Homocysteine and Its Effects on In-Stent Restenosis
Giuseppe De Luca, MD, PhD; Harry Suryapranata, MD, PhD; Giovanni Gregorio, MD; Helmut Lange, MD, PhD; Massimo Chiariello, MD, PhD

Despite the significant reduction in restenosis observed with the use of bare metal stents,1,2 results are still unsatisfactory for high-risk subsets of patients, such as those with diabetes, long lesions, small vessels, bifurcations, and restenotic lesions,3,4 with a restenosis rate up to 30% to 50%. Mounting interest emerged about hyperhomocystinemia as an independent risk factor for atherothrombotic disease,5–9 and several experimental studies have shown that it may affect in-stent restenosis.10–12

Homocysteine is an intermediary amino acid formed by the conversion of methionine to cysteine (Figure 1). Normal homocysteine plasma levels range between 5 and 15 μmol/L, and hyperhomocystinemia levels have been classified as moderate (15 to 30 μmol/L), intermediate (30 to 100 μmol/L), or severe (>100 μmol/L).13 However, normal basal homocysteine does not exclude an abnormality of this metabolic pathway. Such subtle abnormalities can potentially be uncovered by the use of methionine-load test.14

Severe hyperhomocystinemia is a rare genetic disorder characterized by marked elevations in plasma and urine homocysteine concentrations that are associated with osteoporosis, ocular abnormalities, developmental delay, thromboembolic disease, and severe premature atherosclerosis. Less marked elevations in plasma homocysteine (15 to 30 μmol/L) are much more common, occurring in 5% to 7% of the population.14

An example of a patient with mild hyperhomocystinemia is a 50-year-old man who was hospitalized for non-ST-segment elevation myocardial infarction. There were no major risk factors for coronary artery disease. Angiography showed a long subocclusive stenosis (>50 mm) in the proximal-mid right coronary artery. The patient underwent stent implantation of the right coronary artery. After 5 months, he was rehospitalized for new-onset angina. Repeat angiography showed a significant in-stent restenosis. Homocysteine was screened and found mildly elevated (22.1 μg/dL). Two major questions might emerge from this clinical case: (1) Was restenosis due to mildly elevated homocysteine? (2) Would in-stent restenosis have been prevented by homocysteine-lowering therapy?

Underlying Cause of Hyperhomocystinemia
Two major pathways may be identified in the metabolism of homocysteine: transsulfuration and remethylation (Figure 1), with the involvement of several vitamins. Elevations in the plasma homocysteine concentration (Table) are mostly due to conditions described as follow.

1. Genetic Defects in the Metabolic Enzymes
A thermolabile variant of methylene tetrahydrofolate reductase (MTHFR) with reduced enzymatic activity (T mutation) is the most common form of genetic hyperhomocysteinemia.15 The responsible gene is relatively common in the population (estimated to be between 5% to 14%).16,17 Homozygosity for the thermolabile variant of MTHFR (TT genotype) is a common cause of mildly elevated plasma homocysteine levels in the general population.17,18

2. Nutritional Deficiencies in Vitamin Cofactors
Elevated homocysteine may be a consequence of deficiency of folate, vitamin B₆, and/or vitamin B₁₂.6 In fact,
these vitamins are major determinants of the homocysteine concentration.

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**Experimental Evidence**

Histopathologic hallmarks of atherothrombosis related to elevated homocysteine levels include intimal thickening, elastic lamina disruption, smooth muscle hypertrophy, platelet accumulation, and the formation of platelet-enriched occlusive thrombi. Several studies have demonstrated the involvement of homocysteine in the process of in-stent restenosis. Three mechanisms have been proposed: (1) leukocyte recruitment by upregulation of monocyte chemoattractant protein-1 and interleukin-8 expression and secretion; (2) increased smooth muscle cell proliferation and enhanced collagen production; and (3) marked platelet accumulation due to either direct proaggregatory effects of homocysteine or an impairment in endothelium-mediated platelet inhibition.

**Clinical Evidence**

Despite the potential involvement of hyperhomocysteinemia in the restenotic process suggested by experimental studies, almost all available clinical trials have shown that hyperhomocysteinemia is not associated with in-stent restenosis. One study was conducted by Kosokabe et al., who in 67 patients analyzed the impact of MTHFR genotypes and levels of homocysteine on in-stent restenosis evaluated by intravascular ultrasound. Even though neointimal hyperplasia was related to MTHFR genotypes, no relation to plasma homocysteine levels was observed. Several additional studies have investigated the relation between homocysteine, genotypes of MTHFR, vitamins (levels of B6, B12, and folate), and angiographic restenosis after stent implantation, confirming the absence of any relation between hyperhomocysteinemia and restenosis.

**Figure 1.** Graph shows vitamin coenzymes and substrates involved in homocysteine metabolism. THF indicates tetrahydrofolate; VIT B6, riboflavin; VIT B12, vitamin B12; VIT B12, methyl cobalamin; DMG, dimethylglycine; MS, methionine synthase; MTHFR, methylene tetrahydrofolate reductase; CS, cystathionine-beta synthase; CL, cystathionine-gamma-lyase; and BHMT, betaine homocysteine methyl transferase.
ratio 0.91, 95% confidence interval 0.70 to 1.18; \( P = 0.47 \).

