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# Toxic Effects of Hyperhomocysteinemia in Chronic Renal Failure and in Uremia: Cardiovascular and Metabolic Consequences

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**Hyperhomocysteinemia, highly prevalent in well-nourished patients with chronic renal failure and in uremia, causes toxic effects that can be envisioned in terms of cardiovascular risk increase. However, its effects on cellular metabolism and on gene expression, not to mention receptor regulation, only recently are being evaluated. For example, it has been shown that hypomethylation induced by hyperhomocysteinemia can alter erythrocyte membrane protein repair and gene expression. In addition, increased plasma protein L-isoaspartyl content, related to hyperhomocysteinemia and uremic toxicity, determines specific effects on protein function, with a reduced binding of homocysteine to albumin. We propose that uremia is a state in which proteins present a widespread derangement of structure-function relationships.**
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Homocysteine levels are high in chronic renal failure and in uremia; normal levels are approximately 10 μmol/L; diabetic patients, for reasons related to the hormonal pattern, have lower plasma levels. Mild increases in homocysteine levels have been linked to cardiovascular risk. In chronic renal failure and end-stage renal disease homocysteine levels are between 30 and 40 μmol/L on average if patients are not taking any folate supplements. However, it is possible to encounter much higher levels in uremic patients, even more than 100 μmol/L, which are similar to those present in homocystinuria (Fig 1). High homocysteine levels and premature cardiovascular mortality characterize this genetic disease.

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## Cardiovascular Disease and Hyperhomocysteinemia

Consequences of hyperhomocysteinemia in chronic renal failure and in uremia usually are seen essentially in terms of cardiovascular disease, and, ultimately, patient mortality. At present, a controversy exists between those advocating that, as in the general population, high homocysteine levels correlate with patient mortality and therefore are harmful, and those who find a positive correlation between low homocysteine levels and mortality, so-called *reverse epidemiology*.

Looking at the mortality issue, a study by Mallamaci et al[1] found a significant correlation between higher homocysteine levels and mortality. Patients were followed-up prospectively for 2.5 years and those with higher levels showed a decreased survival rate. In this report, homocysteine levels were adjusted for albumin.

Kalantar-Zadeh et al[2] proposed that when homocysteine levels are low there is a higher mortality rate. Unfortunately, they did not adjust their data for albumin levels. If homocysteine levels and albumin levels both are low then this is not a coincidence of events. Homocysteine and albumin levels are


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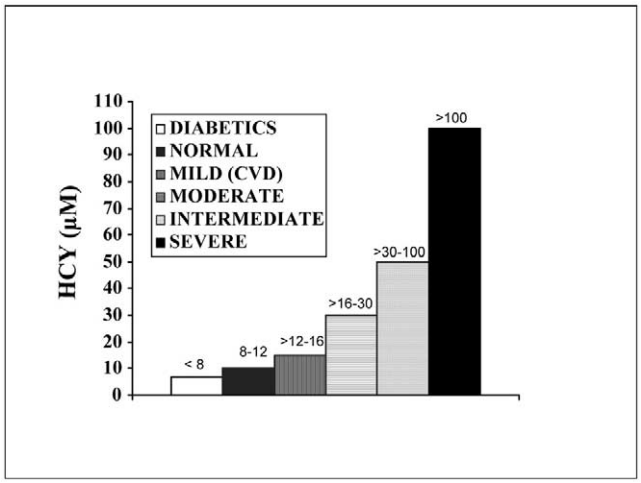


**Figure 1** Homocysteine concentrations in health and in disease. □, Diabetic; ■ , normal; ■ , mild (cardiovascular disease); ■ , moderate; □ , intermediate; ■, severe.

correlated tightly. Homocysteine is itself an amino acid and a derivative of methionine, which is an essential amino acid. Therefore, if albumin and homocysteine levels are both low, it simply could mean that the low homocysteine levels are caused by malnutrition, which will influence mortality powerfully. In this study more than half of the patients were diabetic, and in the deceased group 75% were diabetic. This is also important in the evaluation of these data because diabetic patients have lower homocysteine levels, even if they are uremic.

