editorial

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# Homocysteine Species as Components of Plasma Redox Thiol Status

The incidence of premature cardiovascular disease is high in children or young adults with the inborn errors, homocystinuria, who have extremely high concentrations of homocysteine in blood (severe hyperhomocysteinemia >100 μmol/L) (*1*). This clinical observation has promoted a series of clinical studies in the general population on the relation between mild increases of plasma homocysteine (moderate hyperhomocysteinemia, <30 μmol/L) and cardiovascular disease (*2–4*). It is now established that moderate hyperhomocysteinemia is an important cardiovascular risk factor. The evidence has been summarized in a recent editorial in *Clinical Chemistry* (*5*) and in several review articles (*3, 4, 6*).

Plasma homocysteine is also a clinically useful indicator of vitamin function. Most patients with folate or cobalamin deficiency have moderate to intermediate hyperhomocysteinemia (30–100 μmol/L), which is corrected after supplementing the deficient vitamin (*7, 8*).

Most clinical studies of plasma homocysteine are based on measurement of total homocysteine, which encompasses the predominant protein-bound fraction, the free oxidized fraction (symmetric or mixed disulfides), and the small amounts of free reduced form (the sulfhydryl form) (*8*) (Fig. 1). The total homocysteine concentration is not influenced by the rapid oxidation and redistribution of homocysteine species that take place in freshly prepared plasma. Reliable blood sampling procedures for total homocysteine have been established, and the analysis is sufficiently robust to be included in routine laboratory testing (*8*).

Mansoor et al. (*9*) recently developed techniques for quantifying the separate forms of homocysteine in plasma. These assays are based on trapping the reduced species by collecting blood directly into tubes containing sulfhydryl-reactive reagents such as monobromobimane or *N*-ethylmaleimide and then immediately removing the blood cells (*9*).

Recently, Andersson et al. (*10*) described an alternative strategy to measure reduced, free oxidized, and protein-bound homocysteine and other aminothiols in human plasma. The blood was immediately chilled, and the blood cells were removed by centrifugation within 1 min. The sulfhydryl species in the plasma fraction were protected against oxidation by adding sulfosalicylic acid, thereby lowering pH (*10*). Now, in this issue of *Clinical Chemistry* (*11*), the same authors study the kinetics and temperature dependence of the changes of aminothiol concentrations in whole blood in vitro and the rapid oxidation of the reduced species in the isolated plasma fraction. Based on their results, firm recommendations on blood sampling and processing can be made (*11*). Notably, under optimal sample collection and processing, they obtained values for aminothiols in plasma that agreed with those measured by the method of Mansoor et al. (*9, 11*). Consistent data based on different procedures to prevent rapid oxidation of sulfhydryl groups validate these techniques, but do not exclude the possibility that further differentiation of aminothiol species may be obtained by detailed assessment of reaction kinetics of sulfhydryl groups in freshly prepared plasma.

The complex terminology and the plasma concentrations of the separate homocysteine forms in healthy subjects are summarized in Fig. 1.

The analytical techniques referred to also measure reduced, free oxidized, and protein-bound concentrations of cysteine, cysteinylglycine, and glutathione in plasma (*9–11*). In healthy subjects, cysteine is the most abundant plasma aminothiol (total concentration, ~250 μmol/L); about 65% is protein-bound, 30% is free oxidized, and 3–4% is reduced. The plasma concentration of cysteinylglycine is less (total, ~30 μmol/L); about 60% is protein-bound, 30% is free oxidized, and 10% is reduced. Most (65%) plasma glutathione (total concentration, 6 μmol/L) is in the reduced form (*9–11*), but its high intracellular concentration (*12*) makes the determination unreliable in the presence of hemolysis. The concentrations of these compounds plus homocysteine species in plasma comprise the plasma redox thiol status (Fig. 2).

