Homocysteine - Red signal to cardiovascular diseases: A Systematic Review

Pranita Ashok[1], Advait Teli[2*], Anuradha R Joshi[1]

[1]*Department of Physiology, Bharati Vidyapeeth Medical College and Hospital, Katraj, Pune*

[2]*Bharati Vidyapeeth Medical College and Hospital, Katraj, Pune*

** = Equivalent Authorship*

Corresponding Author

Advait Teli

E-mail ID: teliadvait@gmail.com

Submission : 03.07.2021 Acceptance : 02.08.2021 Publication : 22.12.2021

https://www.doi.org/10.56136/BVMJ/2021_00029

Abstract

Over the past few decades, increased plasma homocysteine has proven itself to be named as an independent marker for developing cardiovascular diseases. Its increase can be due to genetic changes in MTHFR, deficiency in CBS, folic acid and vitamin B6 and B12, etc. It also causes apoptotic endothelial cell death and ceases the growth of damaged endothelial cardiac cells. May cause an increase in inflammasome and activate caspases leading to apoptosis.

Increased oxidative stress and mast cell degranulation have been shown to cause cardiac hypertrophy; higher plasma homocysteine levels have lengthened the QTc interval, which causes ventricular arrhythmia. It has also been correlated to juvenile thrombosis and deep venous thrombosis. Higher homocysteine levels have also proven to deprive the endothelial NO, which leads to enhanced platelet activation. Also, how malondialdehyde modified LDL acts as an oxidative stress factor.

Folate supplementation and Mast cell stabilizers prove to be useful in lowering the mortality induced by Hyperhomocysteinemia. Also, a diet rich in vitamin B6 and B12 should be considered to control homocysteine levels.

Keywords: Homocysteine, Pyroptosis, Inflammasome, Thrombogenic, endothelial stress, Malondialdehyde, Folic acid, mast cell degranulation

Introduction

Since its discovery in 1932 by an American biochemist Vincent Duvigneaud, homocysteine, an amino acid similar to cysteine, it gained a lot of medical importance in its role as a cardiovascular risk factor[(1)]. This product was obtained when methionine was reacted with concentrated acids causing its demethylation. It is a sulfhydryl-containing non-essential amino acid that is an intermediate product in methionine and cysteine biosynthesis. Since its discovery, it has been under many speculations whether or not it is a marker for various cardiovascular diseases.

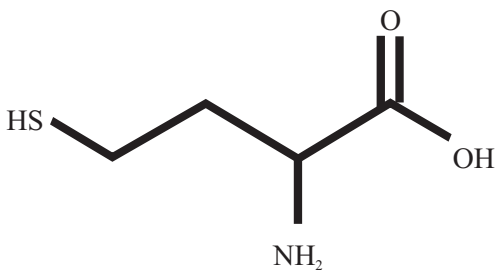

Fig 1: Structure of Homocysteine

As it is a non-essential amino acid, its intake is via diet; studies show animal protein intake increases homocysteine levels; on the contrary, plant protein has shown a protective effect towards an increase in its level named hyperhomocysteinemia[2]. Various studies have demonstrated the correlation of high homocysteine levels with vascular disease and celiac disease, diabetes mellitus, metabolic syndrome, and many more[3,4].

This review article will look at the various detrimental changes in the cardiovascular system brought upon by increased homocysteine levels. Also, what factors cause an increase in its levels, and what can be done to prevent this.

Homocysteine in blood

It is present in plasma in four forms: about 1%circulates as the free thiol; 70-80% is disulfide bound to plasma proteins (mainly albumin); remaining 20-30% combines with itself to form dimer homocysteine or with other thiols, including cysteine, forming homocysteine-cysteine disulfide[5].

Metabolism of homocysteine

From figure 2, we can infer that homocysteine is at the intersection of two metabolic pathways: remethylation and transsulfuration.

In remethylation, homocysteine acquires a methyl group from 5-methyl or from betaine to form methionine. This reaction occurs in all tissues and is driven by vitamin B12, whereas the reaction with betaine occurs only in the liver and is independent of vitamin B12. ATP then activates methionine to form (SAM). SAM formed as a universal methyl donor donates methyl to SAH, which regenerates homocysteine by hydroxylation (important to note that this reversible hydroxylation).

