

REVIEW ARTICLES

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The role of homocysteine in endothelial dysfunction

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Abstract

Background: Homocysteine is a sulfur-containing intermediate product in the normal metabolism of methionine, an essential amino acid. Hyperhomocysteinemia defines the state in which concentrations of homocysteine exceeds normal level. Homocysteine is located at a metabolic branch point and can either be irreversibly degraded to cysteine via the transsulfuration pathway, or conserved by remethylation back to methionine. Folic acid, vitamin B₁₂, and vitamin B₆ deficiencies and reduced enzyme activities inhibit the breakdown of homocysteine, thus increasing the concentration of intracellular homocysteine. Being cytotoxic, homocysteine is increasingly exported from the cell to become detectable in plasma. In recent years the amino acid homocysteine has achieved the status of an important factor in vascular disease, diseases of aging, and other fundamental processes in biology and medicine. Hyperhomocysteinemia may alter vascular morphology, stimulate inflammation, activate the endothelium and the blood clotting cascade, and inhibit fibrinolysis. As a result, hyperhomocysteinemia is associated with loss of endothelial antithrombotic function and induction of a procoagulant environment. The role of homocysteine in endothelial dysfunction is thought to be mediated by mechanisms including oxidative stress. Vascular injury could be caused by an imbalance between nitric oxide production from dysfunctional endothelial cells and homocysteine concentrations.

Conclusions: Hyperhomocysteinemia is associated with alterations in vascular morphology, loss of endothelial antithrombotic function, and induction of a procoagulant environment.

Key words: homocysteine, endothelial dysfunction, hyperhomocysteinemia, endothelium, oxidative stress.

Introduction

In recent years the amino acid homocysteine has achieved the status of an important factor in vascular disease, diseases of aging, and other fundamental processes in biology and medicine [25]. After its discovery in 1932, homocysteine was characterized as an important intermediate in methionine metabolism. Little was known about its biomedical significance until 1962, when children with mental retardation, accelerated growth, and propensity to thrombosis of arteries and veins were found to excrete homocysteine in the urine [19,25]. The cause of homocystinuria in most of these cases is deficiency of the enzyme cystathionine synthase, a pyridoxal phosphate-dependent enzyme [25]. In 1968, a second case of homocystinuria caused by deficiency of a different enzyme, methionine synthase, a folate and vitamin B₁₂-dependent enzyme, was critical in the discovery of the atherogenic potential of homocysteine [25]. McCully and Wilson proposed the "homocysteine theory of arteriosclerosis" in 1975 on the basis of pathological examinations of autopsy material from children with homocystinuria [20]. However, only within the past 5 years has homocysteine taken its place among other major risk factors such as cholesterol, smoking, and obesity [20]. Homocysteine is now widely accepted as a major independent risk factor for cardiovascular, cerebrovascular, and peripheral vascular disease [13,17,20,33]. However, the precise mechanisms underlying this association, although intensively studied, are still incompletely solved [30].

Homocysteine metabolism

Homocysteine is located at a metabolic branch point and can either be irreversibly degraded to cysteine via the transsulfuration pathway, or conserved by remethylation back to methionine (fig. 1) [12,30].

First, methionine is activated by the enzyme methionine adenosyltransferase (MAT) and ATP to form S-adenosylmethionine (SAM) [30]. SAM is the primary methyl group donor for many vital biological processes, including methylation of DNA, RNA, proteins, lipids and neurotransmitters [10,11,13,16,28,29,30,31,32,33,345]. Upon transmethylation, SAM is converted to S-adenosylhomocysteine (SAH), which is further hydrolyzed by the enzyme adenosylhomocysteine hydrolase (SAHH) to homocysteine and adenosine. This reaction is reversible and favors SAH synthesis [12,30].

The transsulfuration pathway, mainly limited to liver and kidneys, is initiated with the condensation of homocysteine and serine to form cystathionine, in a reaction catalyzed by the enzyme cystathionine β-synthase (CBS), with pyridoxal phosphate (vitamin B₆) as co-factor [12,13,30,31,34]. Cystathionine is further metabolized to produce cysteine by another enzyme – cystathionine γ-lyase (CGL). Besides protein synthesis, cysteine is used in the synthesis of glutathione, an important cellular antioxidant also involved in detoxification of many xenobiotics [30].

