https://doi.org/10.4082/kjfm.21.0227 • Korean J Fam Med 2022;43:277-289

KJFM
Korean Journal of Family Medicine

eISSN: 2092-6715

■ Review Article

Serum Homocysteine and Vascular Calcification: Advances in Mechanisms, Related Diseases, and Nutrition

Susie Jung[1], Beom-Hee Choi[2], Nam-Seok Joo[1,*]

[1]Department of Family Practice and Community Health, Ajou University School of Medicine, Suwon, Korea
[2]Greencross IMED Gangbuk Center, Seoul, Korea

Identifying and preventing modifiable risk factors for cardiovascular disease is very important. Vascular calcification has been studied clinically as an asymptomatic preclinical marker of atherosclerosis and a risk factor for cardio-cerebrovascular disease. It is known that higher homocysteine levels are associated with calcified plaques and the higher the homocysteine level, the higher the prevalence and progression of vascular calcification. Homocysteine is a byproduct of methionine metabolism and is generally maintained at a physiological level. Moreover, it may increase if the patient has a genetic deficiency of metabolic enzymes, nutritional deficiencies of related cofactors (vitamins), chronic diseases, or a poor lifestyle. Homocysteine is an oxidative stress factor that can lead to calcified plaques and trigger vascular inflammation. Hyperhomocysteinemia causes endothelial dysfunction, transdifferentiation of vascular smooth muscle cells, and the induction of apoptosis. As a result of transdifferentiation and cell apoptosis, hydroxyapatite accumulates in the walls of blood vessels. Several studies have reported on the mechanisms of multiple cellular signaling pathways that cause inflammation and calcification in blood vessels. Therefore, in this review, we take a closer look at understanding the clinical consequences of hyperhomocysteinemia and apply clinical approaches to reduce its prevalence.

Keywords: Homocysteine; Vascular Calcification; Vitamins; Cardiovascular Diseases

Received: December 3, 2021, Revised: April 5, 2022, Accepted: April 8, 2022
*Corresponding Author: **Nam-Seok Joo** https://orcid.org/0000-0001-5895-1800
Tel: +82-31-219-5324, Fax: +82-31-219-5218, E-mail: jchcmc@aumc.ac.kr

INTRODUCTION

Cardiovascular disease (CVD) is an important health concern, while heart attacks and strokes account for 85% of the total deaths and medical costs worldwide.[1] Most CVDs can be prevented by eliminating behavioral risk factors, such as smoking, improper lifestyle (eating and physical activity), obesity, and alcohol abuse. However, since risk factors such as high blood pressure, diabetes, and hyperlipidemia are common within the population, it is important to identify and prevent them.[2-4]

Therefore, countries with established preventive medicine are educating and discovering the importance of early detection of arteriosclerosis using noninvasive clinical tools. Representative examples include the ankle-brachial blood pressure index,[5,6] non-contrast coronary computed tomography (CT),[7-9] and carotid ultrasound.[10,11] The ankle-brachial index is a tool for predicting the degree of atherosclerosis in peripheral arterial vascular disease, carotid ultrasound for cerebrovascular disease, and non-contrast coronary CT for CVD. However, coronary angiography CT can be used to determine the extent of vascular stenosis; however, is considered inappropriate for screening due to the administration of contrast agents.

Recently, several histological and clinical studies have been conducted on coronary artery and peripheral atherosclerosis, including studies on the relationship between coronary atherosclerosis and coronary artery calcification (CAC) and biomarkers obtainable from blood samples. Blood tests for diabetes, hyperlipidemia, and renal function are performed as predictors of cardiovascular risk factors; however, these diseases cannot fully explain the incidence and progression of CVD. It is important to identify additional biomarkers in blood samples, as early intervention is expected to improve patient prediction and reduce mortality.

The high-sensitivity C-reactive protein is a marker for vascular inflammation; however, it is not suitable for screening purposes because its level increases rapidly in the pre-rupture stage of plaque, which is the acute stage of atherosclerosis. Additionally, research on various proteins and genetic factors is being performed. It has been reported that the serum homocysteine test is considered a predictable and stable test for vascular inflammation. The association between homocysteine and vascular calcification has long been studied. In one study, 60 atheroma biopsies were obtained from vessels containing atherosclerotic coronary arteries, and calcified plaques had higher homocysteine concentrations than noncalcified plaques. In addition, it was confirmed that the higher the concentration of homocysteine in arterial atheromas, the higher the calcium deposition in arterial atheromas.[12] In this study, we focused on serum homocysteine as a predictor of vascular calcification and further summarized some evidence on nutrients for improving serum homocysteine levels and vascular calcification.

