Colloquium: Homocyst(e)ine, Vitamins and Arterial Occlusive Diseases

Reduced, Oxidized and Protein-Bound Forms of Homocysteine and Other Aminothiols in Plasma Comprise the Redox Thiol Status—A Possible Element of the Extracellular Antioxidant Defense System[1]

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ABSTRACT Reduced, oxidized and protein-bound forms of homocysteine (Hcy), cysteine and cysteinylglycine in plasma interact via redox and disulphide exchange reactions, and these aminothiol species comprise a dynamic system referred to as redox thiol status. Notably, in plasma reduced cysteine is the most abundant low molecular weight sulfhydryl compound. Elevation of plasma Hcy (hyperhomocysteinemia) causes changes in redox thiol status. Protein-bound Hcy increases up to a maximum capacity of about 140 μmol/L, and there is a concurrent displacement of protein-bound cysteine. When the Hcy binding approaches saturation, free oxidized and reduced Hcy show a substantial increase. The resulting increase in reduced/total ratio for Hcy causes a parallel change in this ratio for the other aminothiols. These dynamics were observed during both chronic hyperhomocysteinemia (due to cobalamin deficiency or homocystinuria) and acute hyperhomocysteinemia (induced by methionine or Hcy loading). In addition, changes in redox thiol status have been observed in patients with vascular disease (decreased reduced/total ratio for cysteine), renal failure (low reduced/total ratio for aminothiols) or HIV infection (high level of reduced Hcy), which suggest primary imbalance between prooxidant and antioxidant processes in these patients. In conclusion, redox thiol status is a dynamic system which is probably linked to the extracellular antioxidant defence system. This must be taken into account when designing future experimental or epidemiological studies on Hcy and cardiovascular disease. J. Nutr. 126: 1281S–1284S, 1996.

INDEXING KEY WORDS:

- *homocysteine* • *aminothiol compounds*
- *redox status in plasma* • *vascular disease*
- *renal failure* • *HIV*

Elevated plasma total homocysteine (tHcy) is a risk factor for early onset cardiovascular disease (Boushey et al. 1995, Kang et al. 1992, Ueland et al. 1992) and a clinical useful marker for deficiencies of folate or cobalamin (Allen et al. 1994, Ueland et al. 1993). Impaired function of these vitamins is the most common cause of elevated plasma tHcy. Moderate increase is also observed in renal failure, whereas the inborn errors denoted homocystinuria cause large increase of plasma tHcy (Ueland et al. 1993).

Aminothiol species in normal plasma

The major aminothiols in human plasma are Hcy, cysteine and cysteinylglycine. Hcy in plasma exists in different forms, including the major protein-bound fraction (~65%), free oxidized fraction (~30%) where cysteine-Hcy mixed disulphide predominates and trace amounts (1.5–4%) of reduced Hcy. The total amount of the various Hcy species is ~10 μmol/l in healthy subjects. Plasma cysteine (total concentration of ~250

[1] Presented as part of the colloquium "Homocyst(e)ine, Vitamins and Arterial Occlusive Diseases" given at the Experimental Biology '95 meeting, Atlanta, GA, on April 13, 1995. This symposium was sponsored by the American Institute of Nutrition. Guest editors for the symposium were M. R. Malinow, Oregon Regional Primate Research Center, Beaverton, OR, and M. J. Stampfer, Harvard School of Public Health, Cambridge, MA.