In remethylation, homocysteine acquires a methyl group from 5-methyltetrahydrofolate (5-MTHF) or from betaine

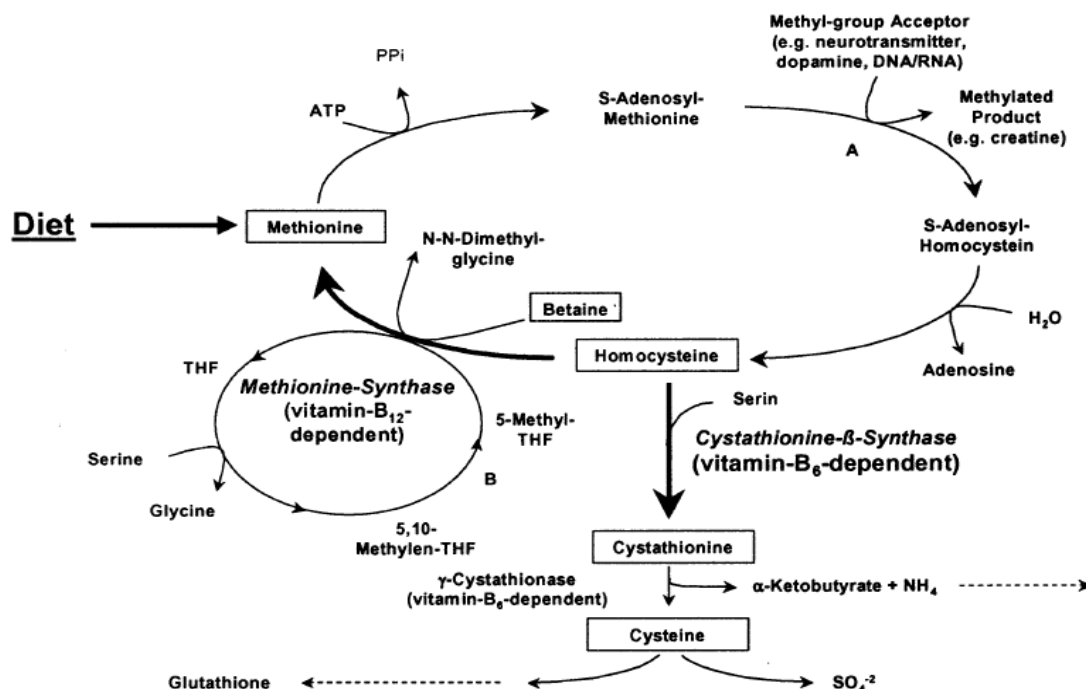


Fig. 1. Homocysteine metabolism (A – enzyme methionine adenosyltransferase, B – enzyme 5,10-methylenetetrahydrofolate reductase) [33].

to form methionine. The reaction with MTHF occurs in all tissues and is vitamin B₁₂-dependent, while the reaction with betaine is confined mainly to the liver and is vitamin B₁₂-independent [31].

Homocysteine remethylation occurs by receiving the methyl group from 5-MTHF, which links the folate cycle with the homocysteine metabolism (fig. 1). 5-MTHF is the active folate derivative and the main circulating form of folate in plasma. It is produced from 5,10-methylenetetrahydrofolate (5,10-MTHF) by the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR), which uses flavine adenine dinucleotide (FAD – the active form of vitamin B₂) as co-factor [1,4,33,34]. The methyl group from 5-MeTHF is transferred via vitamin B₁₂ to homocysteine, in a reaction catalyzed by the enzyme methionine synthase (MTR), with production of tetrahydrofolate (THF) [27,30,33,34]. THF is then recycled to 5,10-MTHF in the presence of serine and vitamin B₆ by the enzyme serine hydroxymethyltransferase (SHMT) [30].

