Importance of Chemical Reduction in Plasma and Serum Homocysteine Analysis

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In 1977, as part of the research on which I based my PhD thesis, I purified S-adenosylhomocysteine hydrolase to homogeneity, and in the years that followed, my colleagues and I studied its role in the metabolism of S-adenosylhomocysteine in cells and animals treated with adenosine analogs. During these experiments, we gained knowledge about the catalytic properties of the enzyme, which enabled us to construct an enzymic assay for homocysteine in rat and mouse tissues as well as in plasma from mice (1). When this methodological work was being carried out in 1982, data on homocysteine were sparse and addressed limited aspects of homocysteine in health and disease, such as homocyst(e)ine in patients with the inborn error of homocystinuria, increased homocyst(e)ine (often the homocysteine-cysteine mixed disulfide) in patients with cardiovascular disease (CVD), and the so-called methionine dependence of some cancer cells (2).

In the paper on homocysteine in tissues (1), we concluded that the method “should stimulate clinical investigations on homocysteine in plasma of humans under various pathological conditions and of patients receiving therapy with drugs interfering with one-carbon metabolism.” To accomplish these goals, we had to modify the assay to be able to measure homocysteine in human plasma or serum, in which this aminothiol undergoes disulfide-exchange reactions with cysteine and plasma proteins. Disulfide-exchange reactions also take place in vitro in plasma or serum samples, causing an artificial decrease in free, non–protein-bound homocysteine during storage. These problems were overcome by use of the radioenzymic assay described in the 1985 report highlighted here. The assay measures total homocysteine (tHcy), which is the sum of all homocysteine species (reduced and mixed disulfides with cysteine and albumin) after conversion to homocysteine by chemical reduction. Later, we and other investigators developed additional assays for tHcy, including methods based on HPLC and fluorescence detection, HPLC-MS/MS, GC-MS, and enzymatic-based immunoassays (3).

The novel methods for tHcy also had higher throughput and better precision than methods based on a conventional amino-acid analyzer. These improvements enabled us to carry out clinical studies using large numbers of stored samples. We first addressed the question of whether tHcy could serve as a marker of folate status in patients with psoriasis, rheumatoid arthritis, or cancer who were treated with a wide range of doses of the antifolate agent methotrexate (3).

From the late 1980s there was a growing interest in tHcy as a marker of folate or cobalamin deficiency, as an indicator of unhealthy lifestyle, and as a risk factor for CVD (3). Numerous case-control and prospective studies on tHcy and CVD were published, and demonstrated increased CVD risk associated with increased tHcy. These results inspired the planning of intervention studies on CVD risk and decreasing tHcy with B-vitamins. A few years later, associations of increased tHcy with other diseases were reported, including dementia, impaired cognitive function, adverse pregnancy outcomes, neural tube defects, and (recently) osteoporosis.

The first intervention studies on decreasing tHcy by B-vitamin supplementation have been completed, but the results are inconsistent (4), most demonstrating no protection against CVD in patients followed for up to 3 years, and possible protection against stroke from folic acid treatment lasting more than 3 years.

The development of methods to measure tHcy in serum or plasma, including the radioenzymic assay, created the methodological foundation for numerous population-based and clinical studies on
the health implications of increased tHcy. The role of tHcy as a useful marker of B-vitamin deficiencies and predictor of chronic diseases has been established, but the question of whether homocysteine itself is the culprit, or merely an epiphenomenon, is still open. tHcy has a place in future studies addressing causality and disease prevention. But when planning such studies, one has to concede the complexity of nature by measuring tHcy in conjunction with other metabolites and vitamins related to one-carbon metabolism (5).

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References


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<thead>
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<th>Structure</th>
<th>Concentration in plasma (μmol/L)</th>
<th>Terminology</th>
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<tbody>
<tr>
<td>Albumin</td>
<td></td>
<td>Protein-bound (bHcy)</td>
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<td></td>
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<td>Protein-homocysteine mixed disulfide (Hcy-albumin)</td>
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<td>Homocysteine-cysteine mixed disulfide (Hcy-Cys)</td>
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<td>Total homocysteine (tHcy)</td>
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<td>Homocysteine (HcyH)</td>
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Homocysteine exists in a number of species in plasma.

In the 1985 paper by Refsum et al., the authors used HPLC gel filtration to demonstrate that albumin was the carrier of homocysteine in plasma. In order to quantify total plasma homocysteine, the reducing agent dithioerythritol was used to release homocysteine from plasma proteins, homocysteine-cysteine mixed disulfide, and homocysteine. Chemical reduction of homocysteine species still remains the standard approach for quantifying total homocysteine. Figure modified from (6).