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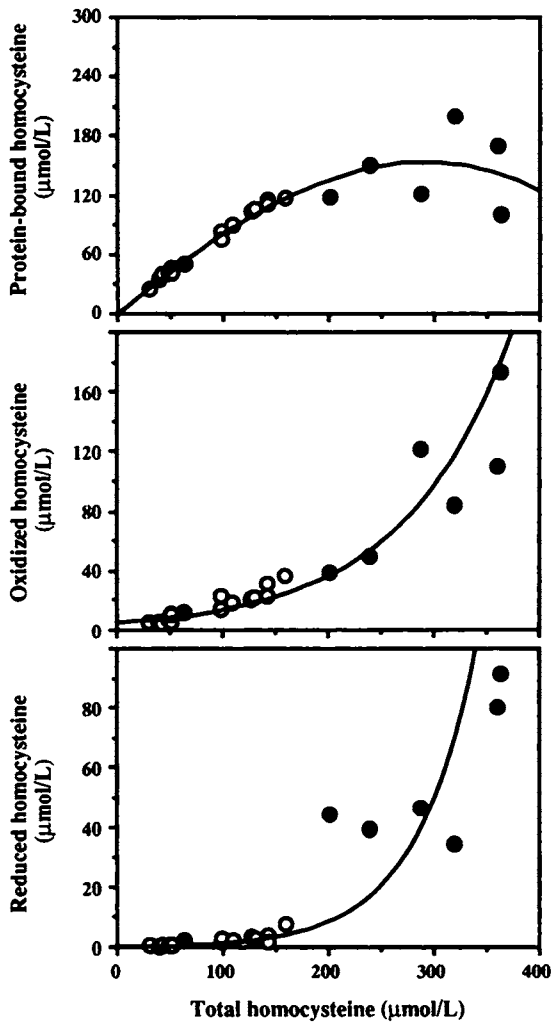

FIGURE 1 Protein-bound, free oxidized and free reduced homocysteine in plasma as a function of total homocysteine. The data are from 8 patients with homocystinuria (adapted from Mansoor et al. 1993) and 13 patients with cobalamin deficiency (adapted from Mansoor et al. 1994). ●, homocystinuria; ○, cobalamin deficiency.

μmol/l) and plasma cysteinylglycine (total concentration of ~30 μmol/l) are also distributed between reduced, oxidized and protein-bound forms (Andersson et al. 1993, Andersson et al. 1995, Mansoor et al. 1992b). These aminothiols and their interrelations comprise an entity referred to as plasma redox thiol status (Ueland 1995).

Dynamics and consequences of changes in redox thiol status during hyperhomocysteinemia

The concept of plasma redox thiol status is based on the assumption of a dynamic relation between plasma aminothiol species through thiol-disulfide exchange and redox reactions. We evaluated this hypothesis by investigating aminothiol species in healthy subjects and in patients with hyperhomocysteinemia. The four subject categories studied include healthy subjects with normal fasting tHcy concentrations subjected to either a methionine (Mansoor et al. 1992a) or Hcy loading (Mansoor et al. 1993) resulting in a transient hyperhomocysteinemia, patients with moderate hyperhomocysteinemia (tHcy <30 μmol/l) and cardiovascular disease (Mansoor et al. 1995) and patients with intermediate (30–100 μmol/l) or severe (plasma tHcy > 100 μmol/l) hyperhomocysteinemia due to cobalamin deficiency (Mansoor et al. 1994) or homocystinuria (Mansoor et al. 1993).

The mechanisms of the hyperhomocysteinemia in these subjects were quite different, i.e., enhanced entry of Hcy into the plasma compartment in patients loaded with Hcy or methionine, impaired Hcy remethylation in cobalamin-deficient patients and inhibition of Hcy catabolism due to cystathionine β-synthase deficiency. We recognized the following phenomena, suggesting homeostatic mechanisms that may operate both under physiological and pathological conditions: *1*) Protein-bound Hcy increases as a function of tHcy but approaches a maximal binding of ~140 μmol/l, suggesting saturation of binding sites (**Fig. 1**). Thus, protein binding may buffer moderate fluctuation in circulating tHcy and thereby protect vulnerable structures including the endothelial cells against high levels of circulating Hcy. *2*) An equivalent reduction of protein-bound cysteine was observed, indicating displacement of cysteine from its binding sites. *3*) Both free oxidized and in particular reduced Hcy increase exponentially as a function of tHcy, and the increase is most pronounced after the binding sites have become saturated (Fig. 1). *4*) There is a linear, positive relation between the reduced:total ratio for Hcy vs. the ratio for cysteine (**Fig. 2**), which in turn is positively related to the reduced:total ratio for cysteinylglycine. This observation may be explained by continuous redox and disulphide exchange reactions in plasma. *5*) Changes in redox equilibrium between the plasma aminothiol species take place within minutes, as judged by data obtained after Hcy injection (Mansoor et al. 1993). These observations indicate that changes in the concentration and redox status of one aminothiol may have rapid and remote

