Why is homocysteine elevated in renal failure and what can be expected from homocysteine-lowering?

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Patients with chronic kidney disease, especially end-stage renal disease (ESRD), exhibit many abnormalities in protein and amino acid metabolism. One of these alterations involves an increased plasma concentration of the sulphur-containing amino acid homocysteine. Hyperhomocysteinaemia has attracted a lot of attention in renal patients, not only because of its close relationship with renal function, but also because it has been implicated as an independent cardiovascular risk factor in these patients [1–3], although some recent studies have found no significant or even an inverse association between plasma homocysteine level and cardiovascular events and mortality in ESRD patients [4–6]. These discordant findings may have been caused by strong confounders which are associated with low homocysteine levels and increased mortality, such as protein energy malnutrition and/or inflammation [7].

Homocysteine and renal function

Plasma homocysteine is strongly correlated with (estimates of) glomerular filtration rate (GFR). Hyperhomocysteinaemia, defined as a plasma total homocysteine level of 12 μmol/l, occurs already at a GFR of about 60 ml/min and when ESRD has been
reached, the prevalence of hyperhomocystinaemia is 85–100%. The association between plasma homocysteine and GFR seems linear and is present even in the hyperfiltrating range [8,9]. The precise mechanism by which GFR is related to plasma homocysteine concentration is not definitively established. There is a reasonably good clinical evidence that hyperhomocystinaemia does not cause renal insufficiency [10–12], although a recent study has linked higher homocysteine levels to a greater decline of GFR [13]. The association between hyperhomocystinaemia and renal dysfunction may therefore be causal, i.e. renal failure causes elevated plasma homocysteine levels, but the relationship may also be due to other confounding factors, which on the one hand lead to renal dysfunction and on the other hand cause hyperhomocystinaemia by different mechanisms. Two, not mutually exclusive hypotheses for the first possibility are: (i) homocysteine disposal in the kidneys themselves is disturbed and (ii) extrarenal homocysteine metabolism is impaired. Factors that cause renal dysfunction and hyperhomocystinaemia by different mechanisms have not been identified.

Renal homocysteine metabolism

The close relationship between plasma homocysteine and GFR suggests that homocysteine is cleared from the body by urinary excretion after glomerular filtration, just like creatinine. However, the amount of homocysteine in the urine is minimal (about 6 μmol/day) [14]. From a normal GFR of 1801/day and a free homocysteine concentration of 3 μmol/l, it can be calculated that 99% of the filtered homocysteine is reabsorbed. The exact location of this uptake and the metabolic fate of homocysteine in the tubules are unknown. Homocysteine transsulphuration and remethylation enzymes are present in human kidney tissue, indicating that metabolism is possible. Studies in the rat have shown that homocysteine is taken up and metabolized by the kidney [15]. Two studies in humans with normal renal function, however, did not find a significant arteriovenous difference in homocysteine concentration across the kidney [16,17] (Figure 1). In the study of Garibotto et al. [17], a small renal homocysteine extraction was found in subjects with a higher renal plasma flow (>500 ml/min), whereas some homocysteine release was found when renal plasma flow was <500 ml/min. Apart from being due to chance, this finding could also suggest that renal homocysteine metabolism, if any, would depend on the renal plasma flow. However, as renal plasma flow does not largely affect GFR in humans (due to filtration disequilibrium), this is somewhat contrary to the general finding that plasma homocysteine is related to GFR. Furthermore, a larger study failed to establish a relationship between plasma homocysteine and effective renal plasma flow [9].

An intrarenal homocysteine disposal, thus, has not yet been proved, but it may exist in balance with an equal rate of homocysteine formation. A preferential defect in homocysteine disposal could hypothetically occur in chronic kidney disease and subsequently lead to hyperhomocystinaemia. For this theory, however, there is no supportive evidence, e.g. from renal arteriovenous studies in renal patients. Also, it should be kept in mind that loss of renal amino acid metabolism in chronic kidney disease does not always predict the consequences on plasma concentrations [18].

Extrarenal homocysteine metabolism

In order to investigate the whole body homocysteine metabolism in chronic kidney disease, one might study plasma concentrations of several metabolites. Homocysteine is generated by demethylation of methionine, which in turn is derived from exogenous or endogenous proteins. S-adenosylmethionine and S-adenosylhomocysteine are the intermediates in this transmethylation pathway. In the remethylation pathway, homocysteine is reconverted to methionine by receiving a methyl group from 5-methyltetrahydrofolate, the active form of folic acid, or betaine. Irreversible disposal of homocysteine occurs through the transsulphuration pathway, in which homocysteine condenses with serine to form cystathionine, which is split into cysteine and alpha-ketobutyrate. There are several metabolic fates of cysteine, such as incorporation in proteins and conversion to metabolites such as 3-mercaptopuruvate, cysteinesulphinate, gamma-glutamylcysteine or cystine. The sulphur end product of cysteine metabolism is sulphate, which is excreted by the kidneys.