Other studies recently have put forward the theory of reverse epidemiology. For example, Wrone et al[3] compared 3 groups of patients taking folic acid supplements of different dosages (1, 5, and 15 mg/d) and found no difference with respect to cardiovascular events after 2 years. In this trial, no placebo was used and patients already were taking folates (no washout), therefore it is difficult to draw inferences about reverse epidemiology or about effects on cardiovascular events (because treatment by itself, regardless of dosage, could affect event rates). Moreover, when adjustment for albumin levels was performed, the reverse epidemiology disappeared (only age, albumin, and race/ethnicity remained significant predictors). Diabetic patients accounted for more than 40% of the total patients in all 3 groups. At present, we believe that we should wait for other carefully designed prospective studies and intervention trials that take potent confounders such as nutritional status into account before we decide to keep homocysteine levels in the high range to increase patient survival chances.

## Metabolic Consequences of Hyperhomocysteinemia

Homocysteine metabolism is shown in Fig 2. Recently, some unforeseen consequences of hyperhomocysteinemia, coming from other laboratories and ours, have been explored that can affect mortality. These include the dynamics between folate receptors and therapy and the complex interplay between homocysteine levels, gene expression, and protein binding and its effects.

A key role for homocysteine has been shown by Antony et al[4] in the regulation of folate-receptor biosynthesis. Folate receptors and reduced folate carriers mediate cellular acquisition of folates. Folate receptors are up-regulated in folate deficiency and down-regulated after folate repletion, but the basis of this regulation was unknown until now. Antony et al[4] proposed the following mechanism: folate depletion inactivates methionine synthase, which will cause an increase in homocysteine levels. Homocysteine in turn determines the synthesis of folate receptors by increasing the interaction between messenger RNA and a nuclear protein. Folate repletion reactivates methionine synthase, and homocysteine levels will decrease. Methionine has no effects on the interaction between RNA and the nuclear protein. In this way, folate-receptor synthesis shuts down. In addition, they showed that during high-dose folate repletion, folates have an indepen-

