Review Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 11/03/2020;
Date Revised: 20/08/2020;
Date Published Online: 25/11/2020;

Authors' Affiliations:
1. School of Health Sciences, University of Management and Technology, Lahore - Pakistan
2. Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore - Pakistan
3. Punjab AIDS Control Program, Primary and Secondary Healthcare Department, Government of the Punjab, Lahore - Pakistan

***Corresponding Author:**
Abida Bano
Email: abidammgpu@gmail.com

How to Cite:
Tanweer A, Bano A, Fatima W, Javed H. Hyperhomocysteinemia: The independent risk factor of cardiovascular diseases. Adv. Life Sci. 8(1): 01-07.

Keywords:
Hyperhomocysteinemia; CVD; Methionine; Homocysteine

Open Access

Hyperhomocysteinemia: The independent risk factor of cardiovascular diseases

Afifa Tanweer[1], Abida Bano[2]*, Warda Fatima[2], Hasnain Javed[3]

Abstract

Hyperhomocysteinemia (Hcy) is an independent risk factor for cardiovascular diseases (CVD) and associated with primary causes of mortality and morbidity throughout the world. Association between Hcy and CVD has been a question for biologists over the last five decades. The circulating levels of Hcy can be increased by defects in enzymes involved in the metabolism of several B and therefore for homocysteine. Although researchers have yielded conflicting findings on the link between homocysteine and CVD risk, but there is convincing evidence on link between B vitamin deficiencies and increased CVD risk. This review identifies the research conducted on this subject matter and provides a framework for the factors associated with Hcy and CVD.

Introduction

The burden of non-communicable chronic diseases, including the CVDs, is rapidly rising around the globe, affecting all parts of world's population [1]. Contrarily to the previous classification of chronic disea ses as "diseases of affluence" they are now arising at a much faster rate in poorer countries than in developed areas of the world [2]. According to cardiovascular diseases account for around 30% of global deaths, and this figure is expected to increase further by 2030 [3]. While deaths caused due to CVD decreased by 31% between 2000 and 2010 [4], it is still projected to continue to be the world's leading cause of death. Given substantial research in the field of its prevention and treatment, CVD infests the developed and developing regions alike. It is accounted for one in every three deaths [1], while 80% of total deaths caused by CVD have been reported to occur in resource limited countries [3]. Asian and African regions of the world are showing highest mortality and prevalence of cardiovascular diseases in the world. People with old age are the main victims of heart disease. Cardiovascular disease is one out of five major causes of "years lived with disability" (YLD) in poor countries [5]. CVD causing premature deaths thus ask for more health care budgets and reducing the GDP by 6.77% together with diabetes [6]. Almost all the parts of World's population are facing a rise in non-communicable chronic diseases. Cardiovascular diseases are one of them which are focusing the underdeveloped population [7]. Research for prevention and treatment is still unable to control cardiovascular diseases in developed and developing countries [8]. This review summarizes the research conducted on Hcy and provides a framework for factors associated with Hcy and CVD.

Methods

Literature search strategy and selection criteria

References included in this review article were searched by using NCBI (PUBMED) and Google scholar, by the use of terms Hcy CVD, Methionine, Homocysteine. Articles resulting from these searches were reviewed and relevant references were cited in this review article. This included articles published in English. Case reports published online were also included in this study. We selected 24 peer reviewed research articles and 35 review articles for this study.

Discussion

Risk factors for development of CVD

The detection of risk factors plays a key role in primary prevention of chronic diseases. Like other non-communicable diseases, several factors which increase the possibility of developing CVD have also been reported, including hypertension, smoking, diabetes, high serum cholesterol and obesity [9].