My colleagues and I have measured the redox status and protein-binding of homocysteine, cysteine, and cysteinylglycine in healthy subjects before and during methionine (*13, 14*) or homocysteine (*15*) loading, in patients with intermediate hyperhomocysteinemia due to cobalamin deficiency (*16*), and in homocystinuria patients with severe hyperhomocysteinemia (*17*). These studies have demonstrated that reduced homocysteine is low under physiological conditions, but increases as a function of total homocysteine; moreover, alteration in the redox status of homocysteine rapidly affects and is related to the redox status of other plasma aminothiols. These observations may be explained by continuous redox and disulfide exchange reactions in plasma. Finally, increasing amounts of homocysteine decrease the amount of protein-bound cysteine, probably by displacement from saturable binding sites in plasma.

Andersson et al. (*11*) here provide preliminary data on perturbed redox thiol status in 19 patients with cerebral infarction. These patients as a group had moderate hyperhomocysteinemia, as has been consis-

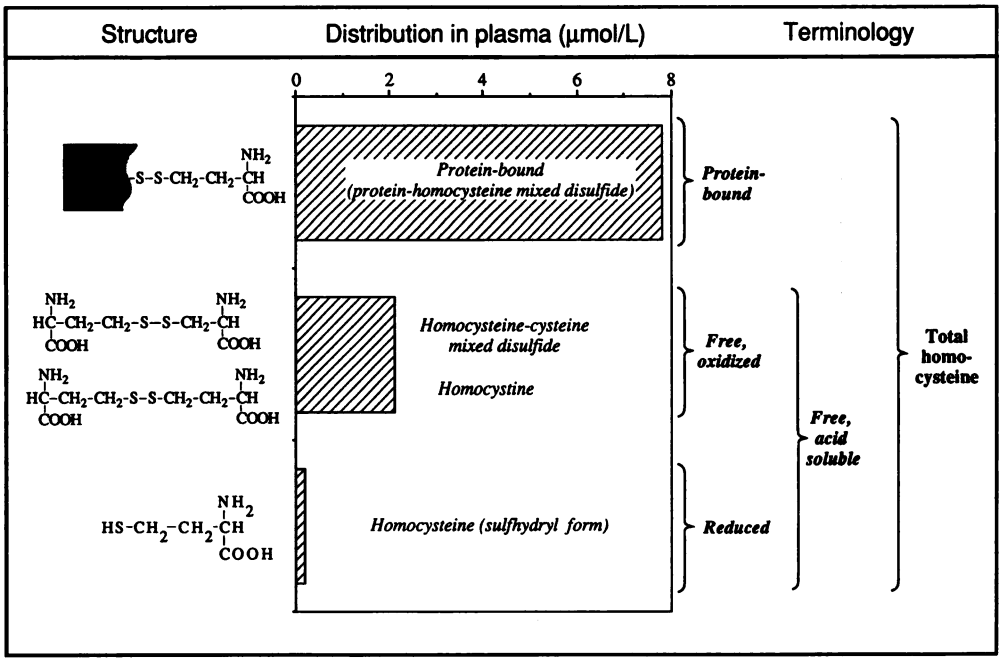


Fig. 1. Structure and distribution of different forms of homocysteine in human plasma.

tently found in several studies (*3, 4, 6*), but also had a high concentration of total cysteine, as previously reported in a Japanese study (*18*). A remarkable finding was a tendency toward lower concentrations of reduced cysteine and cysteinylglycine in these patients than in the controls (*11*). We recently made a similar observation in 60 patients with early-onset peripheral vascular disease. Both during fasting and after methionine loading, our patients had a significantly greater concentration of total homocysteine and total cysteine than did the controls, whereas reduced cysteine and the reduced/total ratio after fasting and postload were significantly lower in the patients (*14*).

Concentrations of both homocysteine and cysteine are consistently reported as increased in patients with renal failure (*8*). Hultberg et al. recently found a low reduced/total ratio for homocysteine and cysteinylglycine and also a trend towards a low ratio for cysteine in patients with renal failure (*19*). We have observed similar trends in 10 renal patients on hemodialysis (Guttormsen, unpublished).

The data on disturbed redox plasma thiol status in cardiovascular (*11, 14*) and renal patients (*19*) are based on a small number of patients, and should be confirmed in larger studies. Other patient categories should be investigated, particularly states characterized by increased formation of reactive oxygen species (*20*), e.g., in AIDS, with certain types of cancer, or during treatment with drugs with antioxidant properties.

