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The Metabolism and Significance of Homocysteine in Cardiovascular Health: Mini Review

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Abstract

Homocysteine conversion to the essential and non-essential methionine and cysteine respectively is not only a decisive step in the normal biosynthesis of these two amino acids, but also dependent upon vitamins of the B complex. Therefore, deficiency of one or more of these crucial co-factors is associated with hyperhomocysteinemia along with innumerable negative consequences on various systems. This mini review will summarize and critically appraise the results of conflicting evidence concerning the effect of hyperhomocysteinemia in the total cardiovascular health. High levels of homocysteine in the blood are known to mediate cardiovascular problems by their adverse effects on cardiovascular endothelium and smooth muscle cells with resultant effects mainly on atherosclerosis and SVS (small vessel stroke). Evidence also suggests, that proper dieting reflects on the low blood levels of homocysteine, which in turn are able to reduce the risk of CVDs by approximately 15-40%. Nevertheless, it should be stated that these evidences are not, under any circumstances, absolute and are being juxtaposed by many studies, including the ones this review will quote. The present study will thus abstract the existing evidence and highlight their controversies that pertain to the relationship between hyperhomocysteinemia and cardiovascular risk.

Keywords: Homocysteine, Metabolism, Vitamin B6, Vitamin B12, Folic Acid, Cardiovascular Health.

INTRODUCTION

As stated by the World Health Organization (WHO) in 2017, cardiovascular diseases (CVDs) are the main killer worldwide [1]. As homocysteine (Hcy) is known as a risk factor since the 1990s this mini review will go into more detail with the metabolism of homocysteine as well as its significance in cardiovascular (CV) Health [2]. Homocysteine, an intermediate product in the normal biosynthesis of the essential amino acid methionine and non-essential cysteine is a sulfhydryl-containing amino acid [3]. It is a key step in the methylation cycle [4]. This mini review will focus on the correlation of deficiencies of the vitamins B6, B12 and folic acid with the blood level of Hcy and their effect of CVDs. Hyperhomocysteinemia, a state where the blood levels of Hcy rise above 15µmol/L [5] (up to 500µmol/L [6]) is associated with nutritional deficiencies of the micronutrients stated above [7]. Decreased levels of homocysteine is associated with osteoporosis or eye lens dislocation, while high levels is mostly correlated with CVDs [6]. Metabolism of homocysteine is done via two pathways: Remethylation and Trans-sulfuration. The two are affected by Methionine (Met) blood levels. Either Met blood levels are high where trans-sulfuration process plays a crucial role, as the remethylation comes into an important role when Met blood levels are low. Vitamin B6, B12 and folic acid are crucial factors determining the enzyme rate as they serve as cofactors in the metabolism in Homocysteine [8].

Metabolism:

Metabolism of Homocysteine

Synthesizing of Hcy is done by transmethylation of the essential amino acid Methionine. Three protein enzymes are involved that function in different tissues: S-adenosyl-L-methionine (SAM) synthase / L-methionine adenosyltransferase, Methyltransferase (MT) and S-adenosyl-L-homocysteine (SAH) hydrolase. Methionine is firstly, with use of ATP, converted to S-Adenosylmethionine by SAM synthetase which is further methylated by MT [7]. Methylation is referred to a mechanism where a specific compound is accepting a methyl group. This process is well known in biosynthesis of different compounds as well as in epigenetic modulations. Finally, to achieve the production of the amino acid compound homocysteine, SAH hydrolase is used. The described process above is referred to the transmethylation. Synthesized Hcy can be

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broken down by several pathways: (i) back to methionine by Methionine synthase, (ii) back to methionine by Betainehomocystein methyltransferase, (iii) to Homocysteine-thiolactone by Methionyl-tRNA synthetase with use of ATP and (iv) to Cystathionine by adding the amino acid serine and by catalyzation of Cystathionine ß-synthetase [Figure 1]. Cystathionine further is broken down to cysteine by Cystathionine γ-lase. In the end cysteine can be degraded to glutathione, proteins, CoA and sulfates. Summarizing the process of degrading Hcy up to Cysteine is stated as Transsulfuration. Remethylation is categorized into Folate/B12 Dependent and Folate/B12 Independent. The former one will be more emphasized in this article than the latter one. Folic acid is firstly converted to tetrahydrofolate and then broken down by Serine hydroxymethyl transferase with the use of serine to glycine, to N-5,10-Methylenetetrahydrofolate and further to N-5-Methyltetrahydrofolate [Figure 1]. The latter compound is catalyzed by Methionine synthetase to methionine and Transmethylation is used to produce Hcy. As stated above, Folic Acid and the Vitamins B6 and B12 are important cofactors in particular enzymes that are involved in either Transmethylation, Folate/B12 Dependent Remethylation or Transsulfuration. Vitamin B6 is a cofactor in Cystathionine ß-synthase, Cystathionine γ-lase and Serine hydroxymethyltransferase [Figure 1]. Vitamin B12 is the main cofactor in the significant enzyme Methionine Synthase [Figure 1]. Lastly, Folic Acid intake is crucial as it is the substrate for the Folate/B12 Dependent Remethylation. [9]