HOMOCYSTEINE AND ITS METABOLISM

Homocysteine is a sulfur-containing amino acid that is produced as a metabolite of methionine, an essential amino acid in dietary proteins. It is an intermediate formed during the amino acid biosynthesis of methionine and cysteine. The biosynthesis and catabolism of homocysteine is well balanced, and its physiological level is ideally maintained at 5–15 μmol/L, and is typically less than 10 μmol/L.[13] Moreover, it is highly reactive and forms albumin-bound homocysteine via disulfide bonds with oxidized homocysteine (homocysteine: homocysteine-homocysteine disulfide and homocysteine-cysteine disulfide). Since it is highly reactive, the body has a homocysteine metabolic system, which metabolizes it in two ways: remethylation to methionine, and transsulfuration to cysteine, with the help of three key enzymes: methionine synthase (MS), methylenetetrahydrofolate reductase (MTHFR), and cystathionine-beta-synthase (CBS). Remethylation pathways include folate-and vitamin B12-dependent and independent pathways. In the vitamin B-dependent pathway, N-5-methyl tetrahydrofolate acts as a methyl donor and is catalyzed by the vitamin B12-dependent enzyme MS. Riboflavin (also known as vitamin B2) helps MTHFR by converting folic acid to the form required for remethylation. In the vitamin B-independent pathway, betaine, a choline derivative, serves as a methyl donor in the methylation process and betaine-homocysteine S-methyltransferase is required as an enzyme. Another pathway, transsulfuration, is catalyzed by the vitamin B6-dependent enzymes CBS and cystathionine-γ-lyase (CES). CBS converts homocysteine and serine into cystathionine, and CES is responsible for producing cysteine.[14,15] As described, homocysteine metabolism requires folic acid, vitamin B12, and vitamin B6 (folic acid, pyridoxine, and cobalamin).

Homocysteine concentration varies according to age, sex, and genetic and regional factors, and is also related to menopause, smoking, nutritional status, and medical condition.[16] Hyperhomocysteinemia is mainly caused by genetic defects, nutritional deficiencies, lifestyle problems and certain medical conditions. Genetic factors are associated with the absence or lack of genetic defects in three key enzymes in the methionine-homocysteine and folate cycle (CBS, MS, or MTHFR). Nutritional and lifestyle problems include vitamin B complex deficiency, excessive consumption of foods containing methionine, smoking, a sedentary lifestyle, and high alcohol intake. Medical conditions include metabolic abnormalities found primarily in patients with diabetes and chronic renal diseases. Among these, genetic disorders of homocysteine metabolism are rare and can cause severe hyperhomocysteinemia (>100 μmol/L).[17]

In nutritional deficiency, the three enzymes (CBS, MS, and MTHFR) are highly dependent on cofactors derived from vitamin B6, vitamin B12, and folic acid; therefore, their deficiency can lead to the accumulation of homocysteine. Deficiencies in cofactors can be caused by nutritional imbalances such as anorexia, high alcohol intake, and chronic renal disease (renal loss). Methionine is abundant in a variety of foods such as meat, dairy, eggs, chicken, and fish and its excessive consump-

tion upregulates the transsulfuration pathway.

It is natural to consider homocysteine as an independent risk factor for vascular disease. Elevated blood homocysteine levels promote atherosclerosis, leading to vascular diseases, such as CVD, cerebrovascular disease, peripheral artery disease, and chronic renal disease.[18]

VASCULAR CALCIFICATION AND CORONARY ARTERY DISEASE AND MORTALITY

Several large-scale studies have shown that CAC scores are correlated with atherosclerotic cardiovascular risk and mortality.[7,19] A prospective study was conducted with 6,000 individuals, including four ethnic groups, with a follow-up period of 3.8 years. Coronary events were 7.73-fold higher in the group with a coronary calcium score of 101–300 and 9.67-fold higher in the group with a coronary calcium score of 300 or greater compared to the group with a calcification score of 0.[19] Another prospective study demonstrated that CAC is a predictor of cardiovascular risk, independent of existing cardiovascular risk factors in asymptomatic groups. The higher the CAC score, the higher the occurrence of coronary events. Compared with a calcium score of 0–100, the incidence of coronary events was 3.1-fold higher in the score range of 101–400, 4.6-fold higher in the score range of 401–1,000, and 8.3-fold higher in scores >1,000.[7] A very low cardiovascular risk is expected in an asymptomatic population without vascular calcification. Additionally, as the calcification area (not density) increases, so does the cardiovascular risk. To determine the risk of coronary events in a healthy, clinically asymptomatic population, atherosclerosis test, coronary calcium score, or carotid ultrasonography could be performed.[20] Although the two clinical tools are comparable in terms of the risk of 10-year atherosclerotic CVD, a non-contrast coronary calcium score is more useful in the prediction of CAC.

Microcalcification is defined as a small calcium deposit (<5 μm) observed within the high-risk lipid core of unstable plaques. Conversely, the healing process of these necrotic or apoptotic plaques results in stable plaques containing macrocalcifications (>5 μm).[21] Macrocalcification is not a simple accumulation of microcalcification. Microcalcification and macrocalcification have different meanings: one is an unstable plaque and the other is a stable plaque, respectively.[22] This is because they are composed of macrophages with different properties. Microcalcification is induced by typical M1-polarized macrophages, and macrocalcification is produced by alternative M2-polarized macrophages.[22] Microcalcification, also called spotty calcification within the enriched lipid pools, implies sustained inflammation by M1 macrophages that secrete pro-inflammatory cytokines such as tumor necrosis factor-(TNF-α) and interleukin-6 (IL-6).[20] Pro-inflammatory macrophages (M1) evoke calcified vesicles and apoptotic bodies through macrophage apoptosis and matrix vesicles from osteoblast-like cells, resulting from vascular smooth muscle cell (VSMC) transdifferentiation. As a result, calcium phosphate is secreted and causes vascular mineralization.[23] At the same time, inflammatory mediators are exposed to the vascular lumen, allowing plaque progression and plaque instability.[24] However, contrarily, fibrous caps were reported to be at a lower risk of plaque formation even though they were rich in calcification.[25] M2 macrophages secrete the anti-inflammatory cytokine IL-10 and promote macrocalcification. The calcification also consists of mineralization from stromal vesicles of osteoblast-like mature VSMCs.[22] Statin therapy has anti-inflammatory effects on plaque and is thought to facilitate plaque regression and increase macrocalcification through the action of M2 polarizing macrophages.[26-28] In this regard, patients with calcified plaque identified on cardiac CT should have a low risk of plaque rupture and CVD; however, clinically, CAC increases the risk of CVD and progresses further. Although calcification is associated with inflammation, the exact mechanisms of microcalcification and macrocalcification remain unclear.