Overview of Homocysteine metabolism

Homocysteine (HCY), a sulfur metabolite-containing essential amino acid, methionine, is formed regularly during methionine metabolism and its low concentrations (5-15µmol/L) are normally present in the plasma. HCY metabolism requires two separate pathways (trans-sulfuration and remethylation), dependent upon B vitamins [10]. The pathway of transsulfuration is triggered at times when methionine is present in excess amounts. During this cycle, HCY is converted to crystathione and subsequently converted to cysteine. Vitamin B_6 (pyridoxine) acts as a cofactor, HCY is largely remethylated during the periods of low methionine to be converted back to methionine [11]. During metabolism of sulfur containing amino acid, homocysteine is produced as a metabolite whose normal plasma concentration is 5-15 µmol/L [12]. In the presence of in excess amount of methionine, transsulfurationpathway is activated to convert homocysteine to crystathione leading to the formation of cysteine. But in the presence of low levels of methionine, re-methylation of homocysteine starts to synthesize methionine [13]. Methyl tetrahydrofolate (from Vitamin B_9) and Vitamin B_{12} act as cofactors in this process [14]. Both of these pathways require the mediation of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH). High levels of HCY are linked to reduced methylation potential, whereas this potential is increased by the presence of Vitamins B_9 and B_{12} [15]. High levels of HCY have been considered a risk factor for cardiovascular disease for nearly half a century, the association between genetic disability to breakdown HCY and premature atherosclerotic disease was reported [16]. The hypothesis discussed above has been researched several times and found conflicting and inconsistent results. Recent research community has observed the linkage between CVD and abnormally high levels of HCY which suggests HCY as a new risk factor for coronary artery disease (CAD) [17]. A cohort study showed the independent association of HCY level with all-cause plus cardiovascular mortality [18]. A study found increased HCY in patients with ischemic heart disease (IHD) as compared to those of controls. It was also demonstrated that increased HCY is a factor for cardiovascular disease as well as atherosclerosis in IHD patients [19]. In 2005, Chua with his team studied the association of HCY and coronary lesion risk in Chinese population with acute myocardial infarction. They suggested the higher HCYconcentration is a major risk factor for coronary atherosclerosis [20]. Vitamin B supplementation to control the increased levels of HCY has no significant improvement in carotid intima medial thickness (CIMT) and flow mediated dilation (FMD) but pooled effect showed little improvement in FMD and CIMT with HCY reduction [21]. A study was reported which showed double-blind placebo controlled for treating increased HCY by folic acid and it was found considerable improvement in study subjects. On the other hand no significant improvement was observed in case of atherosclerosis or carotid distensibility [22]. Another meta-analysis of 8 randomized, placebo-controlled study, folic acid showed decrease in HCY levels by 25% over the period of 5 years on average. No major impact was observed on other cardiovascular events including arterial revascularization [23].

It was found that vitamin B supplementation plays no significant role for the prevention of CVD [24]. Folic acid

is effective for CVD prevention in patients who are also affected by kidney disease [25]. In a meta-analysis studies, it was found high HCY levels as an independent predictor of mortalities including CVD mortalities [26]. The arterial inflexibility has been found to cause occurrence of first CVD in community based Framingham Heart Study [27]. High HCY levels have been observed in IHD patients in Homocystein Slovakia cross-sectional population showing the association between increased homocysteine and CVD [28]. Another study also showed the similar relationship between CVD and hyper-homocysteinemia [29]. Meta analyses of 17 studies showed the increased levels of HCY(5umol/L) as a risk factor for CAD and increase in cholesterol level [30]. Another meta-analysis study did not agree with[31]. In this meta-analysis, no relationship was found between higher HCY and coronary artery disease. Another meta-analysis study showed decrease in homocysteine levels by 3umol/L was shown to decrease the risk of IHD by 16% [32]. It will also decrease the risk of deep vein thrombosis and stroke by 25% and 24% respectively. Including 72 case control studies and 20 prospective studies, it was evident that decrease in HCY levels decreases the cardiovascular disease risk. In HCY collaboration study 2002, increase in HCY levels was suggested to be an independent and modest predictor of IHD and stroke. Other meta-analysis of 24 retrospective and 3 prospective studies, showed some association between increased HCY and venous thrombosis [33]. Despite of a large number of studies on causes, treatment and mechanism of increased levels of HCY, confusions and contradictions exist about the association of HCY and CVD which needs more studies [34].