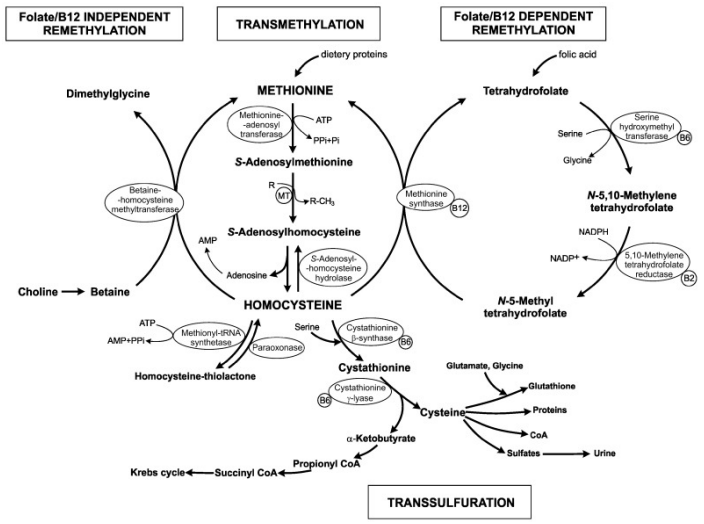

Figure 1: Overview of the Metabolism of Homocysteine. ATP: adenosine triphosphate; AMP: adenosine monophosphate; PPi: pyrophosphate; Pi: orthophosphate; B2/B6/B12: vitamins B2/B6/B12; CoA: coenzyme A; R: acceptor; R-CH3: methylated product; MT: methyltransferases.

Folic Acid

Folate is an essential nutrient, which is appointed to the water-soluble B-Vitamins. Mammals are not able to synthesize Folic Acid and by that they fully have to rely on supplementation in terms of whole grain, liver or green vegetables [10]. Folic Acid is crucially involved in the metabolism of Homocysteine as it acts as a cofactor of enzymes in the Folate/B12 Dependent Remethylation. Folic Acid is further converted to Tetrahydrofolate by Dihydrofolate reductase in order to serve as a substrate for remethylation. Tetrahydrofolate then is further degraded to N-5 Methyltetrahydrofolate which is catalyzed by the Methionine Synthase to Methionine [9]. Low or high levels of Folic Acid would resultingly cause high or low levels of Methionine.

Vitamin B6

Vitamin B6 is an essential nutrient found in pork, fish, whole grain or several vegetables and serves in forms of pyrodoxal 5´-phosphate (PLP) as a cofactor for numerous catalytic functions including the metabolism of Homocysteine [11]. Vitamin B6 is a cofactor in three different enzyme catalyzation. (i) Serine hydroxymethyltransferase in the Folate/B12 dependent Remethylation process and (ii) Cystathionine ß-synthase (CBS) and (iii) Cystathionine γ-lase (CSE) in the Transsulfuration pathway [9]. Nevertheless, the most important role is focused on Transsulfuration pathway. By having moderate Vitamin B6 deficiency CSE activity is decreased while CBS is maintained what will ultimately result in higher cystathionine levels [12]. Cystathionine was elevated in humans with induced marginal Vitamin B6 deficiencies. Preprandial Cystathionine plasma levels were 53% elevated and postprandial by 76% [11].

Vitamin B12

Vitamin B12 or cobalamin is a water-soluble and essential nutrient for humans. It is referred to the general term of all corrinoids that exhibit the biological activity of cyanocobalamin [13]. Cobalamin is found in sources such as meat, eggs or dairy products and is absorbed in the Ileum after combining with intrinsic factor secreted by the stomach [14]. Vitamin B12 serves in forms of 5-Methylcobalamin as a cofactor for the cytosolic enzyme, Methionine Synthase in the remethylation mechanism of Homocysteine to Methionine [9]. Secondary, methionine synthase subsequently converts Methyltetrahydrofolate to tetrahydrofolate in the Folate/B12 dependent pathway [13].