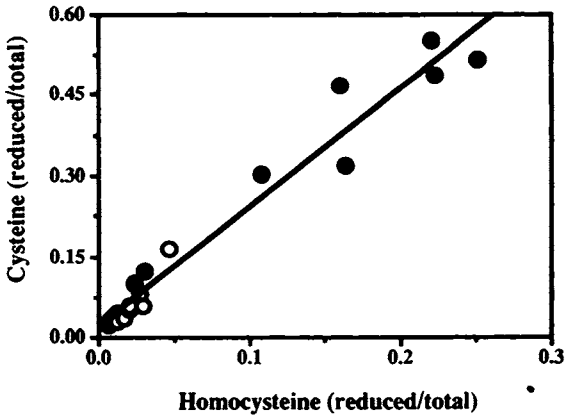

FIGURE 2 The relation between the redox status for homocysteine and cysteine. The data are from 8 patients with homocystinuria (adapted from Mansoor et al. 1993) and 13 patients with cobalamin deficiency (adapted from Mansoor et al. 1994). ●, homocystinuria; ○, cobalamin deficiency.

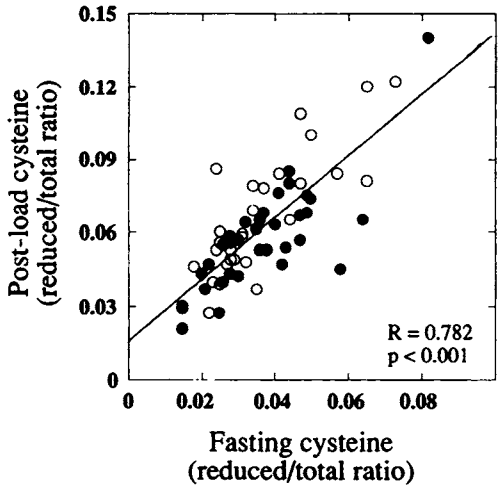

FIGURE 3 Redox status for cysteine during fasting and after methionine loading in 61 patients with early onset peripheral vascular disease (adapted from Mansoor et al. 1995). ●, male patients; ○, female patients.

influence on other sulfhydryl groups, including those essential for the function of enzymes or structural proteins. Such cascade effects may be relevant to mechanism(s) behind the vascular damage observed in patients with hyperhomocysteinemia.

Plasma redox thiol status in cardiovascular disease

There are consistent reports based on more than 4000 patients and a comparable number of controls that atherosclerotic disease in the coronary, cerebral and peripheral vessels are associated with moderate hyperhomocysteinemia (Boūshey et al. 1995), and one Japanese study has reported on high concentrations of total cysteine in patients with cerebral infarction (Araki et al. 1989). Two recent reports confirm and extend these observations by measuring plasma redox thiol status in patients with early onset peripheral vascular disease (Mansoor et al. 1995) or cerebral infarction (Andersson et al. 1995). The most notable findings were lower fasting and postload concentrations of reduced cysteine in 61 patients with peripheral arteriosclerosis compared with healthy controls. A similar tendency was observed in 19 subjects with cerebrovascular disease.

We observed a strong positive correlation between the reduced:total ratios for cysteine in vascular patients before and after methionine loading (**Fig. 3**). This is a notable finding, suggesting that the plasma redox thiol status is a stable biochemical trait that may reflect prooxidant activity in plasma of these patients. We suggest that altered redox thiol status in vascular patients should be considered in the light of the antioxidant-atherosclerosis hypothesis, which has been substantiated by both observational and intervention studies, demonstrating that antioxidants like tocopherol, vitamin C and β-carotene confer protection against cardiovascular disease (van Poppel et al. 1994, Witztum 1994).