Over time, the cobalamin (I) cofactor of MTR is oxidized to form cobalamin (II), leading to inactivation of MTR. Thus, the enzyme methionine synthase reductase (MTRR) is required for reversion of oxidized cobalamin (II) to CH₃-cobalamin (III) to maintain the activity of MTR [22]. The folate-dependent remethylation pathway is present in nearly all cells, except red blood cells [34].

Alternatively, in liver and kidney, methyl groups can also be donated by betaine (also known as trimethylglycine, an intermediate of choline oxidation), in a reaction catalyzed by the enzyme betaine-homocysteine methyltransferase (BHMT) [30].

The intracellular concentration of homocysteine is under

tight control. Once formed in the cell, homocysteine is quickly either metabolized to cysteine or remethylated to methionine. In addition, if one of these pathways is compromised, leading to higher intracellular production than elimination, the excess of homocysteine is rapidly exported to the blood. Hence, cellular export of homocysteine reflects the balance between homocysteine production and catabolism. [12,30].

SAM plays a central role in the regulation of homocysteine metabolism, by coordinating the fate of homocysteine towards remethylation or transsulfuration pathways; it is an allosteric inhibitor of MTHFR and an activator of CBS activity. When the levels of SAM are adequate to sustain methylation demand, the partitioning of homocysteine between both metabolic pathways is approximately equal. In case of excess of methionine supply, an increase in tissue SAM levels occurs, and homocysteine degradation to cysteine is favored. Moreover, SAM also regulates homocysteine remethylation through inhibition of BHMT activity. Conversely, if methionine levels are low, for example during fasting, low SAM levels will neither activate CBS nor inhibit MTHFR, resulting in conservation of homocysteine via remethylation back to methionine [12,16,30,31].

Folic acid, vitamin B₁₂, and vitamin B₆ deficiencies and reduced enzyme activities inhibit the breakdown of homocysteine, thus increasing the concentration of intracellular homocysteine [29,33]. Being cytotoxic, homocysteine is increasingly exported from the cell to become detectable in plasma [33].

Causes of hyperhomocysteinemia

Hyperhomocysteinemia is a terminology suggested to describe the presence of abnormal elevation in total plasma homocysteine levels. In the fasting state, normal plasma levels

of homocysteine are less than 12 $\mu\text{mol/l}$ [1,2,5,6,9,14,21,30]. Pregnant women have lower plasma tHcy than nonpregnant women. The mean tHcy concentration in pregnant women is 5–6 $\mu\text{mol/L}$, and tHcy concentrations >10 $\mu\text{mol/L}$ are rarely observed [28].

Table 1

Determinants of plasma tHcy [28]

Causes	Effect
Genetic factors	
Homocystinuria	↑↑↑
Heterozygosity for CBS defects	↑
Down syndrome	↓
MTHFR 677C→T (homozygosity)	↑
Other polymorphisms	↑
Physiologic determinants	
Increasing age	↑
Male sex	↑
Pregnancy	↓
Postmenopausal state	↑
Renal function, reduced glomerular filtration rate	↑
Increasing muscle mass	↑
Lifestyle determinants	
Vitamin intake (folate, B ₁₂ , B ₆ , B ₂)	↓
Smoking	↑
Coffee	↑
Ethanol intake	↑
Lack of exercise	↑
Clinical conditions	
Folate deficiency	↑↑
Cobalamin deficiency	↑↑↑
Vitamin B6 deficiency	↑
Renal failure	↑↑
Hyperproliferative disorders	↑
Hypothyroidism	↑
Hyperthyroidism	↓
Early stage of diabetes	↓
Late stage of diabetes	↑
Drugs	
Folate antagonists (Methotrexate, Trimethoprim, Anticonvulsants, Cholestyramine)	↑
Cobalamin antagonists (Nitrous oxide, Nitric oxide, Metformin, H2-receptor antagonists, Omeprazole)	↑
Vitamin B6 antagonists	↑
Sulfhydryl compounds	↓
Estrogens, Tamoxifen	↓
Androgens	↑
Cyclosporin A, Diuretics, Fibrates	↑