In transsulfuration, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalyzed with the help of PLP & CBS to form cystathione, which forms cysteine. Excess cysteine is converted into taurine, sulfate, and glutathione.

Biochemical basis of Hyperhomocysteinemia

Hyperhomocysteinemia is defined as an abnormally high level (>15µmol/L) of homocysteine in blood [6]. Hyperhomocysteinemia can occur due to various

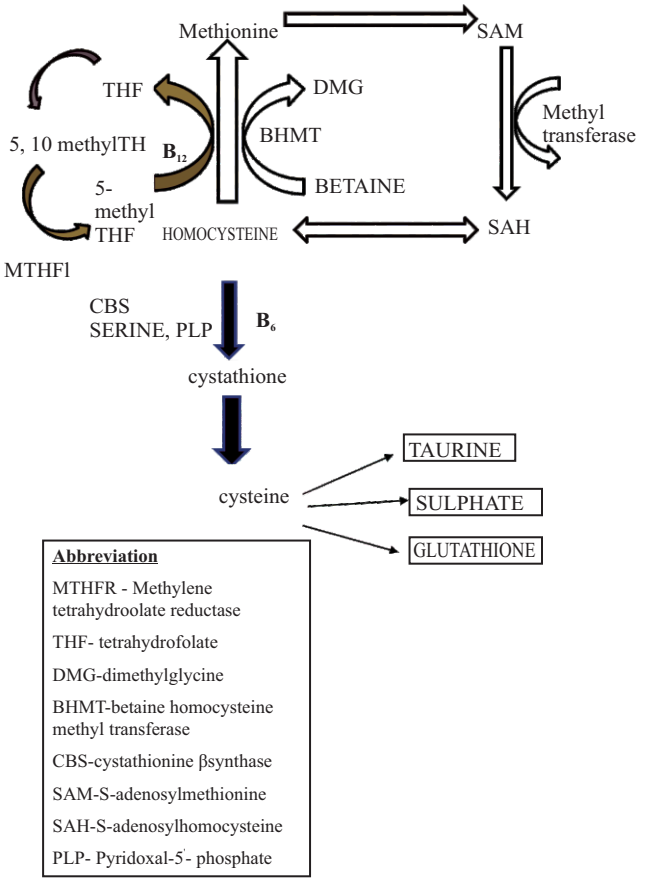

Fig 2: Metabolism of Homocysteine

genetic defects of enzymes in the homocysteine metabolism. The most common genetic defect causing this can be single nucleotide polymorphism of 5,10-methylenetetrahydrofolate reductase associated with mild(13-24µM) and moderate(25-60µM) Hyperhomocysteinemia[6]. Hankey et al. stated that the most common enzyme defect associated with moderately raised total homocysteine is a point mutation in the gene's coding region for MTHFR[7].

Inverse relations have been proved between nutritional deficiencies of folate, vitamin B6, vitamin B12[6], and homocysteine levels. Selhub and colleagues have suggested that inadequate plasma concentrations of one or more of these B vitamins contribute to almost two-thirds of all cases of Hyperhomocysteinemia. The study by Selhub and colleagues found that Homocysteine levels were directly proportional to age if the vitamin concentrations were kept under control. If age, sex, and levels of various other vitamins were kept under control, then homocysteine showed strong negative with plasma folate levels[8].

Rex et al., in their studies, established positive relations between plasma homocysteine levels and advanced chronic kidney diseases & end-stage renal disease, also stating that complications of atherosclerosis increase in these patients, thus resulting in increased mortality. Fasting total homocysteine also increases due to the increase in serum creatinine due to impaired metabolism of homocysteine by the kidney as clearance of homocysteine by plasma is the maximum[9].