**Pharmacological Intervention**

Vitamin administration (a combination of folic acid, vitamins B6, and B12) has been shown to reduce homocysteine levels.\(^{30}\) So far, only 2 randomized studies have investigated the impact of homocysteine-lowering therapy on restenosis after coronary angioplasty and stent implantation. Schnyder and colleagues\(^{31}\) compared placebo with a daily administration of folic acid (1.0 mg), vitamin \( B_{12} \) (400 \( \mu \)g), and vitamin \( B_6 \) (10 mg) in 205 patients (56% of whom received stent implantation). They found that vitamin therapy was most beneficial in patients treated with balloon angioplasty and in those patients with small vessels, whereas a nonsignificant reduction in restenosis was observed in patients treated with stenting (20.6% versus 29.9%, \( P = 0.32 \)). In a larger study that enrolled 636 patients undergoing stent implantation, Lange and colleagues\(^{29}\) randomly assigned patients to placebo or folates. The folate treatment consisted of an intravenous bolus of folic acid (1.0 mg), vitamin \( B_6 \) (5.0 mg), and vitamin \( B_{12} \) (1.0 mg) followed by daily oral administration of folic acid (1.2 mg), vitamin \( B_6 \) (48.0 mg), and vitamin \( B_{12} \) (60 \( \mu \)g) for 6 months. They found a paradoxical harmful effect, with higher restenosis rates associated with folates (34.5% versus 26.5%, \( P = 0.05 \)), particularly in patients with homocysteine levels in the normal range (<15 \( \mu \)mol/L) (36.2% versus 25.3%, \( P = 0.02 \)), whereas slight benefits were observed in patients with elevated homocysteine (27.2% versus 31.7%, \( P = \text{NS} \)). The observed deleterious effects of homocysteine-lowering therapy after coronary stenting may be due to the fact that folate plays a crucial role in the synthesis of DNA and RNA through the formation of 1-carbon units that are needed for the synthesis of purine and pyrimidine.\(^{32}\) The administration of high doses of folate significantly promoted the growth of neointimal cells by providing larger amounts of biochemical precursors for cell duplication.\(^{33}\) Furthermore, by decreasing homocysteine, folate can improve the availability of methyl groups for DNA-methylation,\(^{34}\) which may favor endothelial growth.\(^{35}\)

**Summary and Recommendations**

Mild hyperhomocystinemia does not appear to be a major determinant of in-stent restenosis. It does appear, however, to be an independent risk factor for cerebrovascular, peripheral vascular, and coronary heart disease and for venous thromboembolic disease.\(^{5–9}\) Several randomized clinical trials are underway to address the effect of folate, vitamin \( B_6 \), and vitamin

### HOMOCYSTEINE AND IN-STENT RESTENOSIS

<table>
<thead>
<tr>
<th>Normal homocysteine</th>
<th>OR [95% CI]</th>
<th>Hyperhomocysteine*</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lange et al (^{29})</td>
<td>49/197 (24.9%)</td>
<td>19/60 (31.7%)</td>
<td>13.2%</td>
</tr>
<tr>
<td>Genser et al (^{29})</td>
<td>73/194 (37.6%)</td>
<td>21/68 (30.9%)</td>
<td>22.1%</td>
</tr>
<tr>
<td>Zairis et al (^{29})</td>
<td>69/184 (37.5%)</td>
<td>39/125 (31.2%)</td>
<td>32.3%</td>
</tr>
<tr>
<td>Koch et al (^{25})</td>
<td>118/471 (25.0%)</td>
<td>32/130 (24.6%)</td>
<td>32.4%</td>
</tr>
<tr>
<td>Pooled data</td>
<td>309/1046 (29.5%)</td>
<td>111/383 (29.0%)</td>
<td>100%</td>
</tr>
</tbody>
</table>

Figure 2. Pooled data of clinical trials evaluating the relation between homocysteine and in-stent restenosis (odds ratios and 95% confidence intervals). The size of the data markers (squares) is approximately proportional to the sample size. *Defined as basal homocysteine \(<15 \mu\text{mol/L}.\)
B₁₂ supplementation on cardiovascular disease. Until complete results of these studies become available, screening for hyperhomocystinemia in patients undergoing coronary stenting is only recommended in the case of premature atherosclerotic disease (patients <45 years of age), when there is a paucity of more conventional risk factors, and in patients with a history of unexplained venous thrombosis. However, it should be remembered that homocysteine-lowering therapy might have a deleterious effect in patients treated with stent implantation, with paradoxically more neointimal proliferation and in-stent restenosis, particularly in patients with a homocysteine level within the normal range. Additional studies are needed to investigate the impact of moderate to severe hyperhomocystinemia and vitamin therapy on restenosis in the era of drug-eluting stents. In fact, the vast majority of patients included in randomized trials had homocysteine levels that were within the normal range or were mildly elevated, whereas patients with moderate to severe hyperhomocystinemia might be at higher risk for restenosis and subacute thrombosis, particularly in case of delayed reendothelialization observed with drug-eluting stents. Until these data become available, vitamin therapy cannot be recommended.

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References


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