↓ – decrease in tHcy, ↑ – moderate hyperhomocysteinemia (12 – 30 $\mu\text{mol/l}$), ↑↑ – intermediate hyperhomocysteinemia (30 – 100 $\mu\text{mol/l}$), ↑↑↑ – severe hyperhomocysteinemia (>100 $\mu\text{mol/l}$)

Determinants of plasma total homocysteine (tHcy) include genetic, physiologic, and lifestyle factors; various diseases and drugs (tab. 1) [9,17,28,33,34]. The causes of increased tHcy concentrations vary according to the age of the person

and the degree of tHcy increase [20,27,28,33]. Low folate or cobalamin status or renal impairment account for the majority of cases with increased tHcy [3,11,31,32,33]. In populations eating food fortified with folic acid, renal impairment and cobalamin deficiency are the most important determinants. Homozygosity for the MTHFR 677C→T polymorphism is the most common genetic determinant. Individuals with the MTHFR 677TT genotype usually have higher tHcy than those with the 677CC variant, but it depends on the folate status. Most other genetic polymorphisms in enzymes related to homocysteine have little effect on tHcy concentrations [28,29].

Circulating species of homocysteine in plasma

Homocysteine is present in plasma in various forms in different proportions [20,27,33]. Human plasma contains both reduced and oxidized species of homocysteine [20]. The concentration of free homocysteine in plasma is very low and accounts for less than 2% of total plasma homocysteine in normal subjects [27,33] and the oxidized forms of homocysteine usually comprise 98–99% of total plasma homocysteine in human plasma [20]. Disulfide forms also exist with cysteine and with proteins containing reactive cysteine residues (protein-bound homocysteine). The latter oxidized forms are referred to as mixed disulfides. The concentration of homocystine and homocysteine-cysteine represents approximately 10-15%, and protein-bound homocysteine accounts for over 80% of the measured total homocysteine in normal plasma [27]. Total homocysteine, therefore, is the sum total of all forms of homocysteine that exist in plasma or serum [20]. Only minute amounts of homocysteine are found in the urine of healthy subjects. The term “homocystinuria” should therefore be reserved for inborn errors of metabolism characterized by extremely elevated plasma homocysteine levels and substantially increased excretion of homocysteine in the urine [33].

Mechanisms of homocysteine-mediated vascular damage

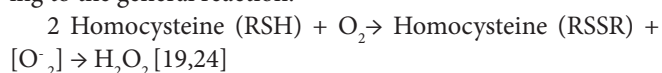
Hyperhomocysteinemia may alter vascular morphology, stimulate inflammation, activate the endothelium and the blood clotting cascade, and inhibit fibrinolysis [1,5,6,9,10,11,12,13,14,18,33,34,35]. As a result, hyperhomocysteinemia is associated with loss of endothelial antithrombotic function and induction of a procoagulant environment. Most known forms of damage or injury are due to homocysteine-mediated oxidative stresses. Chief among these are changes in the intracellular redox potential, interference with the nitric oxide (NO) system, and activation of transcription factors with stimulation of gene expression [33].

Observations in clinical and animal studies have identified potential pathophysiological targets where homocysteine exerts its damaging effect. Those targets include endothelial cells (ECs), vascular smooth muscle cells (VSMCs), connective tissue, platelets, coagulation factors, lipids, and NO signal transduction molecules. Unfortunately, there is not an established, unifying hypothesis by which homocysteine evokes vascular damage. However, there are numerous biological and

biomolecular mechanisms that have been heavily studied and proposed to explain the pathological changes associated with elevated tHcy levels [34].

Oxidative stress is possibly the most detrimental stressor in the pathogenesis of most diseases. Studies have shown that the pro-oxidative homocysteine exerts direct biological damage to vascular cells and tissue through an oxidative mechanism that damages lipids, nucleic acids, and proteins [15,18,19,23,34]. An alternative hypothesis to that of a direct effect of reduced homocysteine on endothelial cell injury is that homocysteine is acting indirectly through its oxidation and the concomitant production of reactive oxygen species [7,24].