A coronary calcium score represents the atherosclerotic burden; however, the presence of plaque is not definitive.[29] Therefore, a coronary artery calcium score is recommended to evaluate the asymptomatic population and can be used to subclassify the CVD risk groups. For example, a CAC score of 0 indicates a very low-risk group and has a CVD risk of 0.1% per year.[30,31] Therefore, CAC 0 can be used as a negative risk marker and patients with such a score rarely need follow-up. If the CAC score is 100 or higher, the 10-year cardiovascular risk can be estimated to be >10%, and patients with such a score need additional cardiovascular examinations such as echocardiography, coronary CT angiography, or intravascular coronary angiography.

When the CAC score exceeded 300, the risk of major coronary events was significantly higher than that of calcium score of 1–100 (hazard ratio [HR], 6.84; 95% confidence interval [CI], 2.93–15.99; HR, 3.89; 95% CI,1.72–8.79).[19] As demonstrated in several large-scale studies, CAC scores are known to indicate increased CVD risk and lower survival rates. In the Multiethnic Study of Atherosclerosis cohort, calcium scores were independently and gradually associated with adverse coronary events over 3.8 years of follow-up.[21] In the Rotterdam Coronary Calcification Study, coronary calcification is an independent predictor of coronary heart diseases (CHDs) and is inversely correlated with new cardiovascular event-free survival.[7] Other large-scale observational data suggest that CAC is an independent incremental risk factor of CVD and mortality during a mean follow-up of 6.8 years[32] and over a median of 11.4 years.[33] Meta-analysis also shows that coronary calcification increases the adverse cardiovascular events and mortality.[18]

The quantitative method for vascular calcification is to calculate the calcium score on CT. Since microcalcifications and macrocalcifications are pathologically and clinically different, additional basic research is needed. Calcification volume has an independent positive correlation with CHD and CVD risk; however, calcification density is inversely proportional to CHD and CVD risks.[34] The Agatston score was weighted with higher calcium density, but did not mean the actual number of calcified plaques in the CAC volume scoring system. On the contrary, volumetric calcification scores are more accurate than standard Agatston scores when volume is considered. Agatston scores were used in most studies, and the results may have been diluted or overestimated due to the presence of macrocalcification.

https://doi.org/10.4082/kjfm.21.0227

MECHANISM OF VASCULAR CALCIFICATION

Vascular calcification is ectopic calcium phosphate deposition in the arterial wall. It is an old concept that vascular calcification is a passive process resulting from aging, or occurs as a simple calcium deposition in the extracellular environment of an abundant environment of calcium and phosphorus products.[35] Nowadays it is considered an active and regulated process wherein calcification activators and inhibitors act, such as in bone mineralization.[36]

Intima and medial calcifications are single spectra and continuous. Based on clinical and histological findings, there are no distinguishing characteristics between intima and media calcification.[37] In addition, calcifying-related proteins that are crucial for bone homeostasis, including osteoprotegerin, receptor activator of nuclear factor (NF)-kappa B ligand (RANKL), TNF-related apoptosis-inducing ligand (an inducer of apoptosis), and messenger RNAs expression patterns, are similarly found in both intima and media calcification.[38] Calcification in both the intima and media is related to VSMCs degeneration and apoptosis, and the matrix vesicles of VSMCs and pericytes are the major initial loci for calcium deposition.[39,40]

Calcification is the summation of hydroxyapatite, which consists of calcium phosphate (hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$). This basic form of calcification is not a simple form of bioapatite crystal. Calcification occurs in matrix vesicles derived from living VSMC and apoptotic bodies of dying cells.[41,42] Matrix vesicles are 20–200 nm-sized small spherical bodies,[43] and are the initial nucleation sites for calcium mineral formation. Matrix vesicles bud from cells, serve as 'cargo', and consist of phosphatidylserine and annexins displaying hydroxyapatite crystals on the inner membrane within the lumen, and/or on the outer membrane of the vesicle.[44] Alkaline phosphatase (ALP) within the membranes (also tissue-nonspecific alkaline phosphatases or TNAP) generates inorganic phosphate in the extravascular space, and leads to an influx of phosphate and calcium into the vesicle via ion channels. Nucleation of hydroxyapatite is facilitated by an annexin-phosphatidylserine complex, which facilitates calcium influx and mineralization.[43,45,46]

Matrix vesicles from VSMCs are similar to those in osteogenesis and bone mineralization, the vesicles are detached from chondrocytes. Osteoblasts, chondroblasts, and transdifferentiated vascular cells have a substantial overlap in mineralization mechanisms and gene expression. Vascular calcification and bone mineralization have in common mineralization-promoting proteins and matrix vesicles derived from osteogenic cells. Paradoxically, osteoporosis patients present with vascular calcification, leading to the assumption that mineralization in the vascular walls and loss from bone occur simultaneously.[47] In another study, patients with chronic kidney disease had lower bone mineral density and higher CAC with an increased incidence of fractures.[48]