Hyperhomocysteinemia and its determinants

Homocysteinuria is characterized by huge elevations in HCY levels (as high as 50 times their normal values), premature thrombosis and cardiovascular illnesses. It results from rare inborn errors in HCY metabolism, in which defects in certain enzymes crucial for HCY metabolism lead to the accumulation of HCY in blood and ultimately passage in urine occur [35]. Slight increments (15-25µmol/L) in HCY concentration may, however, be due to dietary factors associated with its metabolism [36]. Many genetic and environmental factors are now known to determine the HCY levels including lifestyles, behaviors, old age, maleness smoking, caffeine, menopause, chronic alcohol intake, deficiency of vitamins and impairment of renal function. It was indicated that independently of other lifestyle factors, age, and intake of folate and B vitamin supplements, reducing smoking and coffee intake could result in a 0.1- to 1.7-µmol/liter change in plasma total HCY level. This study also suggested that avoiding smoking, caffeine and folate and vitamin B supplementation shows decrease in plasma homocysteine levels [37].

The association between HCY concentrations, demographic and lifestyle factors as well as with B vitamin status was also investigated. Plasma tHCY was found to be higher in non-users of vitamin supplements. A survey was conducted to observe the association of HCY with lifestyle and vitamin B supplementation which showed the supplement users on much safer side [38]. In fact, increased concentrations of HCY are genetically determined. Some genotypes are known to cause rare types of conditions linked with premature atherothrombosis. Gene mutations are responsible for the loss of some enzymes including Methylenetetrahydrofolatereductase (MTHFR) and Crystathione b- synthase (CBS) linked to plasma HCY levels [29]. Certain rare genotypes have shown the association with premature atherothrombosis. The gene mutations in Methylenetetrahydrofolatereductase (MTHFR) and Crystathioneβ-synthase (CBS) have been shown to be associated with hyper-homocysteinimia [29].

Possible mechanisms involved in linking HCY with CVD

In spite of the partial recognition of HCY as a risk factor for CVD, the biological mechanisms involved in this relationship have not been completely identified. However, several mechanisms have been proposed to explain the relationship of hyperhomocysteinemia (HHCY) with the development of vascular disease. These potential mechanisms include endothelial dysfunction, production of reactive oxygen species (ROS) with consequent LDL oxidation, increased monocyte adhesion, activation of coagulation factors and inflammatory pathway and hypofibrinolysis caused by HHCY [39].

Atherosclerosis, being an inflammatory process, has been suggested to exhibit rapid progression due to the role of HCY in chronic inflammation [40]. HCY has been known to enhance the development of several pro inflammatory cytokines, which can contribute to atherogenesis by influencing vascular inflammation [41]. HCY has also been studied to impair normal cellular function, including vascular endothelial cells. This role of HCY has been supported in a study, in which it was found that increments in HCY levels can induce vascular endothelial dysfunction. HHCY hasbeen recognized to impair endothelial vasodilation-an early step in atherosclerotic lesion development. This role of HCY in causing cellular dysfunction has been suggested (not validated) to be due to the involvement of reactive oxygen species (ROS) produced by auto oxidation of HCY. HCY has also been studied to lower nitric oxide bioavailability (the vasodilator) and decreasing the expression of antioxidant enzymes. Vascular injury due to the ability of HCY to cause endoplasmic stress is another mechanism of vascular injury [41]. Another different mechanism for linking CVD and HCY was observed. Reduced mRNA for atheroprotective lipoprotein Apo A-1 and thus reduced concentration of Apo A-1 was identified in MTHFR deficient mice. The findings of their study showed that HHCY may contribute to CVD by decreased Apo A-1 expression, which is the main protein component of HDL [42]. As previously mentioned, HDL is known for its roles in CVD protection

and its negative relation with HCY indicates CVD risk development. This role of HCY in reducing HDL production was also supported in which a strong negative correlation was established between plasma HCY and HDL levels[43]. HHCY secondary to genetic causes as well as dietary HHCY has been found to cause significant increase in HCY-thiolactone, which is an atherogenic metabolite. These studies support that HHCY is associated with several mechanisms which promote CVD, providing a basis for establishing a direct role of HCY in raising cardiovascular disease risk [44].