As understood from figure 2, we know that homocysteine plays a crucial role in the methylation of SAH. An increase in homocysteine levels can increase SAH levels compared to SAH, causing a state of hypomethylation. SAM-to-SAH ratio defines the methylation potential of a cell; a decrease in this potential which will also decrease the ratio is an indicator of Hyperhomocysteinemia[10]. DNA methylation is important as it is essential for normal development &maintenance of cellular homeostasis &functions in adult organisms, such as genomic imprinting[11], inactivation of the X chromosome in females[12], silencing of repetitive DNA elements[13]. Studies have established inverse relations in levels of SAH and DNA methylation in lymphocytes[14]; this means disruption in cellular homeostasis due to DNA hypomethylation due to an increase in SAH caused by the increase in levels of homocysteine. The significance of DNA methylation and atherosclerosis has been widely noted[15,16].

Pyroptotic cell death due to homocysteine

Studies have shown that Hyperhomocysteinemia promotes endothelial apoptosis leading to endothelial dysfunction[17].it has also been proven that Hyperhomocysteinemia promotes systemic and vessel wall inflammation by inducing inflammatory monocyte differentiation[18]. Homocysteine has also proven to induce endothelial pyroptosis, an inflammatory cell death form. Pyroptosis releases fever-producing cytokine interleukin-1. Hang Xi et.al. also established the relation between how inflammasome activation in hyperhomocysteinemia-induced cell death and endothelial dysfunction[19]. Hang Xi et al. also proved that homocysteine activates caspase-1, caspase-8, caspase-9, and caspase-3 in sequential order, thus proving caspase-1(interleukin-1βconverting enzyme or ICE) activation is may be responsible for homocysteine-induced pyroptosis[19]. This promotion in inflammasome assembly and activation of caspase-1 may be the cause of endothelial inflammation.

Homocysteine and cardiovascular diseases

Heart pathological conditions, including diseased vessels, structural problems, blood clots, etc., are called cardiovascular diseases (CVD)[20]. CVD-related deaths are more common than any other, if not the most common in the world. There are various contributing factors for CVD, but this study focuses mainly on homocysteine-induced problems. Various studies regarding CVD have shown that more than 50% of cases of CVD show classical risk factors which do not include homocysteine. The Framingham risk score (FRS) is an important instrument in predicting coronary artery disease in patients with classical risk factors such as dyslipidemia, hypertension, diabetes mellitus, smoking. Still, it does not include homocysteine[21]. Still, Hyperhomocysteinemia in CVD is found in more than 40% of the cases, which is significant. Thus, proving it to be an independent risk factor.

Left ventricular hypertrophy

Studies have shown that LVH is associated with Hyperhomocysteinemia in rural northeast China, more so the presence of metabolic syndrome (syndrome X) and Hyperhomocysteinemia increases the risk of LVH[22]. The possible reason for hyperhomo cysteinemia-induced cardiac hypertrophy may be an increase in the density of mast cells and their degranulation[23]. Mast cells on degranulation release histamine[24]. Histamine, in turn, induces the expression of c-fos, an oncogene involved in the development of hypertrophy[25]. This chymase stored in the granules of mast cells as a complex with heparin proteoglycan is also released during degranulation of mast cells converting angiotensin 1 to angiotensin 2. This, angiotensin 2, provides signals for immediate early c-fos, jun B, Egr-1, and c-myc in both myocyte and non-myocyte; Angiotensin II also induces the fetal program (induction of skeletal a-actin and ANF) and induces

expression of the angiotensinogen gene and TGF-β1 (transforming growth factor) gene. The hypertrophy is mainly mediated by the angiotensin receptor 1(AT-1)[26,27]

Mast cells (cardiac) secretes TNFα, a proinflammatory cytokine that activates nuclear factor-kappa β, inducing hypertrophy[28]. Mast cells also release TGFβ, which activates TGFβ active kinase; this, in turn, activates mitogen-activated protein kinase to induce hypertrophy[29].

Higher plasma homocysteine is also associated with long QTc intervals (corrected QT interval = QT /square root of RR interval)[30], normal QTc is less than 0.46 seconds if more it is called a prolonged interval which is related to ventricular arrhythmia, which may result in cardiac death.