Under aerobic conditions (in the presence of molecular oxygen as an electron acceptor) and at physiological pH, thiols such as homocysteine, oxidize to form disulfides, according to the general reaction:



In plasma, this reaction can be catalyzed by transition metals such as copper and cobalt. Homocysteine can autooxidize readily via general thiol oxidation mechanism described above and form homocystine, or oxidize other thiols such as cysteine and glutathione to form mixed disulfides, or oxidize free cysteine residues on proteins and peptides to form mixed disulfides [19]. Homocysteine itself has the ability to generate potent reactive oxygen species (ROS) when oxidized due to its highly reactive sulfhydryl group. In the circulation, this thiol undergoes rapid metal-catalyzed sulfhydryl auto-oxidation, leading to the generation of superoxide and hydrogen peroxide [15,19,20,27,34,35].

In addition to auto-oxidative ROS production, decreased homocysteine clearance and resultant accumulation further increases the production of harmful oxidants that collectively spurs on the negative cascade of vascular complications. Homocysteine has a detrimental effect on vascular cells and tissues as a result of oxidative damage to lipids, nucleic acids, and proteins. The injurious oxidative stress exerted by homocysteine relies either on auto-oxidation of the highly reactive thiol group of homocysteines or on the formation of intracellular superoxide and peroxy radicals with concomitant inhibition of cellular antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase [13,34].

As a consequence of homocysteine-produced oxidative radicals, there is a critical impairment in the production of endothelium derived NO by several different mechanisms [34,35]. Homocysteine decreases the bioavailability of NO, one of the major endothelium-dependent vasodilators that is produced by the endothelial isoform of nitric oxide synthase (NOS). This effect is caused either by an accelerated oxidative inactivation of NO and/or eNOS or by an increase in serum asymmetric dimethylarginine (ADMA), an endogenous inhibitor of eNOS, thus decreasing NO production [13,34,35]. Also, studies have shown that homocysteine suppresses NO production without altering NOS protein levels or enzyme activity and the homocysteine-induced endothelial injury and

subsequent reduction in NO production are primarily associated with increased ROS levels [34].

In the walls blood of vessels, NO contributes to the regulation of systemic blood flow and pressure by activating intracellular signaling pathways that modulate calcium levels in VSMC resulting in vasodilation. Homocysteine is known to decrease vascular function by the oxidative depletion of biologically active NO. Homocysteine has also been shown to cause striking changes in vessel wall structure by inducing extracellular matrix alterations that fragment the arterial internal and medial elastic lamina. Unlike the growth retardation of the endothelium, homocysteine has been associated with myointimal hyperplasia and VSMC hypertrophy [2,4,6,8,9,17,20,27,34,35]. It has been shown that homocysteine stimulates VSMC proliferation by the activity of homocysteine-generated oxygen radicals that activate cytokines that are active in the initial phase of the proliferative process. It has also been reported that the homocysteine-mediated reduction in NO synthesis and release by injured ECs causes the release of growth factors that provoke proliferation of nearby VSMC [34].

Homocysteine has been shown to induce vascular inflammation by enhancing the expression of pro-inflammatory cytokines, such as monocyte chemoattractant protein 1 (MCP-1), which regulates migration and activation of monocytes/macrophages, and interleukin 8 (IL-8), which is an important chemoattractant for neutrophils and T-lymphocytes [13]. Homocysteine has been shown to initiate the process by increasing the expression and plasma levels of the inflammatory cytokine, tumor necrosis factor alpha (TNF- α), and enhancing the activation of a redox-sensitive nuclear inflammatory transcription factor, nuclear factor-kappa B (NF- κ B), in the vasculature [19,34,35].

It has long been believed that homocysteine may cause lipid peroxidation by an oxidation-dependent pathway [2,4,34,35]. Homocysteine generates oxidative radicals that initiate oxidative degradation of lipids on the EC surface, which causes loss of membrane function and increased permeability. Clinical data have shown that patients with hyperhomocysteinemia have an increase in end products of lipid peroxidation such as F₂-isoprostanes and malondialdehyde [34].

