HOMOCYSTEINE IN PLASMA

June 2013

Introduction

Homocysteine (Hcys) is a non-essential, thiol-containing amino acid which is produced from the intracellular de-methylation of dietary methionine [1]. Hcys is exported from cells into the extracellular fluid at a slow but consistent rate and circulates in the blood mainly in the oxidised form, bound to plasma proteins. There are two major pathways for its further metabolism – trans-sulphuration to cysteine (by a vitamin B6-dependent pathway) or re-methylation to methionine (by a vitamin B12 and folic acid-dependent pathway). Vitamin B2 is also required for homocysteine metabolism on account of its role in the re-cycling and maintenance of folic acid levels. Excess homocysteine accumulates in the plasma when any of these pathways is not operating at optimum activity.

Homocysteine is an unusual amino acid in that it is not used for protein synthesis but is produced as a metabolic intermediate, high levels of which may disturb aspects of cell physiology.

Vitamins and homocysteine

The following vitamins are required for homocysteine metabolism:

- Folic acid (5-methyltetrahydrofolate) – as a methyl donor,
- Vitamin B_{12} (methylcobalamin) – as a cofactor for the enzyme methionine synthase (MS), [which re-methylates Hcys back to methionine],
- Vitamin B_6 (pyridoxal phosphate) - as a cofactor for the enzymes cystathionine b-synthase [on the trans-sulphuration pathway; Hcys to cystathionine], cystathionase [also on trans-sulphuration pathway; cystathionine to cysteine], and serine hydroxymethyltransferase [conversion of serine to glycine],
- Vitamin B_2 (FAD) – as a cofactor for methylenetetrahydrofolate reductase (MTHFR) [5,10- methyl THF to 5-methyl THF, which is part of the folate cycle].

The inverse relationship between plasma homocysteine and folic acid, B12, B2 (riboflavin) and B6 (pyridoxine) has been known for some years. The current concern is that deficiencies of folic acid, vitamin B12 and vitamin B6 can contribute to moderate hyper-homocysteinemia, which may be an independent risk factor for coronary heart disease and for a variety of other conditions, including cognitive decline [2,3].

Pathways for homocysteine metabolism:

- Irreversible catabolism to **cysteine** by trans-sulphuration - a vitamin B_6-dependent pathway (i.e. pyridoxine is a co-factor)
- Re-methylation to **methionine** by methionine synthase - a vitamin B_{12}- and folate-dependent pathway (methylcobalamin is required and is generated by the methylation of cob(I)alamin by 5-methyltetrahydrofolate).

Excess homocysteine which cannot be directly metabolized is removed from the cell and accumulates in the plasma when the mechanisms described above are impaired. This is a potential cause of the endothelial dysfunction which is characteristic of subjects with moderate to high plasma homocysteine levels.

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Homocystinuria (cystathionine-beta-synthase deficiency)

McCully [4] made the original clinical observation linking elevated plasma homocysteine concentrations with vascular disease. He reported autopsy evidence of extensive arterial thrombosis and atherosclerosis in two children with elevated plasma homocysteine concentrations and homocystinuria. In homocystinuria there is deficiency of the enzyme cystathionine-beta-synthase, a high plasma methionine and clinical symptoms that may take some years to develop, but which include lethal venous thromboses. On the basis of this observation, he proposed that elevated plasma homocysteine can cause atherosclerotic vascular disease.

Subsequent work confirmed Mc-Cully's hypothesis and showed that hyper-homocysteinemia is an independent risk factor for atherosclerosis and atherothrombosis [5,6]. Although severe hyper-homocysteinemia is a rare condition, mild hyper-homocysteinemia has been reported in between 5 and 7 percent of the general population [7,8]. Subjects with mild hyper-homocysteinemia are typically asymptomatic until the third or fourth decade of life when premature coronary artery disease develops, together with recurrent arterial and venous thrombosis.

Homocysteine and cognitive dysfunction.