Thrombosis induced by Hyperhomocysteinemia

Venous thrombosis: - Studies have shown that mild Hyperhomocysteinemia may cause venous thrombosis, also suggested that an increase in the plasma homocysteine levels increased the risk of thrombosis, suggesting that there must be a threshold above which homocysteine has thrombogenic effects[31]. Falcon et al. reported that elevated homocysteine levels were a risk factor for juvenile thrombosis[32], hyperhomocysteinemia in adults can as well cause thrombosis[31]. Rodgers's study can understand the mechanism by which homocysteine produces thrombosis, which suggested that homocysteine directly interacts with thrombomodulin-thrombin and thrombomodulin-protein C interactions and impairs them; his study also suggested that homocysteine may act as a competitive inhibitor to Thrombin[33]. This pathological effect seems to have more effect in women than in men, even though women have lower plasma homocysteine levels[34].

Arterial thrombosis

Experiments have shown that the occurrence of thrombotic occlusion of the carotid artery is 50% more in hyperhomocysteinemic mice than in control mice[35]. A Possible explanation could be the lack of endothelial-derived nitric oxide causing enhanced platelet activation[36]. Another possible explanation can be the decrease in the activity of thrombomodulin due to Hyperhomocysteinemia, which is found in the luminal surface of endothelium and is responsible for the activation of anticoagulant protein C[37,38].

Endothelial cell dysfunction

The term "endothelial dysfunction" refers to the impairment of the normal homeostatic properties of vascular endothelium, including endothelium-dependent regulation of vascular tone, hemostasis, and inflammation[39]. Studies have shown that Hyperhomocysteinemia induces endothelial dysfunction[40,41]. However, it depends on various other factors such as hypercholesterolemia and hypertension[40]. Studies have shown that homocysteine induces endoplasmic reticulum (ER) stress[42], also induces proinflammatory responses[43]. The mechanism by which homocysteine produces endothelial stress maybe because of the increase in expression and synthesis of GRP78 (ER-resident chaperon & a member of 70- Kd heat shock protein family). This GRP78 is induced by agents which produce ER stress[44]. Thus, suggesting that homocysteine alters cellular redox state, leading to ER stress. The Study by TSAI and colleagues also proved that homocysteine decreases DNA synthesis in endothelial cells (whereas it increases DNA synthesis in vascular smooth muscle cells) and negatively affects the regeneration of damaged endothelial cells[45].

Tyagi et al., in their study, proved that homocysteine causes oxidative redox stress in vascular cells, mainly in the endothelium[46]. From figure 3, we understand that homocysteine induces the production of Thrombin and the induction of Latent MMPs, which help in the differential expression of PARs, specifically PAR-4[47,48,49]. Homocysteine causes a decrease in thioredoxin, the reactive oxygen species (superoxide-O2.) reacts with nitric oxide (NO) generates peroxynitrite (OONO-), which forms nitro tyrosine by reacting with tyrosine residues, thus increasing the levels of nitrotyrosine[50]. Homocysteine also decreases NO bioavailability by aiding in ADMA (endogenous inhibitor) production, thus inhibiting e-NOS. It also uncouples e-NOS which results in ROS production, thereby further decreasing the bioavailability of NO.

Production of ADMA is regulated by DDAH, which gets inactivated by homocysteine. This further increases the levels of ADMA. Thus, homocysteine induces oxidative stress by up regulation of PAR-4 (G-protein coupled receptor), decreasing thioredoxin, increasing iNOS & NADPH oxidase expression[51].

Hyperhomocysteinemia has also proven to cause a decrease in EC-SOD levels (most abundant isoenzyme of superoxide dismutase – SOD) in the vascular wall. SOD scavenges the anions which damage the endothelium; this, the function gets significantly impaired and thus potentiates oxidative stress[52].