Several mineralization activators and inhibitors have been proven in *in vitro* studies. Mineralization activators include an overload of Pi and Ca, RANKL, osteopontin (non-phosphorylated), and bone morphogenetic protein-2 (BMP-2), which include matrix γ-carboxyglutamic acid (Gla) protein (MGP), pyrophosphate, fetuin-A, osteoprotegerin (OPG), and osteopontin (phosphorylated).[49] These inhibitors are localized in matrix vesicles from VSMC or circulate in blood vessels. Matrix vesicles normally contain components of mineralization inhibitors; however, concentrated pre-formed basic calcium phosphate and mineralization activators are found in environments with increased calcium and phosphate levels.[50] Noncalcifying vesicles contain inhibitors of calcification, such as fetuin-A and MGP. In contrast, calcifying vesicles contain osteogenic markers, such as runt-related transcription factor 2 (Runx2), Smad1, osterix, TNAP, chaperones, and pro-inflammatory factors.[51,52]

Moreover, these bone-related proteins are expressed not only in matrix vesicles but also in atherosclerotic plaques. *In-vitro* studies are proving the activity of these bone-related proteins, including BMP-2,[36] osteopontin,[53,54] MGP,[55] and OPG.[56] These proteins act through the RANKL/OPG pathway which is the main mechanism of mineralization both in osteoporosis and vascular calcification.[57] BMPs are a subclass of transforming growth factor-beta superfamily, and BMP-2/4 can induce mineralization and local inflammation. On the contrary, BMP-7 delays vascular calcification. Runx2 is a key osteogenic transcription factor downstream of BMP-2, which controls severe osteoblastic differentiation-related proteins such as osteocalcin (OC), osteopontin, and type 1 collagen.[58]

Homocysteine, mostly associated with vascular calcification, is thought to induce early endothelial dysfunction, thereby regulating several genes and proteins previously described, ultimately leading to apoptosis, which is thought to regulate the production and composition of matrix vesicles. Apoptosis preceding vascular calcification and apoptotic bodies derived from VSMCs initiate vascular calcification, which serves as the nucleation core.[41,59]

HOMOCYSTEINE AND OXIDATIVE STRESS AND INFLAMMATION

The overall content of the subheading is diagrammed in Figure 1. Homocysteine is easily oxidized and produces hydrogen peroxide and superoxide radicals, which in turn oxidize low-density lipoprotein (LDL) cholesterol and proteins. Homocysteine is an indicator of oxidative stress, indicating that hyperhomocysteinemia induces abundant reactive oxygen species (ROS). One of the highly reactive compounds, homocysteine thiolactone, is a byproduct of homocysteine auto-oxidation, and its production is usually low at the physiological level of homocysteine; however, it is easily produced under hyperhomocysteinemia conditions. Homocysteine thiolactone is a highly reactive molecule that can react with LDL or proteins,[60] resulting in protein acylation (homocysteinylation) of lysine residues and LDL oxidation after homocysteinylation. Homocysteine thiolactone induces endothelial cell apoptosis independently from the caspase pathway.[61]

Hyperhomocysteinemia also increases ROSs differently by decreasing the activity of antioxidant enzymes, such as GPx1 and superoxide