In general, patients with renal failure have normal plasma levels of methionine, betaine and B-vitamins, elevated levels of S-adenosylmethionine, S-adenosylhomocysteine, cystathionine, cysteine and sulphate,
and low serine levels [19–24] (Figure 2). Plasma homocysteine shows the strongest relationships with plasma folate, S-adenosylhomocysteine and cysteine. The two most obvious explanations for these findings would be a block in homocysteine remethylation and a disturbance in cysteine disposal. Support for the first theory is based on trials showing that successful homocysteine-lowering regimens in chronic kidney disease always include folate. Randomized trials have shown that different forms of folates and different routes of administration are equally effective in lowering plasma homocysteine in ESRD patients [25,26], which has substantially weakened suggestions that a disturbed folate metabolism may cause hyperhomocysteinaemia in chronic kidney disease [27]. Based on high cysteine and low taurine levels, Suliman et al. [28] proposed that a block in decarboxylation of cysteinesulphinic acid, the intermediate between cysteine and taurine, contributes to hyperhomocysteinaemia in CRF. Among subjects with normal to severely impaired renal function, Nakanishi et al. [24] found that plasma homocysteine was independently associated with plasma sulphate and they speculated that retention of sulphate somehow leads to hyperhomocysteinaemia in chronic kidney disease.

Results from studies that rely on measurement of plasma levels of metabolites must be interpreted with caution. Amino acid metabolism takes place intracellularly and varies between organs and tissues. Plasma contains only a small fraction of total body free amino acids and the plasma concentration of an amino acid depends on the supply from the cellular and/or interstitial compartment and the disposal from plasma. Elevated or decreased concentrations are indicative of a disturbed metabolism, but normal concentrations do not exclude abnormalities, because the rates of synthesis and elimination may be altered in the same direction. In addition, regulation of amino acid metabolism is influenced by feeding. Measurements in the post-absorptive state may therefore not provide a complete picture of amino acid metabolism.

Our group has used a stable isotope method to study the whole body sulphur amino acid metabolism in ESRD patients and healthy individuals [29–31]. The method involves the use of a primed, constant infusion of tracer methionine, containing a 13C-carboxyl and a 2H3 methyl label. The methyl label is removed during methionine transmethylation and the carboxyl label appears as labelled CO2 after transsulphuration and oxidation of homocysteine. At isotopic steady-state, the whole body fluxes of transmethylation, transsulphuration and remethylation can be estimated from sample enrichments at plateau. The main findings of the experiments were that total remethylation and transmethylation flux were decreased in ESRD patients, whereas transsulphuration rate was similar compared with the values of the control subjects [29–31] (Figure 2). The observation that total transsulphuration was not affected in ESRD, is probably the result of a similar dietary protein and therefore, methionine intake in both groups. Two mechanisms may explain the elevated plasma homocysteine level and decreased flux through the methylation cycle in ESRD. First, the primary defect in the sulphur amino acid metabolism may be an impairment of homocysteine transsulphuration, which is offset by a higher plasma homocysteine after which the daily methionine load can again be metabolized through the transsulphuration pathway. The higher homocysteine level would slow down the methylation cycle by inhibiting transmethylation. Second, the primary defect may be a block in
homocysteine remethylation, which can only be partially compensated for by an increase in plasma homocysteine, resulting in a further slowing down of the methylation cycle without compromising transulfuration. In the latter model, the elevated cysteine levels and the more favourable response to folate compared with vitamin B6 therapy in ESRD may be better explained. To reconcile the observations of a close relationship between homocysteine and GFR on the one hand and a primary defect in remethylation or transulfuration on the other hand, it can be hypothesized that an unknown compound, which is eliminated by glomerular filtration, regulates homocysteine clearance by remethylation or transulfuration.

**Consequences of hyperhomocysteinaemia**

Several biochemical mechanisms have been proposed to explain the presumed vasculotoxic effects of homocysteine. The main theory is that high homocysteine levels lead to endothelial dysfunction. Impaired endothelial vasomotor responses have been ascribed to a reduced bioavailability of nitric oxide due to autooxidation of homocysteine in plasma which leads to oxidative inactivation of nitric oxide [32]. Alternatively, homocysteine may lead to the accumulation of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, by inhibiting its cata catalyzing enzyme dimethylarginine dimethylaminohydrolase. In patients with renal insufficiency, hyperhomocysteinaemia and impaired endothelium-dependent vasodilatation are both present, but are not significantly related to each other [33–35]. In another study, there was no significant relationship between plasma homocysteine and antibodies against oxidized LDL, a marker of oxidative stress, in haemodialysis patients [36].

Other potential consequences of hyperhomocysteinaemia include general hypomethylation due to inhibition of the transmethylation pathway, posttranslational protein modification and/or damage by homocysteine-thiolactone, a highly reactive compound formed by methionyl-tRNA synthetase, and enhanced endoplasmic reticulum stress, which involves disruption of the folding and the processing of the newly synthesized proteins in the endoplasmic reticulum.

