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Homocysteine and Heart Disease. Does Inadequate Vitamin B_6 and Folate Consumption Increase the Risk for Atherosclerosis?

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Abstract: Cardiovascular disease (CVD) is the leading cause of mortality in most industrialized countries. Folate and vitamin B_6 have essential role in methionine metabolism. Folate provides a methyl group for the remethylation process of homocysteine (Hcy) back to methione. Vitamin B_6 acts as a cofactor for cystathione-β-synthase, which catalyzes the conversion of tetrahydrofolate to methylene-tetrahydrofolate (CH_2-THF). Recent studies have shown that hyperhomocysteinemia is an independent risk factor for CVD. Deficiency of folate and vitamin B_6 results in increase in Hcy concentration and decrease in intermediary product, *S*-adenosylmethione (SAM). This compound is the principal methylation agent and plays role in nucleic acid and protein synthesis and integration and proper function of cells. Homocysteine alters the surface properties of endothelial cells. Reduced form of homocysteine undergoes oxidation process. End products of this process are hydrogen peroxide, superoxide and hydroxyl radicals. These products interfere with vascular function. The objective of this study was to present relevant studies concerning the metabolism of Hcy, its role in development of atherosclerosis and its relation to vitamin B_6 and folate status.

Key words: Homocysteine, vitamin B_6, folate, atherosclerosis

INTRODUCTION

Cardiovascular diseases (CVD) are the leading causes of mortality in most industrialized countries. Cardiovascular disease is a general term and covers coronary artery, cerebrovascular and peripheral vascular diseases. More specifically, CVD can include atherosclerosis, thrombosis and embolic diseases. There are numerous major risk factors associated with occurrence of CVD. They include smoking, obesity, high cholesterol intake and alcoholism. These individual risk factors may involve as a complex in the etiology of CVD and complicate diagnosis, pathogenesis, treatment and prognosis. On the other hand, recent investigations have shown that elevated total homocysteine (tHcy) in blood plasma involves in the etiology of atherosclerosis as a major independent risk factor. This study presents metabolism of Hcy in relationships to atherosclerosis and vitamin B_6 and folate status.

Metabolism of Homocysteine: Folate and vitamin B_6 deficiencies alter methionine metabolism and increase homocysteine concentration through playing central role in one-carbon metabolism (Fig. 1). Folate drives Hcy metabolism, with 5-methyltetrahydrofolate serving as substrate for remethylation process back to methionine. Deficiency of folate causes failure in intracellular methylation process due to accumulation of CH_3THF (metil-tetrahydroflate). N-5-methyl-THF is a poor substrate for polyglutamation. Vitamin B_6 catalyzes the entry of Hcy into the transsulfuration pathway as a cofactor for cystathione-β-synthase and serine transhydoxymethylase, which catalyze the conversion of THF into CH_2THF. Inhibition of these enzymes adversely affects remethylation of Hcy, which results in deficiency of S-adenosylmethinone (SAM). This compound is the principal methylation agent and necessary for nucleic acid and protein synthesis and integrity and function of cells. Depletion of SAM also causes cell membrane injury in the liver and leads to persistence of damaged proteins that involve in atherogenesis[1]. Moreover, impairment of transsulfuration leads to decreased production of glutathione (GSH), a major protective agent in the liver.

Homocysteine can be either in reduced (sulphydryl) or in oxidized (disulfide) form. Homocysteine refers to reduced form and homocystine refers to oxidized form. Reduced form is account for 1% of tHcy in plasma. Oxidized form can exist as homocystine (5-10%), mixed-disulfides (80-90) and cysteine-Hcy (5-10%). Most of studies did not distinguish form of tHcy in plasma. More than 10 μmol L^{-1} of tHcy in plasma is accepted as hyperhomocysteinemia and causes vascular dysfunction.