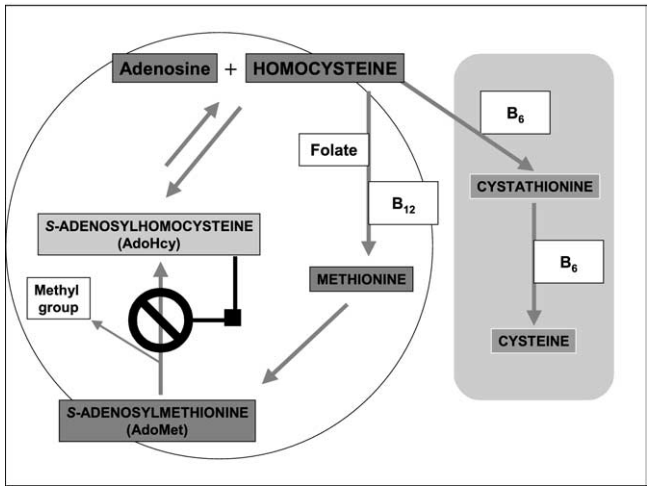


**Figure 2** Homocysteine (Hcy) metabolism. Methionine is activated by reacting with adenosine triphosphate, resulting in the formation of AdoMet (enzyme: AdoMet synthetase). AdoMet is the methyl donor for approximately 40 methyltransferases, the methyl-accepting substrate being small molecules (amino acids, phospholipids, amines, and so forth) and macromolecules (DNA, RNA, proteins). AdoHcy is the demethylated product of AdoMet and a powerful competitive inhibitor of all AdoMet-dependent enzymes. AdoHcy is hydrolyzed rapidly to Hcy and adenosine (enzyme: AdoHcy-hydrolase) under physiologic conditions. This is the only source of Hcy in human beings. However, this reaction is fully reversible, and thermodynamics actually favor biosynthesis over hydrolysis. Hcy is metabolized through almost equally partitioning into transsulfuration to cysteine or remethylation into methionine. Transsulfuration requires pyridoxalphosphate as the active form of vitamin $B_6$ (rate-limiting step is catalyzed by cystathionine-$\beta$-synthase). Remethylation is catalyzed mostly by methionine synthase, which requires methylcobalamin, the active form of vitamin $B_{12}$, as an essential cofactor. Methyltetrahydrofolate is the active folate derivative, which functions as the methyl donor in the latter reaction. Methyltetrahydrofolate is formed from methylenetetrahydrofolate (enzyme: methylenetetrahydrofolate reductase). An alternative pathway for Hcy remethylation requires betaine as the methyl donor.

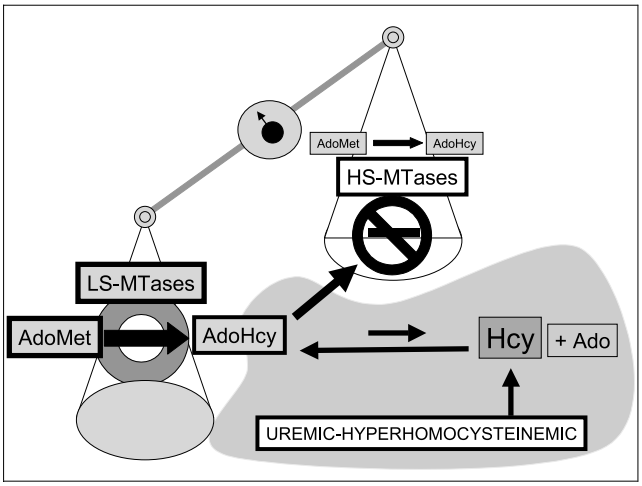


**Figure 3** The unbalanced methylation hypothesis of homocysteine (Hcy) action. Under conditions in which Hcy builds up, AdoHcy hydrolysis is hampered, with consequential inhibition of methyl transfer reactions. The extent of this inhibition depends on the intracellular concentration ratio of AdoMet/AdoHcy, and on the individual $K_i$ and $K_m$ values of the individual enzymes for AdoMet and AdoHcy, respectively. Methyltransferases that have low sensitivity to AdoHcy inhibition (LS-MTases) will continue to function at a relatively high rate, even when hyperhomocysteinemia is present, thus contributing to AdoHcy build-up. This will create conditions for the inhibition of activity of methyltransferases that are highly sensitive to inhibition by AdoHcy (HS-MTases). The mechanism for AdoHcy build-up and methyltransferase inhibition is indicated with boxes and arrows. Protein and DNA methylation are the methyl transfer reactions that have been found to be inhibited in uremic hyperhomocysteinemic patients.

dent effect on the interaction between RNA and the nuclear protein, down-regulating folate receptors.

In uremia, a resistance to folate therapy has been shown consistently. Plasma and red-cell folate levels are higher than normal in the face of hyperhomocysteinemia, as shown by Robinson et al.[5] Little is known about the relationship between folate levels, homocysteine levels, and folate-receptor synthesis in uremia but it is worth studying. It is possible that these findings by Antony et al[4] represent the basis for understanding this folate resistance in uremia.

In the past few years we have proposed and explored the unbalanced methylation hypothesis in uremia (Ingrosso D, et al,[8] Fig 3). The accumulation of the homocysteine precursor S-adenosylhomocysteine (AdoHcy), which occurs when homocysteine levels are high, leads to an inhibition of those methyltransferases that are more sensitive to the inhibitor AdoHcy (high-sensitive methyltransferases). The low-sensitive methyltransferases continue to consume adenosylmethionine (AdoMet) and produce AdoHcy to an almost normal extent, thus further maintaining inhibition of the high-sensitive methyltransferases.