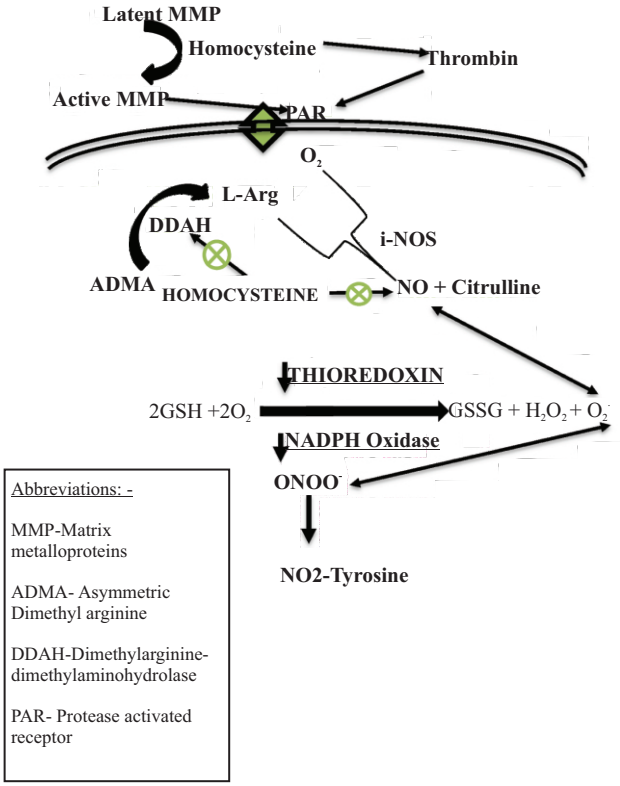

Fig 3: Increase in oxidative stress due to activation of latent matrix metalloproteins and Protease activated receptors.

Acute coronary syndrome

Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acid peroxidation. Increased levels of free radicals will lead to overexpression of MDA[53]. MDA causes lipid peroxidation by in-vitro oxidation of LDL, leading to hydro peroxides that are converted to aldehydes. These aldehydes react with lysine residues in the Apo lipoprotein B-100 moiety rendering the LDL more negative in its charge; this result in a decreased affinity for the LDL receptor and an increased affinity for scavenger receptors is called MDA-modified LDL (MDA-LDL). MDA-LDL is a very significant systemic oxidative stress marker[54]. Homocysteine, as we discussed, increases the production of reactive oxygen species and thus free radicals. MDA-LDL being immunogenic and bound to the antibodies enters the intima of the vessel and binds with macrophages. This is called a foam cell which accumulates in the vessel forming an atherosclerotic plaque. Therefore, MDA-LDL levels are high in acute coronary syndrome along with high homocysteine levels[55].

Treatment

Treatment varies with respect to the underlying cause. However, Folic acid supplementation has proved to reduce homocysteine levels because: -

1. Folate is a methyl donor, thereby reduces homocysteine levels,
2. Folate reduces the oxidant stress by its antioxidant actions,

5-MTHF can reduce superoxide generation by acting on two generation systems, namely xanthine oxidase/ hypoxanthine & action on endothelial nitric oxide synthase[47].

1. Folate acts as an electron donor, enhances binding of BH4 to e-NOS (endothelial nitric oxide synthase), and increases BH4 availability, thus helping in the generation of endothelial nitric oxide[47].

Mast cell stabilizers like sodium cromoglycate & ketotifen have also proven to attenuate homocysteine-induced hypertrophy. They decrease the heart's oxidative stress, protect mast cells from degranulation, and increase mast cell density[56].

Conclusion

Various theories have been proposed to explain the effects of Hyperhomocysteinemia and its effects on cardiovascular diseases by causing Endothelial dysfunction, Ischemic heart diseases, Thrombosis,S ER stress, etc. All these theories have again and again proven the fact that homocysteine has been playing a role in causing various cardiovascular defects. Thus,

homocysteine should be named as an independent marker for causing cardiovascular diseases.