Plasma redox thiol status in other diseases

Several studies show elevated plasma tHcy and cysteine in patients with renal failure (Ueland et al. 1993), but only sparse data exist on redox thiol status in these patients. Hultberg et al. (1995) recently demonstrated low reduced:total ratio for Hcy and cysteinylglycine and a similar trend for cysteine in patients with renal failure. We made similar observations in 10 patients with renal failure. The reduced:total ratios for cysteine and cysteinylglycine were low compared with healthy subjects. After hemodialysis, which markedly reduced plasma tHcy and total cysteine, the ratios were increased by 75% (Guttormsen 1995, unpublished). Perturbed redox thiol status in uremic patients may reflect accumulation of oxidative waste (Canestrari et al. 1994, Costaglio et al. 1989).

There is evidence of impaired cobalamin absorption in AIDS patients (Herbert et al. 1990), but previous studies demonstrated normal concentrations of the cobalamin markers serum methylmalonic acid (Hagelskjaer et al. 1991) and plasma tHcy (Jacobsen et al. 1990). We recently confirmed the observation of normal tHcy in 22 patients with HIV infection but found a remarkable elevation of reduced Hcy that was 3 times higher (0.49 μmol/l) than in healthy subjects (0.15 μmol/l). Only one HIV-infected patient had a concentration of reduced Hcy within the range of controls (Müller et al. 1996). Whether plasma Hcy in these patients is trapped as a particular chemical form reacting with the derivatizing agent or exists as authentic reduced Hcy in the circulation is uncertain (Müller et al. 1995). Increased concentration of reduced Hcy may be related to the increased production of reactive oxygen species and the impaired antioxidant status in HIV-infected patients (Baker and Wood 1992, Staal et al. 1992).

Future studies should investigate the possible relation between plasma redox thiol status and development of cardiovascular disease in renal patients and its relation to disease progression in HIV-1 infected patients. Other patient categories should also be investigated, in particular those with diseases characterized by increased formation of reactive oxygen species or impaired antioxidant defence (Halliwell 1994).

Possible mechanisms of altered plasma redox thiol status

Diverse biochemical conditions may cause altered plasma redox thiol status, and at least two different mechanisms can be distinguished on the basis of data summarized above. It has been unequivocally demonstrated that hyperhomocysteinemia induces rapid changes in plasma redox thiol status, probably via thiol-disulphide exchange and redox reactions. These changes seem independent of whether the tHcy elevation is due to increased formation or inhibition of Hcy remethylation or catabolism. The moderate increase in

tHcy in cardiovascular patients and increased tHcy and total cysteine in renal failure may result from impaired aminothiol metabolism or clearance. However, disturbed balance between reduced and oxidized species of aminothiols in vascular, renal and HIV patients suggests additional mechanisms. Conceivably, low reduced:total ratio of plasma aminothiols may reflect increased prooxidant activity due to impaired function of extracellular antioxidants, including vitamin C, protein thiol, bilirubin, ureate and alpha-tocopherol (Halliwell 1994). A selective increase in the reduced species may suggest increased amount of thiol reactive component(s) converting sulfhydryl groups into forms available to derivatization without prior reduction.

Conclusion

Reduced, free-oxidized and protein-bound forms of Hcy, cysteine and cysteinylglycine comprise the plasma redox thiol status. Altered concentration of one species causes instant effect on other components, probably via displacement, thiol-disulphide exchange and redox reactions. In addition, components of the extracellular antioxidant defense system as reviewed by Halliwell and Gutteridge (1990) and the plasma aminothiol species are probably mutually interactive. Notably, reduced cysteine is the most abundant low molecular weight thiol in plasma and may represent an important extracellular antioxidant. These concepts should influence future experimental as well as epidemiological studies on Hcy and other aminothiols as risk factors for early onset cardiovascular disease.

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