The Homocysteine and Cardiovascular Health

According to current concepts, Homocysteine damages cells and tissue of arteries by inciting the release of cytokines, and other mediators of inflammation of cell division [15]. By affecting the smooth muscle cells, Hcy tends to cause changes in the connective tissue of the arteriosclerotic plaques, causing fibrosis, calcification, damage to the elastic tissue layer and proteoglycan deposition. Degradation of these aggregates leads to a disposition of cholesterol and other fats in the developing plaques. Since the discovery and development of the homocysteine theory of arteriosclerosis numerous clinical and epidemiological studies have observed elevation of blood homocysteine as a potent risk for vascular diseases [16]. A meta-analysis of published studies has suggested that elevation of Hcy blood levels is a casual factor in atherogenesis. The same literature states that by lowering the Hcy blood levels, a decrease in 15-40% risk of vascular diseases can be observed [17]. The results of the health studies, Hordaland homocysteine study and the European concerted action study all support the validity of homocysteine theory of CVD. However, the causality of correlation between Hcy and coronary heart diseases is still not certain [18].

Correlation with Vitamin B6, B12 and Folic Acid

A report of the Framingham Heart Study in 1993 showed that in a group of 1160 elderly participants between the ages of 67-96 years, the elevation of blood homocysteine has been caused by dietary deficiencies of vitamin B6, folic acid and a decrease in the absorption of vitamin B12 in the ileum [19]. In addition, genetic variations of the methylenetetrahydrofolate reductase, 677TT, affects about 12% of the population, which leads to an increased risk of CVD if dietary folic is marginal. The amounts of dietary B vitamins consumptions which is needed for the body to maintain normal blood Hcy levels are 3 mg vitamin B6 and 400 µg folic acid per day, as shown by the Framingham Heart Study. The daily intake of Vitamin B12 is denoted by 14µg but it is generally adequate, except in vegans who consume no meat, fish or dairy foods [20]. However recently three prospective trials of supplementation with B vitamins in patients with advanced vascular disease concluded that moderate doses of folic acid and vitamins B-12 and B-6 over a 3–5 y period have little effect on risk of recurrent heart attack or stroke. The vitamin intervention for stroke prevention (VISP) study observed that participants without renal impairment, without malabsorption of vitamin B-12, or without taking non-study vitamin B12

supplements had a reduction of 21% in vascular events from B vitamin therapy [21]. In the heart outcome prevention evaluation (HOPE2) trial of patients with advanced vascular disease, a reduction of 24% in stroke from B vitamin therapy was concluded. Yet, lower of blood Hcy levels was not significant in this trial [22]. The Norwegian Vitamin Trial (NORVIT) study had shown that treating with cardiac bypass or angioplasty (47.7% decrease of recurrent CVDs) was more efficient that the vitamin B comparing group (42.2% decrease of recurrent CVDs) (23). Concluding, vitamin B6, B12 and folic acid may have an effect on lowering the risk of CVD but their relationships has to be investigated further in clinical trials.

Lowering of Homocysteine

Homocysteine blood levels between 4-8 μmol/L decrease the risk of getting a cardiovascular disease. However, this blood level should be maintained by a healthy diet with an abundance of vitamin B6, folic acid and vitamin B12 [17]. Vitamin B6 is essential for the metabolism of carbohydrates, proteins and fats in addition to its significant role in the creation of erythrocytes. It helps in preventing blocked arteries and reduces heart disease risk [24]. If the homocysteine concentration is in the range of 8-12μmol/L, an effort should be made to improve the diet by providing a sufficient amount of vitamin B6, B12 and folic acid to keep the homocysteine low and to minimize the risk of a cardiovascular diseases. Aging may cause a decrease in the ability to absorb these vitamins, which ultimately results in elevation of the Hcy blood level concentration. Above the age of 60 years, it should be considered to consume 3 mg vitamin B-6, 400μg folic acid, and 100μg vitamin B-12 as dietary supplements, most conveniently in a daily multivitamin pill, in addition to consuming the Heart Revolution Diet [25]. The Heart Revolution Diet consists of fresh vegetables, fruits, whole-grain foods, seafood and meat, nuts, eggs, and diaries. Highly processed foods should be minimized, because they are partially diminished of vitamin B-6 and folic acid. In addition to an improved diet, supplements of 10mg vitamins B-6, 1000μg, folic acid and 100μg of vitamin B-12 should be considered to decrease the blood levels of Hcy.