Increased concentrations of homocysteine induce secondary effects on redox status and protein binding of other aminothiols in plasma. Notably, these changes can be demonstrated within minutes (after homocysteine loading), and the secondary changes are essentially independent of the cause of hyperhomocysteinemia, i.e., enhanced homocysteine formation, or inhibition of homocysteine remethylation or homocysteine catabolism (*13–17*). These alterations are probably mediated by disulfide exchange and displacement reactions within the plasma compartment.

Conditions causing increased oxidative stress have been reported to consume extracellular antioxidants, including vitamin C, protein thiols, bilirubin, urate, and α-tocopherol (*21*). Little attention has been paid to the high amount of reduced cysteine as a component of the extracellular antioxidant defense system (*22*). Reduced cysteine represents the most abundant low-molecular-mass thiol component in plasma, amounting to ~10 μmol/L in healthy fasting subjects (*9*) (Fig. 2). Low concentrations in patients with vascular disease (*11, 14*) or possibly in patients with renal failure (*19*) may reflect increased overall pro-oxidant activity, which may convert reduced forms of cysteine and other aminothiols to their corresponding disulfides. Notably, substantial evidence indicates that progression of atherosclerosis is related to enhanced pro-oxidant activity (*23*), and consistent reports on redox imbalance in uremic patients possibly reflect accumulation of oxidative waste (*24, 25*).

Thus, altered plasma thiol redox status may be brought about by at least two different mechanisms: (*a*) A primary hyperhomocysteinemia will directly affect the redox status and protein binding of other aminothiols; (*b*) conditions creating a pro-oxidant environment in plasma may influence thiol–disulfide redox cycling by favoring disulfide formation. It is feasible that both mechanisms can operate simultaneously.

Changes in redox status of aminothiols in plasma probably affect the sulfhydryl residues of available proteins or enzymes via thiol–disulfide exchange reactions and may thereby alter their function. Conversely,

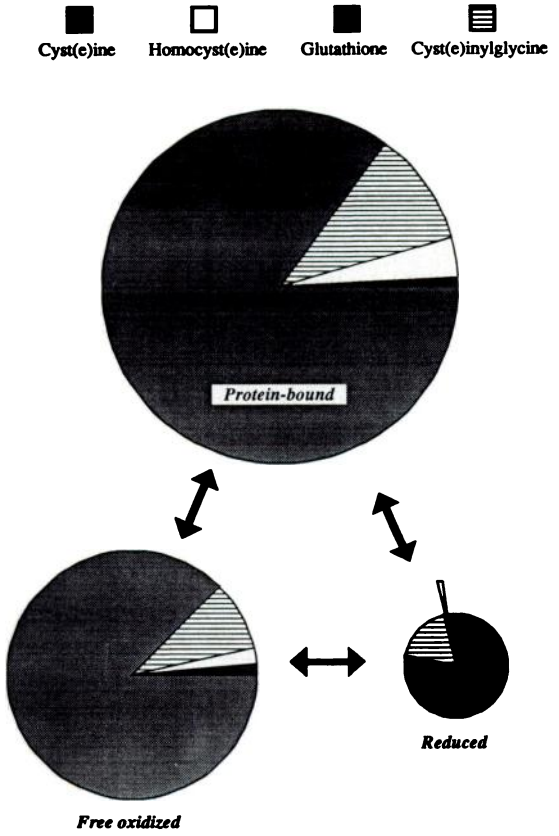


Fig. 2. Redox thiol status in human plasma.

The pie charts illustrate the amount of protein-bound aminothiols, free oxidized aminothiols, and free sulfhydryl species existing in normal plasma. The areas within each circle and sector are proportional to the plasma concentration of the separate species. The *arrows* indicate interconversion between aminothiol forms by thiol–disulfide exchange reactions.

the redox status of aminothiols in plasma is probably an integral part of the extracellular antioxidant defense system and may be linked to intracellular redox status. A picture of a complex interactive system emerges, where changes of one component may cause multiple defects of cellular function.

Knowledge of the remote effect of hyperhomocysteinemia on the redox status of other aminothiols should guide future research on the mechanism behind the vascular lesions. Furthermore, the concentration of reduced cysteine in plasma should be addressed in both epidemiological and mechanistic studies of cardiovascular disease.

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