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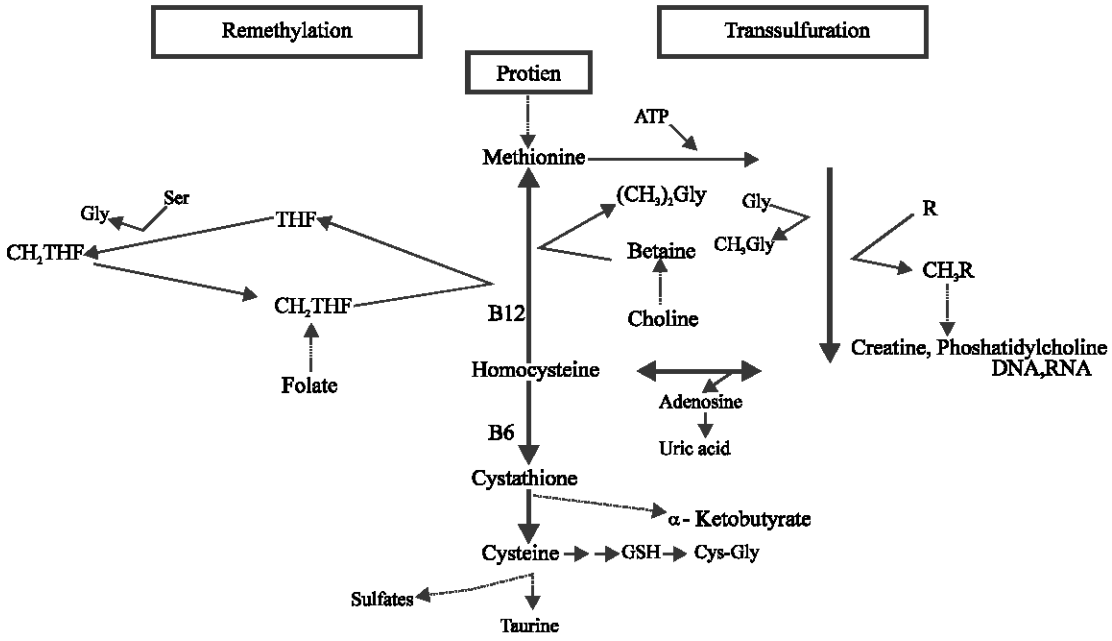

Fig. 1: Role of folate and vitamin B_6 in methinone metabolism

This concentration may increase 20 to 50-fold in end-stage renal disease. Analytical procedure of determination of tHcy concentration in plasma and/or red blood cells is based on reduction of disulfide bonds by 2-mercatoethanol, dithiothreitol, sodium borohydride, phosphine tris.

Hiperhomocysteinemia causes atherosclerosis: It is very important to elucidate mechanism that high plasma Hcy concentration is related atherosclerosis and folate and vitamin B_6 supplementation lower tHcy concentration. Because of the fact that better understanding of pathogenesis improves approaches to treat diseases. The endothelium produces components of extracellular matrix and regulatory mediators such as nitric oxide, prostanoids, endothelin, angiotensin-II, tissue-type plasminogen activator (tPA), von Willebrand factors, adhesion molecules and cytokines (interleujin-1 and tumor necrosis factor-α.). These products are responsible for regulation of vascular tone and fibrinolysis, the extravasation of leukocytes and the proliferation of vascular smooth muscle cells[2].Therefore, the endothelium is a major structural component that controls vascular function.

In molecular perspective, vascular dysfunction due to high tHyc is attributed to increase in oxidative stress[3]. Homocysteine represents a part of redox thiol system because it contains sulphydryl group. In spite of being such as low proportion of tHcy concentration in plasma (1%), reduced Hcy is the atherogenic form. Because reduced form (sulphydryl group) of Hcy undergoes oxidation process *in vivo* and generates hydrogen peroxide and super oxide and hydroxyl radicals that are injurious agents. Superoxide and hydroxyl radicals initiate lipid peroxidation on cell membrane and alter endothelial production of nitric oxide, with formation of peroxynitrite. Consequently, decreased nitrous oxide facilitates proliferation of vascular smooth muscle cells. Hydrogen peroxide freely passes cell membrane and decreases activity of glutathione peroxidase[4,5]. Glutathione is an intracellular reducing agent keeping sulphydryl group of protein and necessary for regulation of nuclear factor κB (NK-κB). Apoptopic effect of Hcy has not been elucidated yet, but decrease in this immunmodulatory factor may cause vascular dysfunction as well[3].