Clinical Implications of Hyperhomocysteinemia on CVDs

Most studies state a relationship between high levels of homocysteine in the blood and cardiovascular events [33],[35],[37]. Furthermore, a prospective study [27] names homocysteine as a strong-and independent of traditional confounders- predictor for ischemic stroke [31], providing evidence that the median of differences in total homocysteine concentrations between matched pairs was 2.1 μmol/L higher in cases contrary to the matched controls, the 75th percentile of difference in total homocysteine was 5.3 μmol/L higher in cases and the odds ratios associated with total homocysteine concentration found altogether were lower in controls, although this paper lacks discrimination of hyperhomocysteinemia between a risk factor or a biomarker. Homocysteine was also affiliated with an indirect effect on the vessel endothelial wall through eliciting damage via oxidative stress, especially in the small vessels with an odds ratio of 1.34 [24] and therefore increasing the risk for atherosclerosis [26],[28],[30]. A large meta-analysis [29] of 72 studies showed a causal relationship between hyperhomocysteinemia and cardiovascular diseases, for every 5 mmol/ml increase of blood homocysteine, like ischemic heart disease (odds ratio= 1,42), deep vein thrombosis (odds ratio=1,60) and stroke (odds ratio=1,65), along with numerous other meta-analyses and prospective studies [31],[32],[38]. In a large meta-analysis of folate supplementation and CVD risk, in 20 RCTs, 3.164 events of stroke were stated among 77.816 participants. The stroke rate in the folate supplementation group was 3,8%, whereas in the control group was 4.4% and in addition with these evidence, the Relative Risk (RR) pooled from this meta-analysis showed a 10% decrease in the risk for stroke in the individuals administered with folic acid [38]. In a mendelian randomization analysis of 2019, the OR of SVS per 1 SD increment in folate and vitamin B_6 levels were 0.49 (95% CI, 0.34–0.71, $p = 1.3 \times 10^{-4}$) and 0.70 (95% CI, 0.52–0.94, $p = 0.02$), respectively, whereas such a correlation did not reach statistical significance with other major severe stroke subtypes [24]. Despite these evidence, a systematic review and meta-analysis of 2011 comprised 16 trials upon 44.841 and alleged that folic acid supplementation as compared to placebo had no effect on major cardiovascular events (RR, 0.98; 95% CI, 0.93-1.04), stroke (RR, 0.89; 95% CI,0.78-1.01), myocardial infarction (RR, 1.00; 95% CI, 0.93-1.07) [36]. Finally, further randomized controlled trials argue that folate supplementation is not effective at decreasing the risk of CV diseases [30],[34].

CONCLUSION

This paper reviewed the importance of B vitamins as essential cofactors in the physiological metabolic pathways of homocysteine, an intermediate product in the formation of both amino acids methionine and cysteine. Nonetheless, the debate for the contribution (or not) of B complex vitamins like folate (B9), B6, B12 and Hyperhomocysteinemia in the development and protection from cardiovascular diseases has been ongoing in the past years. Although the majority of the studies ecompassed in this review support a causal relationship between hyperhomocysteinemia and CVDs, accompanied by oft-quoted risk reducing intervention by folate, the aforementioned conflicting evidence along with other meta-analyses, RCTs as well as the authors of this article, suggest that further clinical trials with large sample sizes could potentially provide definite answers to these topics.