A more recent concept concerns activation of the unfolded protein response (UPR) that is triggered when unfolded or misfolded proteins accumulate in the endoplasmic reticulum (ER). This ER stress induces the expression of several molecular chaperones and other stress response proteins, which are aimed at restoring correct protein folding or retranslocating defective proteins back to the cytosol for degradation in the proteasomes. In case of a prolonged ER stress, the UPR extends to the activation of apoptosis by various signaling pathways. This is precisely what happens in human endothelial cells after exposure to homocysteine in vitro: while inducing misfolding in the ER by altering the local redox potential and interfering with disulfide bond formation, homocysteine activates UPR and, subsequently, growth arrest and apoptosis (fig. 2) [13].

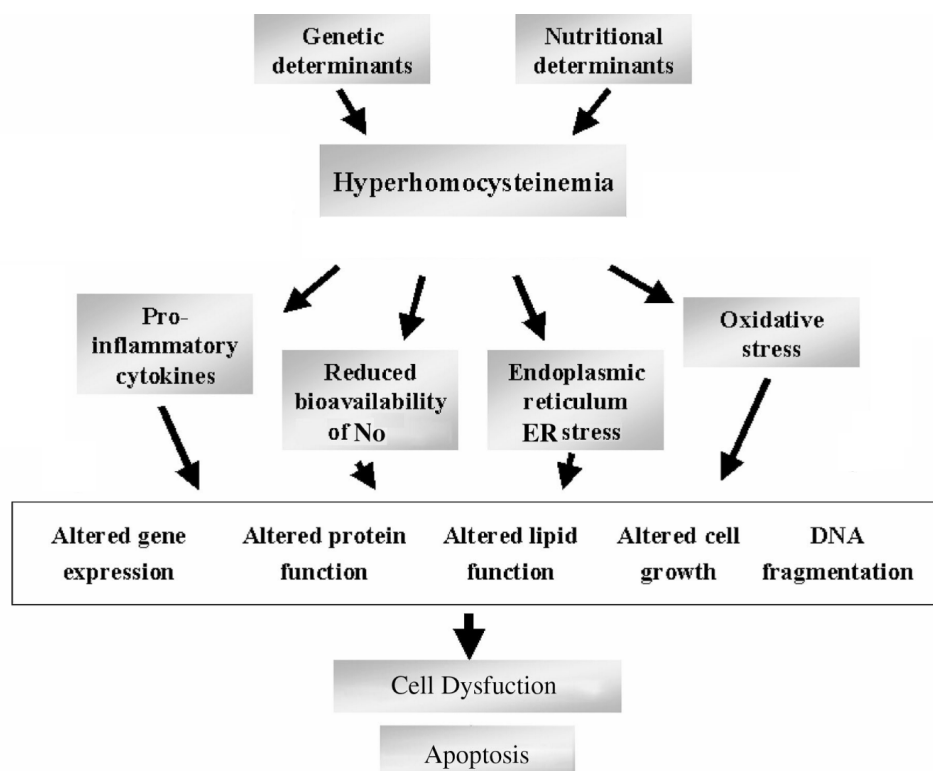


Fig. 2. Cellular and molecular mechanisms of hyperhomocysteinemia induced cell dysfunction [13].

These actions can be in connection with the widely believed capability of homocysteine to alter the surface properties of endothelial cells by changing their phenotype from anticoagulant to procoagulant [10,18,21,24,26].

Endothelial cells also possess several antithrombotic mechanisms to protect against intravascular thrombosis. However, elevated plasma homocysteine levels have been reported to cause an imbalance in coagulant and clotting properties toward a prothrombotic state in coronary and peripheral disease that is primarily mediated by the endothelial dysfunction [34].

In fact, physiological levels of homocysteine may enhance the binding of lipoprotein to fibrin. On the other hand, high levels of homocysteine reduce protein C activation, thus inhibiting its anticoagulant activity; induce a great inhibition, by more than 75%, of antithrombin III; inhibit the synthesis of anticoagulant heparan sulphate through an induced alteration of the redox potential; suppress thrombomodulin and inactivate its co-factor activity; block tissue plasminogen activator binding to endothelial cells; and activate tissue factor transcription [10,18,24,26].

