated with the loss of renal function, as assessed by the serum creatinine concentration ($r = 0.62$; $P < 0.01$).

Soria et al. studied total homocysteine levels (= free + protein-bound homocysteine) in 53 patients with different degrees of chronic renal failure [27]. Group I consisted of 25 patients with moderate renal failure (serum creatinine ≤ 300 μmol/l). Group II comprised 18 patients with serum creatinine levels between 300 and 600 μmol/l. The remaining 10 patients constituted Group III and had serum creatinine values above 600 μmol/l. Plasma homocysteine in Group I was substantially elevated: 18.2 ± 1.7 μmol/l versus 8.0 ± 0.3 μmol/l (mean $\pm$ SEM) in 45 controls.

Group II had even higher homocysteine levels: 27.3 ± 3.2 and group III had values comparable with group II, i.e. 27.3 ± 1.7 μmol/l. Again the homocysteine concentration was correlated with the serum creatinine concentration ($r = 0.76$).

Recently, a third study was published on hyperhomocysteinaemia in patients with various degrees of chronic renal failure [28]. Again, three groups were distinguished. The first one ($n = 28$), having a creatinine clearance (C_{cr}) of 30–75 ml/min, had a mean ($\pm$SD) total plasma homocysteine concentration of 16.2 ± 8.1 μmol/l. In the second group ($n = 29$), with C_{cr} ranging from 10–30 ml/min, homocysteine levels were 23.3 ± 14.7 μmol/l, while in the third group ($n = 22$), with advanced renal failure ($C_{cr} < 10$ ml/min), homocysteine values of 29.5 ± 14.4 μmol/l were observed. All groups had significantly higher levels than the 45 controls (8.2 ± 2.2 μmol/l). Linear regression analysis showed a negative correlation between C_{cr} and total plasma homocysteine ($r = 0.40$; $P < 0.01$). All patients received moderate protein restriction (0.6–1.0 g/kg/day) according to the degree of renal failure. Folic acid supplementation was given in none of these three studies.

Plasma homocysteine in dialysis patients

Wilcken et al. studied 19 chronic haemodialysis patients [29]. The cysteine-homocysteine mixed disulphide concentration (CHMD) before dialysis was 6.3 ± 3.7 μmol/l. After haemodialysis CHMD was 2.7 ± 1.2 μmol/l, even lower than the values obtained in 22 normal subjects (3.1 ± 1.0 μmol/l).

Smolin et al. reported elevated CHMD and protein-bound homocysteine concentrations in chronic haemodialysis patients taking 1 mg folic acid and 10 mg pyridoxine per day [30]. Haemodialysis substantially lowered the unbound fraction from 14.7 ± 6.4 to 7.9 ± 4.9 μmol/l, leaving the protein-bound fraction largely intact. Pre-dialysis protein-bound homocysteine of 349 $\pm$ 77 nmol/g protein in the renal patients remained high after dialysis, i.e. 312 ± 71 nmol/g protein, versus 153 ± 50 nmol/g protein in fasting controls.

Kang et al. determined the protein-bound homocysteine in 13 haemodialysis patients on 5 mg folic acid daily [31]. Before dialysis they measured 197.7 ± 19.6 nmol/g protein, after dialysis 153.9 $\pm$ 19.1 nmol/g protein, constituting a decrease of 23%. Protein-bound homocysteine in 13 controls was 53.4 ± 3.6 nmol/g protein.

The mechanism of hyperhomocysteinaemia in CRF

The renal handling of homocysteine is incompletely understood. Since about 30% of plasma homocysteine is not protein-bound, this fraction freely passes the glomerular basement membrane; the daily filtered load can be calculated to be about 550 μmol. Thus, a large part of the circulating unbound homocysteïne is delivered to the proximal tubular cells. The quantity of homocysteine in 24-h urine samples varies from 3.5 to 10 μmol [3]; urinary homocysteine clearance is only 0.3% of creatinine clearance [7]. The low urinary excretion is accounted for by the avid reabsorption taking place in the cortical tubule: more than 99.5% of the filtered homocysteine is reabsorbed by the proximal tubular cells [32]. Reabsorption in the proximal tubule occurs via two saturable systems. The first, a high-affinity, low-K_m (0.17 mM) system, is shared with cystine, arginine and lysine [32]. Urinary homocysteine excretion can be stimulated by infusion of these amino acids [33]. The second system with low-affinity, high-K_m (7.65 mM) characteristics, seems to be shared with cystine only [32]. The fate of the reabsorbed homocysteine is unknown. In the human kidney cystathionine β-synthase activity is low [34], whereas the activity of both the homocysteine remethylation enzymes is high [35,36]. Assuming that the filtered and reabsorbed homocysteine is catabolized in the proximal tubule cell, the kidney may normally play a substantial role in homocysteine metabolism. In chronic renal failure, renal catabolism may be hampered because homocysteine, due to the decreased glomerular filtration, cannot reach its renal metabolic site, i.e. the proximal tubular cell. Moreover, tubular cell dysfunction itself in renal failure may contribute to the raised homocysteine levels due to the fact that homocysteine is passing the tubular cell into the peritubular capillaries without being catabolized.