For example, we showed some years ago that methylation-dependent membrane protein repair, a process in which a methylation reaction is involved, is inhibited in erythrocytes of uremic patients.[6,7]

In addition, we have shown that total DNA methylation is reduced in dialysis patients and the levels of decrease correlate significantly with plasma homocysteine levels.[8]

DNA methylation is viewed as a mechanism for gene silencing and regulation as, for example, in the case of imprinted genes. Considering the way through which genes are passed from 1 generation to another, the allele coming from 1 of the parents generally is shut off through methylation. Under normal conditions, the gene mode of expression therefore is termed *monoallelic* for these genes (the gene coming from either the mother or the father is expressed, the other is silenced in a nonrandom manner).

SYBL1 (a pseudoautosomal gene, X or Y inactivated) and H19 (an imprinted gene with maternal expression) are regulated as described previously. The allelic expression of these genes was used to test the functional outcome of DNA hypomethylation in uremic patients. Results show that, for SYBL1, gene expression in patients is biallelic: both alleles are expressed. For H19, only in patients with high homocysteine levels ($\gtrsim$60 $\mu$mol/L) is gene expression biallelic.

After folate therapy, gene expression returns to monoallelic and total DNA methylation improves in parallel with a decrease of homocysteine levels, thus testifying that homocysteine modifies DNA methylation in a reversible fashion. Therefore, we can state that in patients with higher homocysteine levels there is a transcriptional activation of the normally repressed allele caused by DNA hypomethylation. Folate treatment is able to revert the biallelic expression into monoallelic in the patients who had biallelic expression.

Plasma proteins in hemodialysis patients show a significant increase in the content of L-isoaspartyl residues; they are significantly altered, or damaged.[9]

This alteration can be repaired under normal conditions by a mechanism depending on a specific methyl transfer reaction.[10] This particular methyltransferase has been shown to be inhibited in uremia and, therefore, this kind of protein damage is increased.[6,9] This inhibition depends partially on high homocysteine levels and therefore methylation inhibition because folate therapy is able to reduce the level of damage.

However, the pathogenesis of this alteration, when considering the plasma protein compartment, depends mostly on uremic toxicity. Several uremic toxins from different chemical groups can induce protein damage. However, we found that guanidine in particular is able to elicit this protein damage in a dose-dependent manner. Deamidated albumin, that is, in vitro–damaged albumin, was prepared with a standard protocol, and the binding capacity of various substances to this damaged albumin were tested. A reduced binding of homocysteine to serum albumin was found.

We therefore can conclude that increased protein damage caused by the uremic milieu and hypomethylation induces protein damage, with reduced homocysteine binding to proteins and a possible increase in free homocysteine levels.[11]

Among the possible consequences of hyperhomocysteinemia, there is protein homocysteinylation, which is the binding of homocysteine to proteins, that occurs as a postbiosynthetic acylation of free amino groups (protein-N-homocysteinylation, mediated by homocysteine thiolactone). This protein modifica-

tion in in vitro experiments leads to functional derangements such as a loss of enzymatic activity.[12] Another type of protein homocysteinylation is through the formation of a covalent disulfide bond found primarily with cysteine residues (protein-S-homocysteinylation). This type of protein alteration, which is likely to occur in uremia because of the high homocysteine levels present in this condition, has not been investigated. If proven to be present in uremia it could be another example of the widespread presence of a derangement of the peptide link in uremia.

In conclusion, it can be stated that in chronic renal failure and end-stage renal disease both altered gene expression and the alterations in protein structure, dependent on hyperhomocysteinemia and acting through an increase of a homocysteine-related metabolite, may play a crucial role in terms of macromolecule functional derangement. We believe that very little is known about the interactions between high blood homocysteine levels and the genes, proteins, and receptors underlying homocysteine toxicity in uremia.

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