**Homocysteine-lowering treatment**

From the many, mostly short-term, homocysteine-lowering intervention studies in renal failure patients, one can conclude that folate compounds are the most effective and consistent in lowering plasma homocysteine compared with other therapies. Although direct comparisons are scarce, the maximal homocysteine-reducing capacity of folic acid in dialysis patients seems to have already reached a low dose (1–2 mg daily) [37–39]. Vitamin B6 does not seem to have a significant impact on plasma homocysteine levels in dialysis patients, whereas vitamin B12 may lower homocysteine somewhat further when added to folic acid, especially in patients with subclinical B12 deficiency. Other therapies that have been tested (with moderate success at best) include supplementation of betaine, serine, creatine or acetyl cysteine and dialysis with superfilt or protein leaking filters. A common finding of all intervention studies is that final on-treatment homocysteine levels remain above normal in the majority of patients.

Surprisingly, most studies have examined the effect of homocysteine-lowering treatment only on plasma homocysteine itself (or related metabolites). The influence of homocysteine-lowering treatment on vascular or other pathogenic factors has been addressed in only a few studies. We have shown that one year of folic acid therapy in haemo- and peritoneal dialysis patients did not improve carotid artery stiffness or endothelial dysfunction, which was assessed as flow-mediated vasodilatation in the brachial artery and with biochemical markers [37,38,40]. Subsequently, it was also shown that folic acid therapy did not improve endothelium-dependent vasodilatation in patients with pre-dialysis renal failure [35]. Using the stable isotope method with labelled methionine, we have shown that folic acid treatment normalizes transmethylation and remethylation fluxes in haemodialysis patients, in spite of a persistent elevation of plasma homocysteine [31]. Ingrosso et al. [41] demonstrated that folate treatment in ESRD patients ameliorates DNA hypomethylation and restores altered expression of genes that depend on DNA methylation, again without normalization of plasma homocysteine. Together, these interesting findings suggest that folate therapy may have biological effects in ESRD patients which are not mediated by the lowering of plasma homocysteine per se, but rather by the improvement of the transmethylation pathway, in which many important methylation reactions take place [42].

So far, only one prospective randomized intervention trial in renal patients with clinical endpoints has been published [6]. In this study, 510 ESRD patients (mostly on haemodialysis) were randomized to 1, 5 or 15 mg folic acid daily. After a mean follow-up of 2 years, there was no significant difference in mortality or cardiovascular events between the three groups. Beneficial effects of homocysteine-lowering, however, cannot yet be excluded because there was no true placebo group in this study. Currently, there are two other clinical endpoint trials underway in patients with chronic kidney disease. In the FAVORIT trial, about 4000 renal transplant recipients will be randomized to a combination of folate, vitamin B12 and vitamin B6 or placebo, and in the HOST trial, more than 2000 patients with advanced renal disease are randomized to a high dose of a combination of folate, vitamin B12 and vitamin B6 or to placebo [43].

In conclusion, there is no clear evidence that homocysteine-lowering treatment with folic acid at higher doses than 1 mg per day will lower the risk of
vascular events or mortality in patients with ESRD. The ongoing trials will hopefully further clarify the value of homocysteine-lowering treatment in chronic kidney disease. Further research on the effects of an improved transmethylation by folic acid beyond vascular biology is also warranted.

Conflict of interest statement. None declared.

References

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**Vascular calcification—a matter of damage limitation?**

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**Introduction**

Vascular calcification has now been recognized as an important determinant of cardiovascular mortality in patients on dialysis. Recent cell biological studies, using phenotypically modulated, human vascular smooth muscle cells (VSMCs) *in vitro*, have highlighted the importance of vascular damage, leading to vesicle release, combined with loss of function of inhibitory proteins, as the major events in the calcification process. VSMC calcification is a regulated process, therefore the potential exists to inhibit progression or more significantly, induce regression. Identification of damage-inducing agents and calcification inhibitors is now quite advanced. The next challenge will be in determining ways to limit damage and induce expression and/or efficacy of inhibitors. Although new therapeutics have shown the potential to act on these pathways, there is still much to be learnt about how the complex ‘uraemic’ milieu appears to favour vascular calcification at the expense of bone mineralization.

**Vascular calcification—two sites with different consequences**

Vascular calcification or ‘hardening of the arteries’ has long been recognized as a complication of ageing and disease, yet until recently, little attention was paid to its clinical consequences. However, with the realization that vascular calcification is a time-dependent and widespread complication of patients on dialysis, and most likely contributes to their high cardiovascular mortality, much attention has now been focused on its aetiology, consequences and mechanisms [1,2].

Vascular calcification occurs at two anatomical sites in the vessel wall, the media and the intima. Dialysis patients have an increased prevalence of both forms, however, it is the extreme medial calcification that is...