Role of dietary factors

As already mentioned, HCY being an intermediate produced during methionine metabolism, is normally present in the plasma. But the concentrations are maintained by the mechanisms responsible for its conversion into either cysteine or into methionine itself. The role of B vitamins as cofactors in HCY metabolism, predispose a vitamin deficient person to HHCY and thus to CVD development. Administration of methionine and provision of vitamin (cobalamin, choline, folate and / pyridoxine) deficient diets have been used as model for inducing experimental HHCY, in addition to genetic alterations of enzymes involved in HCY metabolism. The use of these approaches indicates the important role of certain dietary factors in influencing plasma HCY levels [45]. A study reviewed by Elmadfa concluded that vegetarians, especially vegans, are at risk of developing HHCY due to low dietary intake of Vitamin B12 which is found predominantly in animal foods [46]. The authors recommended the use of well-planned vegetarian diets to avoid such complications. Folate is available in variety of plant and animal foods including liver, mushrooms and green leafy vegetables (especially spinach, broccoli). The food sources of vitamin B6 include meats, whole grains, vegetables and nuts, with greater bioavailability of pyridoxine derived from animal foods. Vitamin B12 is found in foods bound to protein and thus, high protein foods including egg, fish, meat, organ meats, milk, and cheese are its richest sources [47]. As folate and vitamin B12 do not share the same food sources, therefore a careful planning is required to fulfill the requirements of these vitamins. A well-balanced diet including all food groups can be of benefit [48]. High dietary protein intakes have also been found to increase circulating HCY levels, as HCY is an amino acid metabolite. In a dietary controlled study, a high protein diet (containing 21% energy from proteins or 4-4.5g methionine per day) was demonstrated to increase total HCY levels throughout the day. Fasting HCY levels were not affected by one week high protein diet, indicating the effects of overnight fast on normalizing HCY levels [49].

A large body of research support the role of folate in decreasing HCY levels and folic acid supplementation has now been suggested as a safe preventive measure in high risk individuals. Dietary deficiency of Vitamin B6, B9 and absorptive insufficiency of vitamin B12 are important factors responsible for HHCY. Therefore, dietary improvement by providing the food sources of B vitamins in abundance may prevent vascular disease by lowering serum HCY [40]. tHCY levels can be reduced by increasing the intake of fruits and vegetables by enhancing dietary folate intake. Patients with hypercystenemia and cardiovascular disease have shown improvement with a daily dose of folic acid at a concentration of 0.65-10mg [50]. However, supplemental folate can mask the neurological symptoms of B12 deficiency if it occurs at the same time. Therefore, simultaneous supplementation of both vitamins is recommended. Administration of recommended dose (1mg/day) of vitamin B12 can cause 10-15% reduction in HCY levels. The effect of B12 supplementation has been found to increase nitric oxide concentrations and an improvement in endothelial function. Pyridoxine (vitamin B6) in a dosage of 10-250 mg/day along with folatetherapy, has a greater effect on HCY lowering than folate administration alone. However, chronic administration of B6 may result in peripheral neuropathy [51]. The relationship of folate and HCY concentration in Mediterranean IHD patients was investigated, IHD patients had lower folate intakes and serum folate levels as compared to the controls. Dietary folate intake was found to have a strong influence on fasting HCY levels [49]. Combination of vitamins rather than either one alone has been found to have much pronounced effect on HCY lowering. A randomized placebo controlled intervention showed a significant improvement in HCY levels with B vitamin supplementation [21]. A meta-analysis was conducted to assess the Meta-analysis for investigating the effects of different doses of folate supplementation on HCY levels as well as the effects of combination therapy of B12 and B6 with folate. The authors, after analyzing 25 randomized controlled trials, concluded that daily dose of more than0.8 mg/ day of folic acid are required to achieve maximum HCY reduction. Recommended a dose of folate (0.8mg/day) for considerable reduction in high homocysteine levels [52]. Vitamin B12 was found to be associated with additional 7% reduction in HCY levels but B6 supplementation along with folate did not appear to have a significant effect [53]. Therefore, combined administration of vitamins B9 and B12 were found to have greater effect than either one alone [54]. Similar results have previously been found in comparatively smaller meta-analysis [55]. A much lower dose of folate supplementation (0.5- 5mg/day) along with 0.5 mg of B 12 was found to reduce HCY levels by a quarter to a third. This difference might have been due to the inclusion of older data in that meta-analysis. Vitamin B6 was not found to have a significant role in HCY lowering, as was found in 2005 meta-analysis [56]. Although the role of B vitamins in HCY lowering has been established, studies on the effects of this lowering on subsequent cardiovascular events have yielded conflicting results. In a randomized placebo controlled trial, daily folic acid dose of 800µg for three years was found to decrease plasma t HCY levels by 26% in 819 adults with HHCY. In spite of the improvement in HCY concentration, folate supplementation was unable to slow down the development of atherosclerotic lesion or progression of arterial stiffening [22].