Arterial lesions in the vessel of hyperhomocysteinemic patients include fibrous thickening of the intima, fragmentation of the internal elastic lamina, frying and splitting of muscle, elastic fibres within the media and hypetrophy and migration of medial smooth muscle cells of vessels. High tHcy concentration also interferes with collagen cross-linking, which results in decreased dilatation and hyperpulsatility. It has been reported that damage of high tHcy concentration is more severe in cerebrovascular and peripheral arteries than coronary arteries. Woo *et al.*,[6] investigated arterial endothelial function in hyperhomocysteinemic patients and

normohomocysteinemic healthy people. They measured endothelium-dependent (EDD) arterial dilatation of brachial artery using high-resolution ultrasound. After pressure (250 mm Hg for 4.5 min), EDD in brachial artery of healthy and patient subjects were increased 11 and 7%, respectively. High tHcy concentration decreases bioactivity of nitric oxide, which is a potent vasodilator and anti-platelet mediator. Stehouwer and Jakobs[5] reported that high tHcy is associated with decreased expression of thrombomodulin and heparan-sulfate, decreased activity of the protein C and decreased binding of tPA to the endothelial cell membrane. In turn, these decrease protein synthesis and protein folding in endoplasmic reticulum[3]. Increase platelet aggregation due to stimulation of thrombaxane biosynthesis and activation of macrophage-derived tissue factors is primary consequences of folate deficiency and/or hypehomocysteinemia[1]. They provided a pioneer *in vivo* study to demonstrate development of atherogenesis in relation to folate deficiency. They supplemented the diet of male Sprague Dawler rats with 750 and 250 μg kg^{-1}. Plasma tHcy concentrations were 5 vs. 20 μmol L^{-1} for control and treatment groups, respectively. Concentration of plasma conjugated dienes (lipid peroxidation product) was 470 vs. 664 μmol/L for control and folate deficient group, respectively. Moreover, platelet aggregation and thromboxane concentration were 14% and 85 pmol greater in folate deficient rats compare to control group. Folate deficiency led to macrophage tissue factor activity (macrophage-derived procoagulant activity of peritoneal cells).

Nishio and Watanabe[2] hypothesized thatHcy stimulates proliferation of vascular muscle cells (VMC). In order to test this hypothesis, they evaluated the effect of Hcy on VMC growth and on the activity of superoxide dismutase (SOD), catalase and GSH peroxidase in the cells of thoracic aorta of rats. In this study, [^{3}H[-thymidine was used as a marker to determine mitogenesis. Both Hct and platelet derived growth factor (PDGF) were found to be weak mitogen. However, 2-fold increase in concentration of tHcy (from 250 to 500 μM) resulted in 4-fold increase in PDGF, suggesting thatHcy potentiate mitogenic effect of PDGF and Hcy itself is a mitogenic compound. Addition of either N-actyl-Lcysteine (antioxidant) or catalase into medium blocked the mitogenic effect of Hcy, but of SOD did not inhibit incorporation of [^{3}H[-thymidine. Inefficiency of SOD was attributed to formation of hydrogen peroxide. Moreover, addition of aminotriazole (catalase inhibitor) enhanced the incorporation of [^{3}H[-thymidine. These researchers also tested whether the combination of PDGF or Hcy and PDGF alone induced more reactive oxygen species. Hydrogen peroxide and superoxide concentrations were increased in cell media containing 0 to 500 μM of Hcy in a concentration dependent-manner. Their concentrations were significantly higher in media containing greater than 100 μM of Hcy. Meanwhile, the activity of catalase and GSH-peroxidase decreased and of SOD increased. Conclusion in this study, the increase in SOD is a response to increased levels of superoxide. Decreases in catalase and GSH-peroxidase are results of inhibition of antioxidant enzymes generated from Hcy. This study precludes thatHcy enhances the proliferative action of growth factors or cytokines in atherosclerosis.