Furthermore, it has been shown that homocysteine induces the activity of a protease which activates factor V, thus promoting coagulation even in the absence of thrombin. Homocysteine rapidly reacts with nitric oxide to form S-nitroso-homocysteine, which acts as a potent antiplatelet agent; the formation of this adduct may attenuate the production of peroxides from homocysteine, thus protecting against the atherogenic properties of homocysteine [10,18,19,24]. Vascular injury could be caused by an imbalance between

nitric oxide production from dysfunctional endothelial cells and homocysteine concentrations [24,35].

Conclusions

This review focuses on disorders of homocysteine metabolism, on situations in which the metabolic mechanism is impaired and elucidates the mechanisms by which homocysteine can cause endothelial dysfunction. Thereby: (1) Homocysteine may increase oxidative stress; (2) Homocysteine may impair endothelial function and bioavailability of nitric oxide; (3) Homocysteine may impair vascular smooth muscle cell function; (4) Homocysteine may change extracellular matrix, collagen structure and function; (5) Homocysteine may induce a prothrombotic state; (6) Homocysteine may increase lipid peroxidation, and increase the oxidation of lowdensity lipoprotein; (7) Homocysteine may induce inflammation and apoptosis.

References

1. Alan L. et al. Homocysteine metabolism: nutritional modulation and impact on health and disease. *Alternative Medicine Review*, 1997, 4, 234 – 254.
2. Antoniadis C. et al. Homocysteine and coronary atherosclerosis: from folate fortification to the recent clinical trials. *European Heart Journal*, 2009, 30, 6 – 15.
3. Bergen N. et al. Homocysteine and folate concentrations in early pregnancy and the risk of adverse pregnancy outcomes: the Generation R Study. *BJOG: an international journal of obstetrics and gynecology*, 2012, 119 (6), 739 – 751.
4. Cattaneo M., Lussana F. Hyperhomocysteinemia and cardiovascular aging. *Cell Aging and Gerontology*, 2002, 11, 309 – 335.