Heart outcomes Prevention Evaluation 2 determined the prolonged effects of Vitamin B9, B12 and B6

administration on vascular events in high risk population. the results of this large, prospective, randomized clinical trial report that although HCY levels were significantly reduced by daily supplementation of folic acid, pyridoxine and cobalamin over a period of five years but did not reduce the cardiovascular disease incidence [57]. A meta-analysis on the use of Vitamin B supplement was conducted that showed decrease in HCY and improvement in endothelial function in short term trails only [21]. Therefore, despite the well-known role of dietary factors in reducing HCY levels, their effects on lowering the CVD risk are still not validated. The studies have produced inconsistent data on the influence of HCY lowering achieved by vitamin supplementation on reducing cardiovascular disease incidence. Therefore, this subject requires further investigation [58].

Risk factor control as primary prevention of CVD

A large body of data strongly supports investments in prevention as the most sustainable solution for CVD endemic. Clear evidence supports the fact that primary prevention and individual health care interventions play an important role in reducing the disease burden. Reducing cardiovascular risk factors has been responsible for more than 50% decline in CVD mortality [59]. Several modifiable risk factors can be targeted for CVD risk prevention in high risk as well as in general population. Behavioral risk factors, including smoking, physical inactivity, alcohol abuse, and unhealthy diet, have been linked to vascular disease development and progression. Identification of these easily modifiable factors can lead to prevention of majority of fatal cardiovascular events [60]. Promotion of knowledge about the CVD risk factors as well as their control therefore, remains a strategy for rapid decline in the CVD prevalence. High plasma HCY levels have been identified to be linked to CVD and their causal relationship has been identified. Presence of some CVD risk factors can result in HHCY. Reductions in smoking and taking a healthy diet can be used as approaches for lowering HCY concentrations. In addition, certain dietary modifications can also result in improvement in HCY levels. Dietary intake or supplementation of vitamins B9, B6 and B12 can be of benefit [61].

Conclusion

In spite of a variety of knowledge on the subject, the association between HCY and CVD remains conflicting. However, the current evidence suggests a possible role of high levels of HCY in causing CVD and opens new avenues for researches in the field of nutrition, public health and medicine.

Conflict of interest

The author declares no conflict of interest.