Role of folate and vitamin B_6 in atherosclerosis: Alcohol and smoking impair disposal of homocysteine. In general, alcoholism and smoking cause low intake, poor absorption, decreased hepatic uptake and retention and increased urinary excretion of nutrients. Also, they interfere with one-carbon metabolism, for which vitamin B_6 and folate serve as coenzymes. Giraud *et al.*,[7]studied the link between smoking and depressed PLP concentration. They analyzed plasma PLP concentrations in 23 tobacco smokers, 11 chewers and 11 nonuser averaging. Plasma PLP concentrations were 81, 71 and 61 for nonusers, chewers and smokers, respectively. Cravo *et al.*,[8] examined association of low folate and vitamin B_6 intakes with hyperhomocysteinemia in 31 healthy and 32 alcoholic subjects. In alcoholic subjects, ethanol consumption was 2.78 g kg^{-1} BW per day. Plasma folate concentrations, the red cell folate contents and plasma PLP concentrations were lower in alcoholic subjects than in healthy subjects (11 vs. 16 nmol L^{-1}; 129 vs. 162 nmol L^{-1}; and 29 vs. 119 pmol/L^{-1}, respectively). The lower plasma vitamin concentrations (folate and vitamin B_6) were associated with higher plasma tHcy concentration. Concentrations of tHcy in plasma of alcoholic and healthy subjects were 18 vs. 8 μmol L^{-1}, respectively. They concluded that alcoholism lowers folate intake and impairs the disposal of Hcy through the transmethylation and transsulfuration pathways (Fig. 1). Acetaldehyde, an end product of ethanol oxidation, displaces protein-bound PLP.

High plasma homocysteine concentration is typical for folate and vitamin B_6 deficiency and is an independent risk for atherosclerosis. The association of elevated plasma tHcy concentration with increased risk for arterial disease and vascular dysfunction is well documented. A number of case studies have shown that elevated plasma tHcy concentration is directly or indirectly related to deficiency of folate and vitamin B_6, or both. Robinson *et al.*,[9] studied interrelationships among vitamin B_6 and folate status, plasma tHcy concentration and their relations to atherosclerosis in 750 patients with vascular

diseases (case group) and 800 healthy volunteers (control group). Folate and vitamin B_6 deficiencies were defined as a red cell folate concentration less than 372 nmol L^{-1} and plasma vitamin B_6 concentration less than 20 nmol L^{-1}, respectively, based on 10th percentile for healthy subjects. They found that fasting tHcy and after methinone loading tHcy concentrations were higher in case group than in control group. They also observed that there was a significant gender effect on tHcy concentration in both groups. Women had lower tHcy concentration in plasma than men, both in case group (10.2 vs. 11.7 μmol L^{-1}) and in control group (8.6 vs. 10.2 μmol L^{-1}). The red cell folate concentration was lower in case group than in control group. There were also gender effects on red cell folate concentrations. In both groups, the red cell folate concentration in women was lower than in men (727 vs. 819 nmol L^{-1}, in case group and 726 vs. 876 nmol L^{-1}, in control group, respectively). In case group, vitamin B_6 concentration of subjects was lower than in control group. Vitamin B_6 concentration of female subjects was greater than male subjects in both groups. The concentrations of vitamin B_6 in plasma of women and men were 26.4 and 25.7 nmol L^{-1} in case group and 32.1 and 30.7 nmol L^{-1} in control group, respectively. For both genders, the red cell folate and vitamin B_6 were inversely related to tHcy and there was no correlation between folate and vitamin B6 concentrations. Odds ratios (OR) of vascular disease in subjects with high tHcy and low folate and vitamin B_6 relative to their counterparts were 1.8, 1.5 and 1.6, respectively. Odds ratio indicates that how many times one is more likely to be susceptible to vascular disease relative to another. Differences in the concentrations of vitamins were attributed to higher creatine-creatinine synthesis and more efficient transsulfuration in men and more efficient remethylation in women.