REFERENCES

1. WHO (2017). Cardiovascular Diseases (CVDs), [online]. Available at: https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)
2. Shenoy V, Mehendale V, Prabhu K, Shetty R, Rao P. (2014). Correlation of serum homocysteine levels with the severity of coronary artery disease. Indian journal of clinical biochemistry, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/24966483/
3. Faeh D, Chiolero A, Paccaud F. (2006). Homocysteine as a risk factor for cardiovascular disease: should we (still) worry about? Swiss medical weekly, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/17225194/
4. Loscalzo J, Handy DE. (2014). Epigenetic modifications: basic mechanisms and role in cardiovascular disease (2013 Grover Conference series). Pulmonary circulation, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/25006435/
5. Guo H, Chi J, Xing Y, Wang P. (2009). Influence of folic acid on plasma homocysteine levels & arterial endothelial function in patients with unstable angina. The Indian journal of medical research, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/19491420/
6. Tauheed H, Reetika A, Aniket KB, Reshmee B, Gurumayum SS, Lasihram RS. (2019). Disturbed homocysteine metabolism is associated with cancer. Experimental & Molecular Medicine, [online]. Available at: https://www.ncbi.nlm.gov/pmc/articles/PMC6389897
7. CurroM, Gugliandolo A, Gangemi C, Risitano R, Ientile R, Caccamo D. (2014). Toxic effect of mildly elevated homocysteine concentrations in neuronal-like cells. Neurochemical research, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/24867323
8. Ganguly P, Alam SF. (2015). Role of homocysteine in the development of cardiovascular disease. Nutrition Journal, [online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4326479/#CR10
9. Škovierová H., Vidomanová E., Mahmood S., Sopková J., Drogová A., Červeňová T., Halašová E., Lehotský J. (2016). The Molecular and Cellular Effect of Homocysteine Metabolism Imbalance on Human Health. International Journal of Molecular Sciences, [online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5085763/
10. Scaglione F., Panzavolta G. (2014). Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. Xenobiotica; the fate of foreign compounds in biological systems, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/24494987
11. Ueland PM., Ulvik A., Rios-Avila L., Midttun O., Gregory JF 3rd. (2015). Direct and Functional Biomarkers of Vitamin B6 Status. HHS Public Access, [online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5988249/