Authors' Contribution

Afifa Tanweer: conceive the idea and write manuscript

Abida Bano: Proof reading, article setting and submission

Warda Fatima: Data Collection and technical supervision

Hasnain Javed: Writing the article and financial support

References

1. Chrysant SG, Chrysant GS. The current status of homocysteine as a risk factor for cardiovascular disease: a mini review. Expert review of cardiovascular therapy, (2018); 16(8): 559-565.
2. Danaei G, Singh GM, Paciorek CJ, Lin JK, Cowan MJ, *et al*. The global cardiovascular risk transition: associations of four metabolic risk factors with national income, urbanization, and Western diet in 1980 and 2008. Circulation, (2013); 127(14): 1493-1502.
3. Aanensen DM, Feil EJ, Holden MTG, Dordel J, Yeats CA, *et al*. Whole-Genome Sequencing for Routine Pathogen Surveillance in Public Health: a Population Snapshot of Invasive *Staphylococcus aureus* in Europe. mBio, (2016); 7(3): 1-16.
4. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, *et al*. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. Circulation, (2014); 129(3): 933-944.
5. Control CfD, Prevention. Million hearts: strategies to reduce the prevalence of leading cardiovascular disease risk factors--United States, 2011. MMWR Morbidity and mortality weekly report, (2011); 60(36): 1248.
6. Emamgholipour S, Sari AA, Pakdaman M, Geravandi S. Economic Burden of Cardiovascular Disease in the Southwest of Iran. International Cardiovascular Research Journal, (2018); 12(1): 6-12.
7. Margozzini P, Passi A, Kruk M, Danaei G. P3415 Will less developed countries be capable of giving high-quality universal coverage to cardiovascular disease survivors? Results from a national health survey, Chile ENS 2016–2017. European Heart Journal, (2019); 40(Supplement_1): 2035.
8. Recio C, Maione F, Iqbal AJ, Mascolo N, De Feo V. The potential therapeutic application of peptides and peptidomimetics in cardiovascular disease. Frontiers in pharmacology, (2017); 6(7): 526.
9. Hajar R. Risk factors for coronary artery disease: historical perspectives. Heart views: the official journal of the Gulf Heart Association, (2017); 18(3): 109.
10. Ni Y, Xue L, Zhu G, Chen Y. Serum Homocysteine, VEGF and TGF-β1 dynamic change in colorectal cancer patients prior and post-operation. Pteridines, (2019); 30(1): 121-125.
11. Lopes C. Hyperhomocysteinemia: How Does it Affect the Development of Cardiovas-cular Disease. International Archives of Cardiovascular Diseases, (2018); 2(008):1-12.
12. Hasan T, Arora R, Bansal AK, Bhattacharya R, Sharma GS, *et al*. Disturbed homocysteine metabolism is associated with cancer. Experimnental and molecular medicine(2019); 51 (2): 1-13.
13. Kim J, Kim H, Roh H, Kwon Y. Causes of hyperhomocysteinemia and its pathological significance. Archives of pharmacal research, (2018); 41(4): 372-383.
14. Belardo A, Gevi F, Zolla L. The concomitant lower concentrations of vitamins B6, B9 and B12 may cause methylation deficiency in autistic children. The Journal of nutritional biochemistry, (2019); 7038-46.
15. Gao L, Siu PM, Chan S-w, Lai CW. Cardiovascular protective effects of salvianic acid a on db/db mice with elevated homocysteine level. Oxidative medicine and cellular longevity, (2017); 2017.
16. McCully KS. Homocysteine, vitamins, and vascular disease prevention. The American journal of clinical nutrition, (2007); 86(5): 1563S-1568S.
17. Lee KW, Hill JS, Walley KR, Frohlich JJ. Relative value of multiple plasma biomarkers as risk factors for coronary artery disease and death in an angiography cohort. Cmaj, (2006); 174(4): 461-466.
18. Bostom AG, Silbershatz H, Rosenberg IH, Selhub J, D'Agostino RB, *et al*. Nonfasting plasma total homocysteine levels and all-cause and cardiovascular disease mortality in elderly Framingham men and women. Archives of Internal Medicine, (1999); 159(10): 1077-1080.
19. Vrentzos GE, Papadakis JA, Malliaraki N, Zacharis EA, Mazokopakis E, *et al*. Diet, serum homocysteine levels and ischaemic heart disease in a Mediterranean population. British journal of nutrition, (2004); 91(6): 1013-1019.