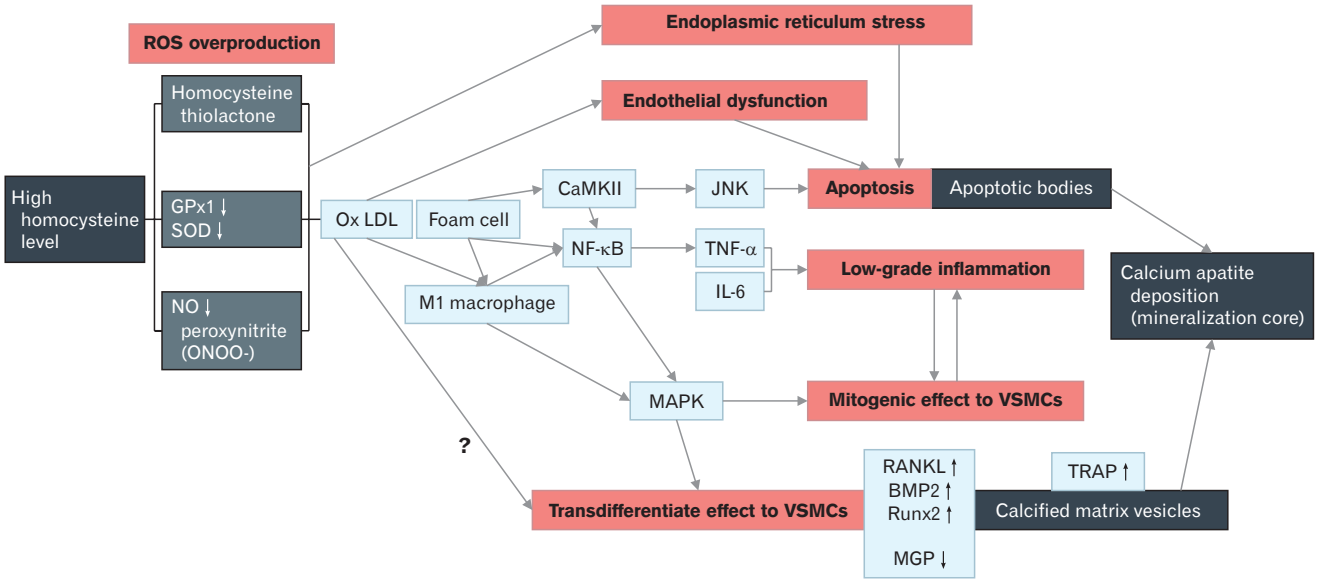

Figure 1. Effects of high homocysteine levels on inflammation and calcification. Hyperhomocysteinemia induces reactive oxygen species (ROS) production in endothelial cells and vascular smooth muscle cells. This results in apoptosis and inflammation through multiple cellular signaling pathways that create endoplasmic reticular stress and endothelial dysfunction. In addition, ROS allow vascular smooth muscle cells to differentiate with mitotic and osteo-like features in the mitogen-activated protein kinase (MAPK) pathway and an uncertain mechanism. Calcium apatite is deposited on the blood vessel wall and released from apoptosis and matrix vesicles of osteo-like vascular smooth muscle cells. GPx1, glutathione peroxidase 1; SOD, superoxide dismutase; NO, nitric oxide; Ox, low-density lipoprotein; CaMKII, Ca2+/calmodulin-dependent protein kinase II; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; JNK, c-Jun N-terminal kinase; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; VSMC, vascular smooth muscle cells; TRAP, tartrate-resistant acid phosphatase; RANKL, receptor activator of NF-κB ligand; BMP2, bone morphogenetic protein 2; Runx2, runt-related transcription factor 2; MGP, matrix Gla-protein.

dismutase.[62] Hyperhomocysteinemia increases the uncoupling of endothelial nitric oxide synthase, resulting in a decreased production of nitric oxide (NO). NO is a protective gaseous lipophilic messenger against endothelial dysfunction.[63] ROSs combine with NO and inactivate it through the resulting peroxynitrite (ONOO-), which is a potent oxidizing agent that accelerates lipid and protein oxidation[64] by increasing reactive oxygen and nitrogen intermediates, resulting in endothelial dysfunction.[65]

In addition to NO, studies on H_2S, a protective gas that affects endothelial function, have been recently published. The level of H_2S is associated with homocysteine levels and is produced during homocysteine metabolism. H_2S dysfunction is also caused by hyperhomocysteinemia. Homocysteine and H_2S concentrations are regulated by each other, and their imbalance is closely linked to CVDs. H_2S and homocysteine are related to the level of endothelial dysfunction and injury.[66,67] H_2S has a protective effect against hyperhomocysteinemia-induced endothelial injury.[68] It is produced by vascular cells and exhibits antioxidant, anti-apoptotic, anti-inflammatory, and vasoactive properties. Hyperhomocysteinemia induces decreased H_2S production as observed in cell- and animal-level studies.[69-71]

Homocysteine induces mitochondrial dysfunction and apoptosis.[61,72] Mitochondria, small organelles inside cells, are crucial for the energy factory and electron transport system, which is the main source of ROS.[73] In a normal physiological environment, ROS formation and antioxidant activity are well balanced. Homocysteine is an unstable amino acid that is easily auto-oxidized to produce free oxygen radicals. Homocysteine and increased free oxygen radicals inhibit cellular antioxidant systems. Therefore, hyperhomocysteinemia is the main cause of oxidative stress. In the case of increased ROS or decreased antioxidant activity, mitochondrial oxidative stress occurs.[74] ROS participates in several steps of the mitochondrial apoptotic process,[75] and homocysteine is the reactive molecule of the vessels, causing ROSs such as hyperglycemia, hyperlipidemia, and uremia. Mitochondrial DNA is susceptible to damage by ROS, and this accumulated mitochondrial DNA damage results in the release of inflammatory cytokines and abnormal proliferation and apoptosis of VSMCs.[76,77] Homocysteine alters mitochondrial (electron transport chain) gene expression,[78] function, and structure. Damaged mitochondria accelerate oxidative stress, inducing VSMC calcification via the induction of Runx2 expression.[79]

These ROSs also convert LDLs to oxidized LDLs, and oxidized LDLs induce inflammatory responses by upregulating adhesion molecules (vascular cell adhesion molecule for monocyte adhesion; and monocyte chemotactic protein 1 and monocyte chemoattractant protein1 for monocyte penetration into subendothelial tissue). Monocytes attach to and penetrate the endothelium and are converted to macrophages, which can engulf a large amount of oxidized LDLs, which are then converted to foam cells. Oxidized LDLs and foam cells accumulate to form a fatty streak and release pro-inflammatory cytokines, such as TNF-α, IL-6, IL-12, and IL-1β.[80-83] They are produced by macrophages (M1) within the progressing plaque lesions and promote microcalcification. From this point of view, vascular calcification is a compensatory response to chronic inflammation in atherosclerosis. In

https://doi.org/10.4082/kjfm.21.0227

addition, homocysteine levels could be a marker of chronic intravascular inflammation. After the resolution of chronic inflammation, macrophages (M2) produce anti-inflammatory cytokines (IL-10) and induce osteogenesis, leading to plaque macrocalcification and stabilization, as well as plaque regression.[22,84] Vascular calcium scoring through X-ray or CT scans detects only macrocalcification, which is found in stabilized and regressed plaques. Statin therapy could reduce atheroma, but could lead to an increase in the calcium score by increasing the macrocalcification.[28,85,86] The initiation and propagation of calcium deposits are accelerated in increased homocysteine, representing a high oxidative stress and low-grade inflammation in the intravascular environment.

