Citation Classic

Total Plasma Homocysteine:
The Mediator/Marker Controversy Continues

Donald W. Jacobsen[1]*

Featured Article: Jacobsen DW, Gatautis VJ, Green R, Robinson K, Savon SR, Secic M, et al. Rapid HPLC determination of total homocysteine and other thiols in serum and plasma: sex differences and correlation with cobalamin and folate concentrations in healthy subjects. Clin Chem 1994;40:873–81.[2]

My interest in homocysteine metabolism began in the late 1960s when I was a postdoctoral fellow in the Department of Biochemistry at Scripps Clinic and Research Foundation (now the Scripps Research Institute) working on B_{12}-dependent enzymes. In 1969 Kilmer McCully reported an association between increased homocysteine and premature cardiovascular disease in 2 patients with homocystinuria (1). Although assays for total plasma homocysteine (tHcy), the sum of reduced and oxidized homocysteine species, were yet to be developed, homocystinuria was generally assumed to be a condition in which blood concentrations of homocysteine were highly increased. In 1985 Refsum et al. described a radioenzymatic assay for tHcy (2) that made it possible to assess the association of mild hyperhomocysteinemia with the risk for the development and progression of cardiovascular disease. During the late 1980s several groups, including ours, independently developed assays for tHcy based on HPLC with fluorescence detection and HPLC with electrochemical detection. It soon became apparent that mild hyperhomocysteinemia was indeed an independent risk factor for coronary artery disease, cerebrovascular disease, and peripheral vascular occlusive disease.

In the featured 1994 report we clearly established that in healthy volunteers tHcy concentrations were higher in serum than in EDTA plasma. We now know that substantial amounts of homocysteine are exported from red blood cells during the 1-h clotting period needed to prepare serum, whereas little or no export occurs in EDTA-treated blood maintained at 4 °C before centrifugation. We also established that serum and plasma homocysteine concentrations are higher in males than in premenopausal females. Finally, we demonstrated an inverse correlation between serum B_{12} and tHcy and between serum folate and tHcy, suggesting that the 2 B-complex vitamins are important determinants of tHcy in healthy individuals. It is now well established that folate deficiency causes hyperhomocysteinemia, and B_{12} deficiency causes both hyperhomocysteinemia and methylmalonic acidemia, which are often combined. Studies from numerous groups, including our own, showed that age was also a major determinant of tHcy. We are born with a tHcy of around 5 μmol/L and, if we live to be 100, our tHcy at that age is likely to be approximately 25 μmol/L.

We developed this assay to establish the incidence of hyperhomocysteinemia in patients with coronary artery disease (3) and end-stage renal disease (4) and in heart transplant recipients (5). These studies established that tHcy is an independent risk factor for cardiovascular disease and that in coronary artery disease the degree of risk increases with increasing tHcy with no threshold effect (3). In 1995 Shipchandler and Moore introduced a fully automated assay for tHcy using the Abbott IMx analyzer (6), allowing many clinical research centers to confirm that an increased tHcy was indeed a risk factor for cardiovascular disease.

Although increased tHcy is an independent modifiable risk factor for cardiovascular disease, complications of pregnancy (preeclampsia and neural tube defects), cognitive dysfunction (dementia and Alzheimer disease), and hip fracture due to osteoporosis, there is considerable controversy surrounding the role of homocysteine in the development of these diverse pathologies. Is homocysteine a mediator of disease, or is it merely a marker of the disease process? Because tHcy concentrations can be decreased by treatment with folic acid or folic acid combined with vitamin B_{12} (cyanocobalamin) and vitamin B_6 (pyridoxine), numerous secondary intervention homocysteine-lowering trials have been completed or are nearing completion. The results with respect to coronary artery disease have been disappointing. However, lowering of homocysteine in patients with known cardiovascular disease appears to reduce the overall incidence of stroke. The mediator-marker controversy surrounding

[1] Cell Biology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH.
[2] This paper has been cited more than 275 times since publication.
* Address correspondence to the author at: Cell Biology, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH, 44195. Fax 216-444-9404; e-mail jacobsd@ccf.org.

homocysteine will continue to drive basic and clinical research on this modifiable independent risk factor.

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