20. Chua S, Wu C-J, Chang H-W, Hang C-L, Chen C-J, *et al*. Impact of elevated plasma total homocysteine concentration on coronary atherosclerosis in Chinese patients with acute myocardial infarction undergoing primary coronary intervention. International heart journal, (2005); 46(2): 181-193.
21. Potter K, Hankey GJ, Green DJ, Eikelboom J, Jamrozik K, *et al*. The effect of long-term homocysteine-lowering on carotid intima-media thickness and flow-mediated vasodilation in stroke patients: a randomized controlled trial and meta-analysis. BMC cardiovascular disorders, (2008); 8(1): 24.
22. Durga J, Bots ML, Schouten EG, Grobbee DE, Kok FJ, *et al*. Effect of 3 y of folic acid supplementation on the progression of carotid intima-media thickness and carotid arterial stiffness in older adults. The American journal of clinical nutrition, (2011); 93(5): 941-949.
23. Clarke R, Halsey J, Lewington S, Lonn E, Armitage J, *et al*. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: meta-analysis of 8 randomized trials involving 37 485 individuals. Archives of Internal Medicine, (2010); 170(18): 1622-1631.
24. Myung S-K, Ju W, Cho B, Oh S-W, Park SM, *et al*. Efficacy of vitamin and antioxidant supplements in prevention of cardiovascular disease: systematic review and meta-analysis of randomised controlled trials. Bmj, (2013); 346f10.
25. Qin X, Huo Y, Xie D, Hou F, Xu X, *et al*. Homocysteine-lowering therapy with folic acid is effective in cardiovascular disease prevention in patients with kidney disease: a meta-analysis of randomized controlled trials. Clinical nutrition, (2013); 32(5): 722-727.
26. Peng H-y, Man C-f, Xu J, Fan Y. Elevated homocysteine levels and risk of cardiovascular and all-cause mortality: a meta-analysis of prospective studies. Journal of Zhejiang university-science B, (2015); 16(1): 78-86.
27. Andersson C, Lyass A, Larson MG, Spartano NL, Vita JA, *et al*. Physical activity measured by accelerometry and its associations with cardiac structure and vascular function in young and middle-aged adults. Journal of the American Heart Association, (2015); 4(3): e001528.
28. Lietava J, Dukat A, Fodor G. Homocysteine Slovakia study: study design and occurrence of hyperhomocysteinaemia and other risk factors. Bratislavske lekarske listy, (2012); 113(2): 80-86.
29. Dwivedi M, Tripathi A, Shukla S, Khan S, Chauhan U. Homocysteine and cardiovascular disease. Biotechnology and Molecular Biology Reviews, (2011); 6(5): 101-107.
30. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. Jama, (1995); 274(13): 1049-1057.
31. Scheer AS, Boushey RP, Liang S, Doucette S, O'Connor AM, *et al*. The long-term gastrointestinal functional outcomes following curative anterior resection in adults with rectal cancer: a systematic review and meta-analysis. Diseases of the colon & rectum, (2011); 54(12): 1589-1597.
32. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. Bmj, (2002); 325(7374): 1202.
33. Christen WG, Ajani UA, Glynn RJ, Hennekens CH. Blood levels of homocysteine and increased risks of cardiovascular disease: causal or casual? Archives of Internal Medicine, (2000); 160(4): 422-434.
34. Kaul S, Zadeh AA, Shah PK. Homocysteine hypothesis for atherothrombotic cardiovascular disease: not validated. Journal of the American College of Cardiology, (2006); 48(5): 914-923.
35. Hoss GRW, Poloni S, Blom HJ, Schwartz IVD. Three Main Causes of Homocystinuria: CBS, cblC and MTHFR Deficiency. What do they Have in Common? Journal of Inborn Errors of Metabolism and Screening, (2019); 7.
36. Wierzbicki AS. Homocysteine and cardiovascular disease: a review of the evidence. Diabetes and Vascular Disease Research, (2007); 4(2): 143-149.
37. Bree Ad, Verschuren WM, Blom HJ, Kromhout D. Lifestyle factors and plasma homocysteine concentrations in a general population sample. American journal of Epidemiology, (2001); 154(2): 150-154.
38. Ganji V, Kafai MR. Demographic, lifestyle, and health characteristics and serum B vitamin status are determinants of plasma total homocysteine concentration in the post-folic acid fortification period, 1999–2004. The Journal of nutrition, (2009); 139(2): 345-352.
39. Ciaccio M, Bivona G, Bellia C. Therapeutical approach to plasma homocysteine and cardiovascular risk reduction. Therapeutics and clinical risk management, (2008); 4(1): 219.