Moderately high plasma homocysteine levels are associated with cognitive decline and the data suggest that homocysteine is a modifiable cause of cognitive dysfunction [9,10,11,12]. Although there is speculation regarding the mechanism through which homocysteine may influence cardiovascular or cognitive health, Hcys has been shown to promote vascular endothelial cell injury and neuronal defects [13,14,15].

Indications

Plasma homocysteine concentrations can therefore be read as a functional marker of the deficiency of folic acid, vitamin B12, vitamin B6 and vitamin B2. Hcys measurement is indicated in subjects with micronutrient deficiencies or at risk from vascular disease or cognitive decline – a substantial proportion of the ageing population.

Specimen requirements

Non-fasting whole blood samples, taken into purple top EDTA tubes containing a special inhibitor (available from Biolab on request). Synthesis of homocysteine continues in red blood cells *ex-vivo* after blood sampling, unless it is inhibited [3]. If posted samples must reach Biolab within 48 hours.

Interpretation

Men, on average, have a slightly higher plasma homocysteine level than women [3]. This is thought to be associated with greater muscle mass, which increases the demand on the labile methyl pool for the synthesis of creatine.

The reference interval is less than 12.0 μmoles/L of plasma homocysteine, but the treatment goal varies (typically less than 7.0 μmoles/L of plasma homocysteine). An elevation of the plasma homocysteine by 5.0 μmoles/L has been associated with an increase of 1.7 in the odds ratio risk of cardiovascular disease (the same as an increase of 500 μmol/l in plasma cholesterol [16].

Methodology: Plasma homocysteine is measured by high-pressure liquid chromatography.

Turnaround time: 5 working days.

References

1. Finkelstein JD. Methionine metabolism in mammals. J Nutr Biochem 1990;5:228-237.
2. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. Br Med J 2002;325: 1202-1208.
3. Perry DJ. Hyperhomocysteinaemia. Baillieres Best Pract Res Clin Haematol 1999;12 :451-477.
4. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56:111-28.
5. Kang SS, Wong PW, Malinow MR. Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. Ann Rev Nutr 1992;12:279-98.
6. Stampfer MJ, Malinow MR, Willett WC, et al. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. JAMA 1992;268:877-81.
7. Ueland PM, Refsum H. Plasma homocysteine, a risk factor for vascular disease: plasma levels in health, disease, and drug therapy. J Lab Clin Med 1989;114:473-501.
8. McCully KS. Homocysteine and vascular disease. Nat Med 1996;2:386-9.
9. Duthie SJ, Whalley LJ, Collins AR *et al.* Homocysteine, B vitamin status, and cognitive function in the elderly. Am J Clin Nutr 2002;75: 908-913.
10. Prins ND, Den Heijer T, Hofman A *et al.* Homocysteine and cognitive function in the elderly: The Rotterdam Scan Study. Neurology 2002;59: 1375-1380
11. Miller JW, Green R, Ramos MI *et al.* Homocysteine and cognitive function in the Sacramento Area Latino Study on Aging. Am J Clin Nutr 2003;78: 441-447.
12. Teunissen CE, Blom AH, Van Boxtel MP *et al.* Homocysteine: A marker for cognitive performance? A longitudinal follow-up study. J Nutr Health Aging 2003;7: 153-159.
13. Austin RC, Lentz SR, Werstuck GH. Role of hyperhomocysteinemia in endothelial dysfunction and atherothrombotic disease. Cell Death Differ 2004;11: S56-S.
14. Suhara T, Keisuke F, Yasuda O *et al.* Homocysteine enhances endothelial apoptosis via upregulation of Fas-mediated pathways. Hypertension 2004;43: 1208-1213.
15. Robert K, Santiard-Baron D, Chasse JF *et al.* The neuronal SAPK/JNK pathway is altered in a murine model of hyperhomocysteinemia. J Neurochem 2004;89: 33-43.
16. Jacobsen DW, Gatautis VJ, Green R et al (1994). 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 40:873-881