Oxidative stress induced by hyperhomocysteinemia also regulates the osteoblastic differentiation of vascular cells. Although homocysteine contributes more to intima calcification than media calcification, it also initiates media calcification in the same manner. ROS upregulation and inflammation induce VSMC differentiation into osteoblast-like cells. IL-1β secreted by macrophages stimulate VSMC calcification,[82] and IL-6 induces VSMC to transdifferentiate into osteoblast-like cells.[80] VSMCs transform through the expression of calcification regulator Runx2 which induces RANKL expression.[87] RANKL-receptor activator of NF-kappa B binding induces TNAP expression which hydrolyzes pyrophosphate ions, which are potent mineralization inhibitors, and creates phosphate entrapment for mineralization.[88] The Runx2 expression can be induced by BMP-2 upregulation in endothelial cells initiated by hypoxia,[89] ROS,[90] inflammation,[91] and BMP-4 upregulation in foam cells caused by oxidized LDL.[92,93]

Increased homocysteine, oxidized LDL, and oxidative stress can lead to a chronically activated unfolded protein response (UPR).[94] Chronic UPR and direct oxidative stress induced by hyperhomocysteinemia can activate the endoplasmic reticulum (ER) stress pathway.[95] Activation of the UPR in macrophages, VSMC, and pericytes leads to increased cytoplasmic calcium and activation of calcium/calmodulin-dependent protein kinase II, resulting in the amplification of apoptosis.[95]

Vascular calcification is a result of matrix vesicles from the cell apoptotic bodies; in short, the apoptosis process controls vascular calcification.[41] Hyperhomocysteinemia mediates VSMC and endothelial cell apoptosis via the ER stress pathway and ROS production.[96] Hyperhomocysteinemia also induces inflammation through the activation of NF-kB and its downstream pro-inflammatory mediator.[97,98] Moreover, hyperhomocysteinemia upregulates pathogenic genes and downregulates protective genes by impairing methylation and increasing homocysteinylation of proteins.[99] Both enhancements of ROS production and apoptosis induce endothelial dysfunction and initiate vascular calcification by apoptotic bodies and matrix vesicles as a nucleating structure for calcium crystal formation.[100] Increased mineralization is derived by matrix vesicles, calcification regulatory proteins such as OC, BMP-2, and uncarboxylated MGP. Additionally, clues to VSMCs transforming into osteoblast-like cells include BMP-2, osteopontin, Msh homeobox 2 (MSX2), and ALP.[101]

Vascular calcification is a consequence of low-grade chronic inflammation in atherosclerosis, which is due to high ROS formation and signal transduction induced by hyperhomocysteinemia. One way to reduce inflammation by hyperhomocysteinemia is to block the activity of NF-kB as a control for other underlying diseases, while another method is to block hyperhomocysteinemia, which causes oxidative stress.[102]

HOMOCYSTEINE LOWERING BY NUTRITIONAL SUPPLEMENTS

High homocysteine levels are strongly associated with the development of various vascular diseases related to atherosclerosis and are independent predictors of uremia, hyperglycemia, and dyslipidemia.[103] The CVD risk is 3 times higher in the top 5% of homocysteine levels compared to the low 90% of homocysteine levels.[104] A high level of homocysteine increases the mortality associated with coronary artery disease and acute myocardial infarction and increases the all-cause mortality.[105] Since homocysteine levels can be easily lowered with vitamin supplementation, homocysteine itself is expected to function as a modifiable risk marker.

Thus, it is natural to assume that lowering homocysteine levels may help lower the risk of CVD. Many interventional studies have been conducted to lower homocysteine levels by replenishing the nutrients related to homocysteine metabolism.

The randomized controlled trials (RCTs) and prospective studies showing that homocysteine levels are lowered by supplementation with B vitamins, which are cofactors in the metabolic pathway of homocysteine, revealed statistically significant results.[106-112] After 6 weeks of folic acid supplementation alone, homocysteine levels decreased significantly.[107] When folic acid, vitamin B6, and vitamin B12 were administered together, there were no significant differences in homocysteine levels compared to those provided by folic acid alone, and in other results, homocysteine levels were significantly reduced compared to when folic acid and vitamin B12 were taken together.[110] Supplementation with vitamin B6 alone for 12 weeks did not lower the homocysteine level regardless of the dose. Taking vitamin B6 in combination with folic acid and vitamin B12 lowered the homocysteine levels by 32% of the baseline.[111] In addition, the higher the concentration of folic acid taken, the lower the homocysteine levels. Additional homocysteine reduction was found when folic acid was administered with vitamin B12, but not with vitamin B6.[112] In patients with acute myocardial infarction, homocysteine levels decreased after 6 weeks of folic acid supplementation, and there was no difference between folic acid dosages of 2.5 mg and 10 mg.[107] Even in the general population other than those suffering from heart disease, when 0.5–5 mg of dietary folic acid[108] or folic acid fortified (folic acid greater than about 0.5 mg) grains are ingested,[109] the plasma homocysteine levels are lowered, and can be lowered to approximately 25%, at which the level of homocysteine is about 9 μmol/L.[108]

The desirable total homocysteine level is estimated to be 9–10 μmol/

L. The overall mortality was lower in the case of plasma homocysteine <9 μmol/L than in the case of 15 μmol/L or more.[105] According to the results of the Vitamin Atherosclerosis Intervention Trial, the homocysteine concentration of 9.1 μmol/L is too low to induce vascular calcification or subclinical atherosclerosis. If fasting homocysteine is 9.1 or higher, supplementation with B vitamins can be considered.[113] Although this is a small observational study conducted in Koreans of Asian ethnicity, the correlation between homocysteine levels and vascular calcification was confirmed, and the cut-off value of homocysteine, which can predict the presence of vascular calcification, was estimated to be 9 μmol/L.[114,115]

Does lowering homocysteine levels in the vitamin B group lower the risk of vascular diseases? Unfortunately, there is insufficient evidence to corroborate whether vitamin supplementation lowers homocysteine levels and, as a result, lowers the incidence or risk of vascular disease.