40. Ambrose JA, Bhullar AS. Inflammation and Thrombosis in Coronary Atherosclerosis: Pathophysiologic Mechanisms and Clinical Correlations. EMJ, (2019); 4(1): 71-78.
41. de Koning AL, Werstuck GH, Zhou J, Austin RC. Hyperhomocysteinemia and its role in the development of atherosclerosis. Clinical biochemistry, (2003); 36(6): 431-441.
42. Mikael LG, Genest Jr J, Rozen R. Elevated homocysteine reduces apolipoprotein AI expression in hyperhomocysteinemic mice and in males with coronary artery disease. Circulation research, (2006); 98(4): 564-571.
43. Liao D, Tan H, Hui R, Li Z, Jiang X, *et al*. Hyperhomocysteinemia decreases circulating high-density lipoprotein by inhibiting apolipoprotein AI Protein synthesis and enhancing HDL cholesterol clearance. Circulation research, (2006); 99(6): 598-606.
44. Chwatko G, Boers GH, Strauss KA, Shih DM, Jakubowski H. Mutations in methylenetetrahydrofolate reductase or cystathionine β-syntase gene, or a high-methionine diet, increase homocysteine thiolactone levels in humans and mice. The FASEB Journal, (2007); 21(8): 1707-1713.
45. Lentz S. Mechanisms of homocysteine-induced atherothrombosis. Journal of Thrombosis and Haemostasis, (2005); 3(8): 1646-1654.
46. Elmadfa I, Singer I. Vitamin B-12 and homocysteine status among vegetarians: a global perspective. The American journal of clinical nutrition, (2009); 89(5): 1693S-1698S.
47. Litchford M. Assessment: laboratory data. Krause's Food and Nutrition Therapy 12th ed Canada: Saunders Elsevier, (2008).
48. Waly MI, Ali A, Al-Nassri A, Al-Mukhaini M, Valliatte J, *et al*. Low nourishment of B-vitamins is associated with hyperhomocysteinemia and oxidative stress in newly diagnosed cardiac patients. Experimental Biology and Medicine, (2016); 241(1): 46-51.
49. Verhoef P, Van Vliet T, Olthof MR, Katan MB. A high-protein diet increases postprandial but not fasting plasma total homocysteine concentrations: a dietary controlled, crossover trial in healthy volunteers–. The American journal of clinical nutrition, (2005); 82(3): 553-558.
50. Tian T, Yang K-Q, Cui J-G, Zhou L-L, Zhou X-L. Folic acid supplementation for stroke prevention in patients with cardiovascular disease. The American journal of the medical sciences, (2017); 354(4): 379-387.
51. Mohammed ZCM, Biomarkers for the Prediction of Incident Coronary Heart Disease in the MESA Cohort: Johns Hopkins University, (2015).
52. Perna AF, Ingrosso Dm, Homocysteine and chronic kidney disease: an ongoing narrative. Springer, (2019).
53. An Y, Feng L, Zhang X, Wang Y, Wang Y, *et al*. Dietary intakes and biomarker patterns of folate, vitamin B 6, and vitamin B 12 can be associated with cognitive impairment by hypermethylation of redox-related genes NUDT15 and TXNRD1. Clinical epigenetics, (2019); 11(1): 139.
54. Collaboration HLT. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. The American journal of clinical nutrition, (2005); 82(4): 806-812.
55. Trialists'Collaboration HL. Blood homocysteine lowering with folic acid based supplements: a systematic overview of the randomised trials. Netherland Journal of Medicine, (1998); 52S33.
56. Wang B, Wu H, Li Y, Ban Q, Huang X, *et al*. Effect of long-term low-dose folic acid supplementation on degree of total homocysteine-lowering: major effect modifiers. British journal of nutrition, (2018); 120(10): 1122-1130.
57. Martí-Carvajal AJ, Sola I, Lathyris D, Dayer M. Homocysteine-lowering interventions for preventing cardiovascular events. Cochrane Database of Systematic Reviews, (2017); (8).
58. Kumar A, Palfrey HA, Pathak R, Kadowitz PJ, Gettys TW, *et al*. The metabolism and significance of homocysteine in nutrition and health. Nutrition & metabolism, (2017); 14(1): 78.
59. Hennekens CH. Increasing burden of cardiovascular disease: current knowledge and future directions for research on risk factors. Circulation, (1998); 97(11): 1095-1102.
60. De Bacquer D, De Smedt D, Kotseva K, Jennings C, Wood D, *et al*. Incidence of cardiovascular events in patients with stabilized coronary heart disease: the EUROASPIRE IV follow-up study. European journal of epidemiology, (2019); 34(3): 247-258.
61. Corella D, Coltell O, Mattingley G, Sorlí JV, Ordovas JM. Utilizing nutritional genomics to tailor diets for the prevention of cardiovascular disease: a guide for upcoming studies and

implementations. Expert review of molecular diagnostics, (2017); 17(5): 495-513.