In CRF, total body catabolism of homocysteine has been thought to be changed, mainly on the basis of the low serine levels measured in this condition. Serine is involved in homocysteine metabolism in two ways (Fig. 1). Firstly, it is converted to glycine by donating a methyl group in the restoration of 5,10-methylenetetrahydrofolate from tetrahydrofolate, and secondly, it condenses with homocysteine to form cystathionine in the transsulphuration route. The low serine values in CRF have been said to point to enhanced catabolism of homocysteine via these two reactions [37]. The normal kidney, however, produces rather large quantities of serine [38]. In CRF, there is a 80–90% reduction of this production, which itself can explain the low serine levels seen in CRF [38,39]. Consequently, low serine levels do not necessarily reflect an increased catabolism of homocysteine. On the contrary, a feedback inhibition of homocysteine metabolism in the liver and other organs by heightened levels of uraemic toxins has been suggested to contribute to the hyperhomocysteinaemia of CRF [37].

Could suboptimal levels of folic acid, vitamin B_6 or B_{12} contribute to the raised homocysteine concentration in CRF patients?

All studies of hyperhomocysteinaemia in dialysis patients have been performed in patients on haemodialysis. Folate deficiency is unlikely to develop in such patients on a regular diet [40–43]. Moreover, Smolin et al. found high homocysteine levels in 24 haemodialysis patients on a daily multivitamin supplement providing 1 mg of folic acid and 10 mg of pyridoxine [30]. All the patients with stable CRF that were included in the published trials exploring the effect of folic acid on the homocysteine concentration had normal baseline serum- and red cell folate levels, as well as normal serum B_{12} concentrations [44,45]. Furthermore, supplementation with B_{12} and with high-dose pyridoxine was shown not to influence the homocysteine levels of these patients [44]. Therefore, it is unlikely that deficiencies of folic acid, vitamin B_{12} or pyridoxine contribute significantly to the elevated homocysteine levels in CRF patients.

A reduction of the protein intake has been shown to retard the progression of CRF. Protein restriction may lower the plasma homocysteine concentration in advanced CRF. However, the reduced intake of methionine that is induced by protein restriction may only level off the homocysteine levels in advanced renal failure [27].

3. Treatment options for hyperhomocysteinaemia in CRF

There is a limited number of publications on the treatment of heightened homocysteine levels in renal patients. In 1981, Wilcken et al. tested the effect of administering the co-factors for homocysteine metabolism to CRF patients [44]. Eleven long-term renal transplant recipients, with a mean ($\pm$SD) serum creatinine concentration of 210 ± 90 μmol/l and a mean ($\pm$SD) cysteine homocysteine mixed disulphide (CHMD) of 7.3 ± 2.1 μmol/l, received folic acid in a daily dose of 5 mg. After 2 weeks CHMD had declined to 5.1 ± 0.7 μmol/l. After 4 weeks of treatment CHMD was 4.3 ± 0.8 μmol/l; continuation of the treatment did not further lower CHMD. Separate or additive effects of pyridoxine (100 mg/day) or vitamin B_{12} (1000 μg im) on plasma CHMD were not detectable. Wilcken later confirmed the effectiveness of folic acid in 21 CRF patients with a mean ($\pm$SD) serum creatinine concentration of 560 ± 240 μmol/l [45]. Baseline free homocysteine was 12.9 ± 6.8 μmol/l for the patients and 4.2 ± 0.8 μmol/l in 24 normal controls. Administration of folic acid 5 mg/day to the patients for 15 ± 6 days decreased the free homocysteine to 6.8 ± 2.8 μmol/l. This fall was linearly related to the pre-treatment level of free homocysteine ($r = 0.92$). The decline of homocysteine concentrations in CRF patients treated with folic acid coincided with a further decrease of plasma serine and a rise in plasma glycine, indicating the stimulation of remethylation by folic acid [45]. In both studies by Wilcken on the treatment of hyperhomocysteinaemia in CRF the patients had normal pre-treatment levels of folic acid and vitamin B_{12}; the concentration of vitamin B_6 had not been determined.

4. Conclusion

There is a significant increase in plasma homocysteine concentration in patients with CRF, hyperhomocysteinaemia being present from the incipient stage of renal failure and increasing further as renal function deteriorates. To date, the origin of hyperhomocysteinaemia in CRF is now largely unclear and awaits further investigation. Decreased catabolism of homocysteine in the proximal tubular cells due to reduced filtration and to tubular dysfunction and decreased extrarenal homocysteine metabolism due to inhibition by retained metabolites may both contribute. Folic acid treatment can lower homocysteine concentrations in these patients. Further research is needed into the optimal dose of folic acid, not only in patients with advancing renal failure, but also in haemodialysis and peritoneal dialysis patients. The possible contribution of betaine, the other known stimulator of remethylation, should also be explored. Lowering the homocysteine concentration in CRF patients could aid in the prevention of cardiovascular disease in CRF patients, which constitutes a major problem in medical practice.

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