Several studies have reported on whether supplementation with B vitamins can suppress the occurrence or progression of vascular calcification by lowering the homocysteine levels. At the observational level, in populations with diabetes or vascular disease at high risk for cardiovascular events, lower folate concentrations were associated with plaque calcification. When homocysteine and folic acid were divided into quartiles, it was confirmed that the higher the homocysteine level and the lower the plasma folic acid concentration, the higher the carotid calcification score was observed to be statistically significant. In this study, it was concluded that there was no association between vitamin B6 and B12 concentrations, and that low folate concentrations (7–36 nmol/L) were associated with increasing calcification scores.[116] A RCT studied the relationship between high doses of B vitamins (folic acid 5 mg, vitamin B12 0.4 mg, vitamin B6 40 mg) for 3 years and carotid intima-media thickness (IMT) along with aortic and coronary calcium. In patients with homocysteine levels above 9.1 μmol/L, vitamin B supplementation decreased the progression of carotid IMT, but in patients with homocysteine levels less than 9.1 μmol/L, vitamin B supplementation had no significance in slowing IMT progression. The coronary artery calcium score was lower in the vitamin B group (1.2) than in the placebo group (3.9), and the incidence of calcium was lower, but the difference in incidence was not statistically significant.[113]

Additional cofactors that participate in the homocysteine metabolism are riboflavin and betaine. Riboflavin supplementation can lower the homocysteine concentrations and blood pressure levels in specific groups.[117,118] Betaine also reduces homocysteine; however, there is not enough evidence that choline supplements affect the CVD risk. Homocysteine is lowered by the supplementation of the vitamin B complex with pyridoxine (100–200 mg/d), folic acid (5 mg/d), and vitamin B12 (intermittent hydroxocobalamin injection) in cystathionine beta-synthase deficiency.[119] In pyridoxine non-responders, the homocysteine levels are not lowered even if pyridoxine is administered. However, addition of 6–9 g of oral betaine (trimethylglycine) could lower the homocysteine concentration. Additionally, although choline intake lowered the homocysteine levels,[120,121] choline and betaine intake did not lower the risk of CVD.[122,123] Notably, the CVD risk increases when betaine levels are insufficient.[124]

Other nutrients that do not appear to be related to homocysteine metabolism have also been reported to lower the vascular calcification, including vitamin D. The group with low vitamin D levels is estimated to have a higher risk of CVDs; however, there is still insufficient research on whether vitamin D supplementation can lower CVD events.

Homocysteine and vitamin D are inversely related to each other.[125] Similar to the results seen with vitamin B supplementation, vitamin D supplementation significantly lowers serum homocysteine levels. In an RCT trial targeting women of reproductive age with obesity, vitamin D3 supplements (50,000 IU/wk) for 2 months decreased their homocysteine levels and increased their serum 25-hydroxyvitamin D (25(OH)D), calcium, and phosphorous levels.[126] However, the trial results did not include the CVD risk or vascular calcification. A prospective study showed that vitamin D deficiency is associated with CAC.[127] The relationship between CAC and vitamin D levels has been examined for polymorphisms in vitamin D-associated genes. After 3 years of follow-up, the incidence of newly developed CAC was 3.3 times higher in patients with vitamin deficiency (25(OH)D <20 ng/mL), and 1.8 times higher in patients with vitamin insufficiency (25(OH)D <30 ng/mL).

Vitamin D is a fat-soluble vitamin that can be ingested via food; however, most of it is synthesized in sun-exposed skin. Subsequently, through hydroxylation in the liver, 25(OH)D is mainly circulated in the blood vessels and converted into 1,25-dihydroxy vitamin D (1,25(OH)2D), which is calcitriol, the most active form converted in the kidney. The main role of vitamin D is to regulate calcium metabolism by increasing calcium absorption in the intestine, and several studies have shown that vitamin D supplementation is necessary not only to prevent osteopenia and osteoporosis, but to also prevent calcium deposits in tissues other than bones, typically blood vessels. Calcium supplementation alone prevents osteoporosis but increases the risk of vascular diseases such as MI and stroke.[128,129] Vitamin D is known to be a protective factor for CVD, and low vitamin D is known to be a CVD risk factor.[130] However, no clear clinical evidence has been obtained for lowering CVD risk with vitamin D supplements.[131] Vitamin D deficiency is associated with a pro-inflammatory status associated with dyslipidemia and oxidative stress,[132] and vitamin D regulates the gene expression of proteins related to homocysteine metabolism.[133]