Source of support: Nil
Conflict of interest: Nil

ORCiD

Advait Teli 0009-0009-9614-3375

References

1. Butz LW, du Vigneaud V. The formation of a homologue of cystine by the decomposition of methionine with sulfuric acid. Journal of Biological Chemistry. 1932 Dec 1;99(1):135-42.
2. Xiao Y, Zhang Y, Wang M, Li X, Xia M, Ling W. Dietary protein and plasma total homocysteine, cysteine concentrations in coronary angiographic subjects. Nutrition journal. 2013 Dec 1;12(1):144.
3. Valente FX, do Nascimento Campos T, de Sousa Moraes LF, Hermsdorff HH, de Morais Cardoso L, Pinheiro-Sant'Ana HM, Gilberti FA, Peluzio MD. B vitamins related to homocysteine metabolism in adults celiac disease patients: a cross-sectional study. Nutrition journal. 2015 Dec;14(1):1-7.
4. Hayden MR, Tyagi SC. Homocysteine and reactive oxygen species in metabolic syndrome, type 2 diabetes mellitus, and atherosclerosis: the pleiotropic effects of folate supplementation. Nutrition journal. 2004 Dec 1;3(1):4.
5. Mansoor MA, Svardal AM, Schneede J, Ueland PM. Dynamic relation between reduced, oxidized, and protein-bound homocysteine and other thiol components in plasma during methionine loading in healthy men. Clinical chemistry. 1992 Jul 1;38(7):1316-21.
6. Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutrition journal. 2015 Dec;14(1):1-0.
7. Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. The lancet. 1999 Jul 31;354(9176):407-13.
8. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. Jama. 1993 Dec 8;270(22):2693-8.
9. Jamison RL, Hartigan P, Kaufman JS, Goldfarb DS, Warren SR, Guarino PD, Gaziano JM, Veterans Affairs Site Investigators. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. Jama. 2007 Sep 12;298(10):1163-70.
10. Handy DE, Castro R, Loscalzo J. Epigenetic modifications: basic mechanisms and role in cardiovascular disease. Circulation. 2011 May 17;123(19):2145-56.
11. Li E, Beard C, Jaenisch R. Role for DNA methylation in genomic imprinting. Nature. 1993 Dec;366(6453):362-5.
12. Singer-Sam J, Riggs AD. X chromosome inactivation and DNA methylation. DNA methylation. 1993:358-84.
13. Goll MG, Bestor TH. Eukaryotic cytosine methyltransferases. Annu. Rev. Biochem.. 2005 Jul 7;74:481-514.
14. Yi P, Melnyk S, Pogribna M, Pogribny IP, Hine RJ, James SJ. Increase in plasma homocysteine associated with parallel increases in plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation. Journal of Biological Chemistry. 2000 Sep 22;275(38):29318-23.
15. Dong C, Yoon W, Goldschmidt-Clermont PJ. DNA methylation and atherosclerosis. The Journal of nutrition. 2002 Aug 1;132(8):2406S-9S.
16. Zaina S, Lindholm MW, Lund G. Nutrition and aberrant DNA methylation patterns in atherosclerosis: more than just

hyperhomocysteinemia?. The Journal of nutrition. 2005 Jan 1;135(1):5-8.