12. Lima CP., Davis SR., Mackey AD., Scheer JB., Williamson J., Gregory JF 3rd. (2006). Vitamin B-6 Deficiency suppresses the hepatic Transsulfuration pathway but increases glutathione concentration in rats fed AIN-76A or AIN-93G diets. The Journal of nutrition, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/16857832/
13. Allen LH, Miller JW, de Groot L, Rosenberg IH, Smith AD, Refsum H, Raiten DJ. (2018). Biomarkers of Nutrition for Development (BOND): Vitamin B-12 Review. The Journal of Nutrition, [online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6297555/
14. Shipton MJ, Thachil J. (2015). Vitamin B12 deficiency – A 21st century perspective. Clinical medicine (London, England), [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/25824066
15. McCully KS, Wilson RB. (1975). Homocysteine theory of arteriosclerosis. Atherosclerosis, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/1191372
16. McCully KS. (1996). Homocysteine and vascular disease. Nature Medicine, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/8597939
17. McCully KS. (2007). Homocysteine, vitamins, and vascular disease prevention. The American journal of clinical nutrition, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/17991676
18. Clarke R, Bennett DA, Parish S, Verhoef P, Dötsch-Klerk M, Lathrop M, et al.. (2012). Homocysteine and Coronary Heart Disease: Meta-analysis of MTHFR Case-Control Studies, Avoiding Publication Bias. PLoS Medicine, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/22363213
19. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. (1993). Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/8133587
20. Herrmann W, Schorr H, Obeid R, Geisel J. (2003). Vitamin B12 status, particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia in vegetarians. The American Journal of clinical nutrition, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/12816782T
21. Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, Sides EG, Wang CH, Stampfer M. (2004). Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. JAMA, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/14762035
22. Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M, McQueen MJ, et al.. (2006). Homocysteine lowering with folic acid and B vitamins in vascular disease. The New England journal of medicine, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/16531613
23. Debreceni B, Debreceni L. (2011). Why do Homocysteine-Lowering B Vitamin and Antioxidant E Vitamin Supplementations Appear To Be Ineffective in Prevention of Cardiovascular Disease? Blackwell Publishing Ltd, [online]. Available at: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1755-5922.2011.00266.x?fbclid=IwAR2-tUdxB-OZkPuXIb_foz_plQZdYFbOQxf1CtrlvD03zzyfw193B7hATyw
24. Streit L. (2018). 9 Health Benefits of Vitamin B6 (Pyridoxine). healthline, [online]. Available at: https://www.healthline.com/nutrition/vitamin-b6-benefits?fbclid=IwAR1agWrrSa6qvLufivdu9uloh-Eej0sIpgUxKn6D0BphK6epXyUw4WqNNmk
25. Kuller HK, Evans RW. (1998). Homocysteine, Vitamins, and Cardiovascular Disease. Ahajournal, [online] Available at: https://ahajournals.org/doi/pdf/10.1161/01.CIR.98.3.196?fbclid=IwAR1tdAS2syOkfRq5yg7IrfAUqWEQKZK-iGDzDU1CvNgyDKwSsEnUaQjUPVk
26. Wang G, Woo CW, Sung FL, Siow YL, O K. (2002). Increased monocyte adhesion to aortic endothelium in rats with hyperhomocysteinemia: role of chemokine and adhesion molecules. Atherosclerosis, Thrombosis, and vascular biology , [online]. Available at: https://www.ahajournals.org/doi/full/10.1161/01.atv.0000035404.18281.37?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed
27. David Tanne, Moti Haim, Uri Goldbourt, Valentina Boyko, Ram Doolman, Yehuda Adler, Daniel Brunner, Solomon Behar, and Ben-Ami Sela. (2003) . Prospective Study of Serum Homocysteine and Risk of Ischemic Stroke Among Patients With Preexisting Coronary Heart Disease. AHA Avaiahttps://ahajournals.org/doi/10.1161/01.str.0000060203.58958.35
28. Aguilar B, Rojas JC, Collados MT. (2004) . Metabolism of homocysteine and its relationship with cardiovascular disease. Journal of thrombosis and thrombolysis, [online] . Available at: https://www.ncbi.nlm.nih.gov/pubmed/15789174
29. David S Wald, specialist registrar in cardiology,a Malcolm Law, professor,b and Joan K Morris, senior lecturer b. (2002) . Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ, [online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC135491/
30. Jeremy Marcus, MD, Mark J Sarnak, MD, and Vandana Menon, MD. (2007). Homocysteine lowering and cardiovascular disease risk: Lost in translation. The Canadian journal of cardiology. [online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2651913/
31. Madalena Barroso, Diane E. Handy, Rita Castro. (2019). The Link Between Hyperhomocysteinemia and Hypomethylation: Implications for Cardiovascular Disease. Journal of Inborn Errors of Metabolism and Screening, [online]. Available at: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S2326-45942017000100701
32. Ueland PM, Refsum H, Beresford SA, Vollset SE.(2000). The controversy over homocysteine and cardiovascular risk. The American Journal of clinical nutrition, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/10919921
33. Yong Huo, MD; Jianping Li, MD, PhD1; Xianhui Qin, PhD. (2015). Efficacy of Folic Acid Therapy in Primary Prevention of Stroke Among Adults With Hypertension in China . Jama Network, [online]. Available at: https://jamanetwork.com/journals/jama/fullarticle/2205876
34. Gervasio A. Lamas, MD, Robin Boineau, MD MA, Christine Goertz, DC PhD, Daniel B. Mark, MD, MPH, Yves Rosenberg, MD, Mario Stylianou, PhD, Theodore Rozema, MD, Richard L. Nahin, PhD, MPH, Lauren Lindblad, MS, Eldrin F. Lewis, MD, Jeanne Drisko, MD, Kerry L. Lee, PhD, and for the TACT Investigators. (2013) . Oral High-Dose Multivitamins and Minerals or Post Myocardial Infarction Patients in TACT. Ann Intern Med, [online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4143134/
35. Yang HT, Lee M, Hong KS, Ovbiagele B, Saver JL. (2012). Efficacy of folic acid supplementation in cardiovascular disease prevention: an updated meta-analysis of randomized controlled trials. European Journal of internal medicine, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/22884409
36. Yu-Hao Zhou, Jian-Yuan Tang, Mei-Jing Wu, Jian Lu, Xin Wei, Ying-Yi Qin, Chao Wang, Jin-Fang Xu, and Jia He. (2011) . Effect of Folic Acid Supplementation on Cardiovascular Outcomes: A Systematic Review and Meta-Analysis . PLoS One, [online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3182189/
37. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. (1995). A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA , [online] . Available at: https://www.ncbi.nlm.nih.gov/pubmed/7563456
38. Yanping Li, PhD, Tianyi Huang, ScD, Yan Zheng, PhD, Tauland Muka, PhD, Jenna Troup, MPH, and Frank B. Hu, MD, PhD. (2016) . Folic Acid Supplementation and the Risk of Cardiovascular Diseases: A Meta-Analysis of Randomized Controlled Trials . J Am Heart Assoc , [online]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5015297/
39. Larsson SC1,2, Traylor M3, Markus HS3. (2019). Homocysteine and small vessel stroke: A mendelian randomization analysis. Annals of neurology, [online]. Available at: https://www.ncbi.nlm.nih.gov/pubmed/30785218.