Felsom *et al.*,[10] conducted a similar case study to demonstrate the association of fasting tHcy with coronary heart disease in 232 patients with cardiovascular problems and 537 healthy people who were in different life style, gender and ethnic background. Before data analyzes, they measured concentration of t Hcy, folate and vitamin B_6 (PLP-pyridoxal 5`-phosphate), total cholesterol, HDL-cholesterol, triglyceride, fibrinogen in plasma. Systolic blood pressure, body mass index (BMI), physical activity index (PAI) and alcohol and vitamin consumption were recorded as well. They pooled data of case and control group and then sorted each measurement based on plasma tHcy concentration within 5 quintiles ranging from 5.3 to 14.2 μmol L^{-1}. Plasma tHcy concentration was inversely related to plasma folate and PLP concentrations. As alcohol consumption increased, folate intake decreased and plasma tHcy concentration increased. Plasma lipid parameters and fibrinogen, PAI, BMI and systolic blood pressure were not different in quintiles. Frequency of people based on ethnic background and smoking habit were equal across the quintiles, suggesting that plasma tHcy concentration was not related to race and smoking. Plasma PLP concentration of subjects in case group was lower than that in control group (19 vs. 32 nmol L^{-1}), but folate concentrations were not different between these main groups (5.38 and 6.12 nmol L^{-1}), respectively. In this study, increased plasma tHcy and decreased plasma folate concentration were found to be risk factors for CVD in women and decreased plasma PLP was found to be a risk factor for CVD in women and men. They concluded that low plasma PLP concentration was associated with high t Hcy, thereby, it was another independent risk factor for CVD. I think that data should have been analyzed based on two main groups. In the statistical model, the role of other factors could have been placed. This would allow us to see possible interactions. Then based on these possible interactions, data could have been analyzed in several grids. In another word, orthogonal contrasts could have been developed. For instance, women and men in case groups; women in case and in control groups; black women in case and control groups; black and white women in case group; and women consuming alcohol and non-users in case group; etc. This approach would not have confounded the data analyses.

Den Heijer *et al.*,[11] investigated Hct-lowering effect of multivitamin complex in a well-designated and controlled experiment. Eighty-nine patients with venous thrombosis and 227 healthy people were received multivitamin for eight weeks. Subjects in this experiment were separated based on pre-experiment plasma tHcy concentrations. Subjects were assigned in a control group if plasma tHcy concentration was less than 16 μmol L^{-1} and in a case group if plasma tHcy concentration was greater than 16 μmol L^{-1}. Then, subjects in each group were assigned randomly one of two treatments (either placebo or multivitamin complex containing 5 mg folic acid, 0.4 mg hydroxycobalamin and 50 mg pyridoxine). Also, they established a subgroup from normohomocysteinemic healthy objects to distinguish t Hcy-lowering effect of individual vitamins. In this subgroup, subjects were received either 5 mg folic acid or 0.5 mg folic acid, or 0.4 mg hydroxycobalamine. Both normo- and hyperhomocysteinemic subjects receiving multivitamin complex in patients had significantly lower plasma tHcy concentration at the end of experimental period than those receiving placebo. There were 20 and 36% reduction for normo- and hyperhomocysteinemic patients receiving

multivitamin and 1 and 3% elevation for those receiving placebo, respectively. In healthy subjects, placebo did not alter plasma tHcy concentration, but multivitamin complex decreased plasma tHcy concentration 30 and 36% in normo- and hyperhomocysteinemic people, respectively. Interestingly, reduction in tHcy concentration in plasma of normohomocysteinemic healthy people receiving both 5 and 0.5 mg folic acid was 25% and reduction in tHcy in plasma of those receiving 0.4 mg hydroxycobalamin was 10%. They concluded that each vitamin has t Hcy-lowering effect and potentiate t Hcy-lowering effect of others. I think they should have tested lowering t Hcy-effect of vitamin B_6 as well.

In summary, homocystein is an intermediary compund in methionine metabolism and is correlated with folate and vitamin B_6 status. In addition to known causes, increased homocysteine concentration may leade to occurrence of atheroclerosis via altering the surface properties of endothelial cells. These facts should be investigated in veterinary medicine.

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