17. Wang H, Jiang X, Yang F, Gaubatz JW, Ma L, Magera MJ, Yang X, Berger PB, Durante W, Pownall HJ, Schafer AI. Hyperhomocysteinemia accelerates atherosclerosis in cystathionine β-synthase and apolipoprotein E double knock-out mice with and without dietary perturbation. Blood, The Journal of the American Society of Hematology. 2003 May 15;101(10):3901-7.
18. Zhang D, Jiang X, Fang P, Yan Y, Song J, Gupta S, Schafer AI, Durante W, Kruger WD, Yang X, Wang H. Hyperhomocysteinemia promotes inflammatory monocyte generation and accelerates atherosclerosis in transgenic cystathionine β-synthase–deficient mice. Circulation. 2009 Nov 10;120(19):1893-902.
19. Xi H, Zhang Y, Xu Y, Yang WY, Jiang X, Sha X, Cheng X, Wang J, Qin X, Yu J, Ji Y. Caspase-1 inflammasome activation mediates homocysteine-induced pyrop-apoptosis in endothelial cells. Circulation research. 2016 May 13;118(10):1525-39.
20. Anderson KM, Odell PM, Wilson PW, Kannel WB. Cardiovascular disease risk profiles. American heart journal. 1991 Jan 1;121(1):293-8.
21. Ungvari Z, Csiszar A, Edwards JG, Kaminski PM, Wolin MS, Kaley G, Koller A. Increased superoxide production in coronary arteries in hyperhomocysteinemia: role of tumor necrosis factor-α, NAD (P) H oxidase, and inducible nitric oxide synthase. Arteriosclerosis, thrombosis, and vascular biology. 2003 Mar 1;23(3):418-24.
22. Yu S, Chen Y, Yang H, Guo X, Zheng L, Sun Y. Hyperhomocysteinemia accompany with metabolic syndrome increase the risk of left ventricular hypertrophy in rural Chinese. BMC Cardiovascular Disorders. 2020 Dec 1;20(1):44.
23. Singh AP, Singh M, Balakumar P. Effect of mast cell stabilizers in hyperhomocysteinemia-induced cardiac hypertrophy in rats. Journal of cardiovascular pharmacology. 2008 Jun 1;51(6):596-604.
24. Dvorak AM. Mast-cell degranulation in human hearts. The New England journal of medicine. 1986 Oct 1;315(15):969-70.
25. Barka T, Van der Noen H, Shaw PA. Proto-oncogene fos (c-fos) expression in the heart. Oncogene. 1987 Jan 1;1(4):439-43.
26. McEuen AR, Sharma B, Walls AF. Regulation of the activity of human chymase during storage and release from mast cells: the contributions of inorganic cations, pH, heparin and histamine. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 1995 Jun 20;1267(2-3):115-21.
27. Sadoshima JI, Izumo S. Molecular characterization of angiotensin II--induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of the AT1 receptor subtype. Circulation research. 1993 Sep;73(3):413-23.
28. Shiota N, Rysä J, Kovanen PT, Ruskoaho H, Kokkonen JO, Lindstedt KA. A role for cardiac mast cells in the pathogenesis of hypertensive heart disease. Journal of hypertension. 2003 Oct 1;21(10):1935-44.
29. Watkins SJ, Jonker L, Arthur HM. A direct interaction between TGFβ activated kinase 1 and the TGFβ type II receptor: implications for TGFβ signalling and cardiac hypertrophy. Cardiovascular research. 2006 Feb 1;69(2):432-9.
30. Li Z, Guo X, Sun G, Zheng L, Sun Y, Liu Y, Abraham MR. Plasma homocysteine levels associated with a corrected QT interval. BMC cardiovascular disorders. 2017 Dec 1;17(1):182.