5. Chen N. et al. Regulation of homocysteine metabolism and methylation in human and mouse tissues. *The FASEB Journal*, 2010, 24, 2804 – 2817.
6. Den Heijer M. et al. Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies. *Journal of Thrombosis and Haemostasis*, 2000, 3 (2), 292 – 299.
7. Di Simone. et al. Effect of folic acid on homocysteine-induced trophoblast apoptosis. *Molecular Human Reproduction*, 2004, 10 (9), 665 – 669.
8. Djuric D. et al. Homocysteine, Folic Acid and Coronary Artery Disease: Possible Impact on Prognosis and Therapy. *The Indian Journal of Chest Diseases & Allied Sciences*, 2008, 50, 39 – 48.
9. Durand P. et al. Impaired Homocysteine Metabolism and Atherothrombotic Disease. *Laboratory Investigation*, 2001, 5, 645 – 672.
10. Eldibany M., Caprini J. Hyperhomocysteinemia and Thrombosis. *Archives of Pathology & Laboratory Medicine*, 2007, 131, 872 – 884.
11. Finell R. et al. Gene-nutrient interactions: Importance of folic acid and vitamin B12 during early embryogenesis. *Food and Nutrition Bulletin*, 2008, 2 (supplement), S86 – S98.
12. Finkelstein J. The metabolism of homocysteine: pathways and regulations. *European journal of pediatrics*, 1998, 157 (supplement 2), S40 – S44.
13. Forges T. et al. Impact of folate and homocysteine metabolism on human reproductive health. *Human Reproduction*, 2007, 13 (3), 225 – 238.
14. Fredriksen A. et al. Large-scale population-based metabolic phenotyping of thirteen genetic polymorphisms related to one-carbon metabolism. *Human Mutation*, 2007, 28, 9, 56 – 865.
15. Gerdes V. et al. Homocysteine and markers of coagulation and endothelial cell activation. *Journal of Thrombosis and Haemostasis*, 2003, 2, 445 – 451.
16. Green R. Indicators for assessing folate and vitamin B₁₂ status and for monitoring the efficacy of intervention strategies. *Food and Nutrition Bulletin*, 2008, 2 (supplement), S52 – S63.
17. Guilliams T. Homocysteine – A Risk Factor for Vascular Diseases: Guidelines for the Clinical Practice. *The Journal of the American Nutritional Association*, 2004, 7, 10 – 24.
18. Harpel P. et al. Homocysteine and hemostasis: pathogenic mechanisms predisposing to thrombosis. *The journal of nutrition*, 1996, Supplement, 1285S – 1289S
19. Hurjui L. ș. a. Impactul clinic al hiperhomocisteiniei: rol, cauze, tratament. Clasic și modern în fiziopatologie. O abordare integrativă în educație și cercetare, 2015, 229 – 235.
20. Jacobsen W. Homocysteine and vitamins in cardiovascular disease. *Clinical Chemistry*, 1998, 44, 8(B), 1833–1843.
21. Key N., McGlennen R. Hyperhomocyst(e)inemia and Thrombophilia. *Archives of Pathology & Laboratory Medicine*, 2002, 126, 1367 – 1375.
22. Li W. et al. Homocysteine metabolism gene polymorphisms (MTHFR C677T, MTHFR A1298C, MTR A2756G and MTRR A66G) jointly elevate the risk of folate deficiency. *Nutrients*, 2015, 7, 6670 – 6687.
23. Malinowska J. et al. Comparison of the effect of homocysteine in the reduced form, its thiolactone and protein homocysteinylolation on hemostatic properties of plasma. *Thrombosis Research*, 2011, 127, 214 – 219.
24. Medina M. Amores-SaÂnchez M. Homocysteine: an emergent cardiovascular risk factor? *European Journal of Clinical Investigation*, 2000, 30, 754-762.
25. McCully K. The Biomedical Significance of Homocysteine. *Journal of Scientific Exploration*, 2001, 15 (1), 5 – 20.
26. Quere I. et al. Homocysteine and venous thrombosis. In: *Seminars in vascular medicine*, 2005, 5 (2), 183 – 189.
27. Rasmussen K., Moller J. Total homocysteine measurement in clinical practice. *Annals of Clinical Biochemistry*, 2000, 37, 627 – 648.
28. Refsum H. et al. Facts and Recommendations about Total Homocysteine Determinations: An Expert Opinion. *Clinical Chemistry*, 2004, 50 (1), 3- 32.
29. Refsum Helga. Folate, vitamin B12 and homocysteine in relation to birth defects and pregnancy outcome. *British Journal of Nutrition*, 2001, 85, Suppl. 2, S109-S113.
30. Rocha M. Crossroads of homocysteine, nitric oxide and asymmetric dimethylarginine metabolisms. Involvement of S-adenosylhomocysteine and impaired cellular methylation. *Lisbon 2012*.
31. Selhub J. Public health significance of elevated homocysteine. *Food and Nutrition Bulletin*, 2008, 2 (supplement), S116 – S125.
32. Shane B. Folate and vitamin B12 metabolism: Overview and interaction with riboflavin, vitamin B6, and polymorphisms. *Food and Nutrition Bulletin*, 2008, 2 (supplement), S5 – S16.
33. Stanger O. et al. Clinical use and rational management of homocysteine, folic acid, and B vitamins in cardiovascular and thrombotic diseases. In: *Zeitschrift für Kardiologie*, 2004, 93, 439 – 453.
34. Steed M., Tyagi S. Mechanisms of cardiovascular remodeling in hyperhomocysteinemia. *Antioxidants & Redox Signaling*, 2011, 15 (7), 1927 – 1943.
35. Weiss N. Mechanisms of increased vascular oxidant stress in hyperhomocysteinemia and its impact on endothelial function. *Current Drug Metabolism*, 2005, 6, 27 – 36.