31. Wang H, Jiang X, Yang F, Gaubatz JW, Ma L, Magera MJ, Yang X, Berger PB, Durante W, Pownall HJ, Schafer AI. Hyperhomocysteinemia accelerates atherosclerosis in cystathionine β-synthase and apolipoprotein E double knock-out mice with and without dietary perturbation. Blood, The Journal of the American Society of Hematology. 2003 May 15;101(10):3901-7.
32. Zhang D, Jiang X, Fang P, Yan Y, Song J, Gupta S, Schafer AI, Durante W, Kruger WD, Yang X, Wang H. Hyperhomocysteinemia promotes inflammatory monocyte generation and accelerates atherosclerosis in transgenic cystathionine β-synthase–deficient mice. Circulation. 2009 Nov 10;120(19):1893-902.
33. Rodgers GM, Conn MT. Homocysteine, an atherogenic stimulus, reduces protein C activation by arterial and venous endothelial cells.
34. Ueland PM, Refsum H, Brattstrom L. Plasma homocysteine and cardiovascular disease. Francis RB Jr Atherosclerotic Cardiovascular Disease, Hemostasis, and Endothelial Function. New York: Marcel Dekker;. 1992:183.
35. Wilson KM, Dayal S, Bottiglieri T, Lentz SR. Hyperhomocysteinemic Mice Have Increased Susceptibility to Carotid Artery Thrombosis.
36. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. New England journal of medicine. 1998 Apr 9;338(15):1042-50.
37. Weiler H, Isermann BH. Thrombomodulin. Journal of thrombosis and haemostasis. 2003 Jul;1(7):1515-24.
38. Lentz SR, Sobey CG, Piegors DJ, Bhopatkar MY, Faraci FM, Malinow MR, Heistad DD. Vascular dysfunction in monkeys with diet-induced hyperhomocyst (e) inemia. The Journal of clinical investigation. 1996 Jul 1;98(1):24-9.
39. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circulation research. 2000 Nov 10;87(10):840-4.
40. Lentz SR. Homocysteine and cardiovascular physiology. Homocysteine in health and disease. 2001:441-50.
41. Faraci FM, Lentz SR. Hyperhomocysteinemia, oxidative stress, and cerebral vascular dysfunction. Stroke. 2004 Feb 1;35(2):345-7.
42. Outinen PA, Sood SK, Pfeifer SI, Pamidi S, Podor TJ, Li J, Weitz JI, Austin RC. Homocysteine-induced endoplasmic reticulum stress and growth arrest leads to specific changes in gene expression in human vascular endothelial cells. Blood, The Journal of the American Society of Hematology. 1999 Aug 1;94(3):959-67.
43. Ungvari Z, Csiszar A, Edwards JG, Kaminski PM, Wolin MS, Kaley G, Koller A. Increased superoxide production in coronary arteries in hyperhomocysteinemia: role of tumor necrosis factor-α, NAD (P) H oxidase, and inducible nitric oxide synthase. Arteriosclerosis, thrombosis, and vascular biology. 2003 Mar 1;23(3):418-24.
44. Lee AS. Mammalian stress response: induction of the glucose-regulated protein family. Current opinion in cell biology. 1992 Apr 1;4(2):267-73.
45. Tsai JC, Perrella MA, Yoshizumi M, Hsieh CM, Haber E, Schlegel R, Lee ME. Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. Proceedings of the National Academy of Sciences. 1994 Jul 5;91(14):6369-73.
46. Tyagi N, Sedoris KC, Steed M, Ovechkin AV, Moshal KS, Tyagi SC. Mechanisms of homocysteine-induced oxidative stress. American Journal of Physiology-Heart and Circulatory Physiology. 2005 Dec;289(6):H2649-56.
47. Al-Obaidi MK, Philippou H, Stubbs PJ, Adami A, Amersey R, Noble MM, Lane DA. Relationships between homocysteine, factor VIIa, and thrombin generation in acute

coronary syndromes. Circulation. 2000 Feb 1;101(4):372-7.

48. Mujumdar VS, Aru GM, Tyagi SC. Induction of oxidative stress by homocyst (e) ine impairs endothelial function. Journal of cellular biochemistry. 2001 Sep 1;82(3):491-500.

49. Sabri A, Muske G, Zhang H, Pak E, Darrow A, Andrade-Gordon P, Steinberg SF. Signaling properties and functions of two distinct cardiomyocyte protease-activated receptors. Circulation research. 2000 May 26;86(10):1054-61.

50. Halliwell B. What nitrates tyrosine? Is nitrotyrosine specific as a biomarker of peroxynitrite formation in vivo?. FEBS letters. 1997 Jul 14;411(2-3):157-60.

51. Verhaar MC, Stroes E, Rabelink TJ. Folates and cardiovascular disease. Arteriosclerosis, thrombosis, and vascular biology. 2002 Jan 1;22(1):6-13.

52. Nonaka H, Tsujino T, Watari Y, Emoto N, Yokoyama M. Taurine prevents the decrease in expression and secretion of extracellular superoxide dismutase induced by homocysteine: amelioration of homocysteine-induced endoplasmic reticulum stress by taurine. Circulation. 2001 Sep 4;104(10): 1165-70.

53. Draper HH, McGirr LG, Hadley M. The metabolism of malondialdehyde. Lipids. 1986 Apr;21(4):305-7.

54. Marnett LJ. Lipid peroxidation—DNA damage by malondialdehyde. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 1999 Mar 8;424(1-2):83-95.

55. Wang G, Mao JM, Wang X, Zhang FC. Effect of homocysteine on plaque formation and oxidative stress in patients with acute coronary syndromes. Chin Med J (Engl). 2004 Nov;117(11):1650-4. PMID: 15569480.

56. Singh AP, Singh M, Balakumar P. Effect of mast cell stabilizers in hyperhomocysteinemia-induced cardiac hypertrophy in rats. Journal of cardiovascular pharmacology. 2008 Jun 1;51(6):596-604.