VITAMIN K SUPPLEMENTATION AND VASCULAR CALCIFICATION

Although its relationship with homocysteine has not been studied, vitamin K is a nutritionally important nutrient that is involved in vascular calcification. Vitamin K is essential for MGP to function as an inhibitor of vascular calcification.[134] Vitamin K is a fat-soluble vitamin and exists in two dominant forms: vitamin K1 (phylloquinone), mainly

in green vegetables, and vitamin K2 (menaquinone), mainly produced in fermented milk products and by intestinal lactic acid bacteria.[135] Vitamin K1 is transported to the liver to regulate the production of coagulation factors, and vitamin K2 regulates the activities of MGP and OC (bone Gla protein) in the extrahepatic organs and blood vessels.[136] These two proteins are vitamin K-dependent proteins and require carboxylation to function, which requires vitamin K2 as a cofactor.[137] When vitamin K2 is deficient, MGP and OC remain in uncarboxylated form (inactivate form), blood vessels are at risk of CVD, and bones have reduced bone mineral density and are at a risk of osteoporosis.[138] Several observational studies have shown that vascular calcification progresses less, and the risk of CVD is lowered when inactive MGP (uncarboxylated MGP) levels are low.[139] Supplementation of K2 (menaquinone), not vitamin K1 (phylloquinone), is associated with reduced CVD outcomes, while vitamin K2 supplementation and CAC are inversely correlated.[140-143] Increasing the menaquinone intake reduces and retards the progression of vascular calcification, while phylloquinone has no such effect.[142-144] and is known to lower the risk of CVD with high vitamin K intake.[139,142]

In the presence of sufficient vitamin K2 (menaquinones), carboxylation of MGP is accelerated in vascular calcification and OC in bone mineralization. Carboxylated MGP inhibits calcium deposits, and carboxylated OC promotes calcium deposits. As described before, vitamin D ensures that calcium is absorbed easily from the food we consume, and vitamin K2 (MK-7) activates the protein OC, which integrates calcium into the bone. Vitamin K2 (MK-7) activates MGP to bind excess calcium and promotes arterial flow and flexibility. In a vitamin K-rich environment, VSMCs possess a natural contractile function and synthesize MGP, which is a carboxylated form. However, in the presence of oxidative stress, such as hyperhomocysteinemia and uremia, the phenotype of VSMCs changes to proliferating and synthesizing calcification.[145] If vitamin K levels are sufficient in such stressful situations, the transdifferentiation of the VSMCs is prevented and mineralization is prevented by the formation of calcified matrix vesicles.[146] It is thought that MGP may be involved in the mechanism of reducing calcification by inhibiting BMP-2 and BMP-4.[147] However, if vitamin K is insufficient, VSMCs are changed to an osteoblast-like cell phenotype, and osteoblastic VSMCs reduce the MGP production and produce bone-related proteins (such as ALP)[148] and MGP is changed to the uncarboxylated form.

CONCLUSION

There is no doubt that high serum homocysteine levels cause endothelial dysfunction and initiate atherosclerosis. Numerous studies have shown that calcified atherosclerosis is associated with a higher homocysteine concentration than non-calcified atherosclerosis, and that vascular calcification indicates the degree of inflammation in atherosclerosis. In addition, recent studies on the gene and protein levels have shown that homocysteine is related to ROS production, oxidative stress, and ER stress, inducing endothelial dysfunction, causing inflammation and cell necrosis, and transforming VSMCs into osteoblast-like cells. From this perspective, the incidence and progression of vascular endothelial damage caused by homocysteine and the continuation of hyperhomocysteinemia may be related. Therefore, serum homocysteine levels can be considered a biomarker of vascular calcification.

If a large-scale clinical trial comparing vascular calcification and homocysteine is conducted, it will be helpful from a preventive point of view to shed light on their relationship and predict the fraction of hyperhomocysteinemia in cardiovascular risk. Moreover, it could be used to predict the degree of calcification in major blood vessels (coronary, cerebral, and peripheral arteries) by examining the homocysteine concentration in blood at the health examination stage along with predicting the 10-year cardiovascular risk.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ORCID

Susie Jung: https://orcid.org/0000-0003-3317-4405
Beom-Hee Choi: https://orcid.org/0000-0002-9723-6161
Nam-Seok Joo: https://orcid.org/0000-0001-5895-1800

REFERENCES

1. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 Study. J Am Coll Cardiol 2020;76:2982-3021.
2. Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, et al. Heart disease and stroke statistics-2021 update: a report from the American Heart Association. Circulation 2021;143:e254-743.
3. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Abate D, Abbasi N, Abbastabar H, Abd-Allah F, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2017: a systematic analysis for the Global Burden of Disease Study. JAMA Oncol 2019;5:1749-68.
4. GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2018;392:1736-88.
5. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). J Vasc Surg 2007;45 Suppl S:S5-67.
6. McDermott MM. Ankle-brachial index screening to improve health outcomes: where is the evidence? Ann Intern Med 2013;159:362-3.
7. Vliegenthart R, Oudkerk M, Hofman A, Oei HH, van Dijck W, van Rooij FJ, et al. Coronary calcification improves cardiovascular risk prediction in the elderly. Circulation 2005;112:572-7.

8. Rumberger JA. Using noncontrast cardiac CT and coronary artery calcification measurements for cardiovascular risk assessment and management in asymptomatic adults. Vasc Health Risk Manag 2010;6:579-91.
9. Bos D, Leening MJ. Leveraging the coronary calcium scan beyond the coronary calcium score. Eur Radiol 2018;28:3082-7.
10. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008;21:93-111.
11. Chambless LE, Folsom AR, Clegg LX, Sharrett AR, Shahar E, Nieto FJ, et al. Carotid wall thickness is predictive of incident clinical stroke: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol 2000;151:478-87.
12. Van Campenhout A, Moran CS, Parr A, Clancy P, Rush C, Jakubowski H, et al. Role of homocysteine in aortic calcification and osteogenic cell differentiation. Atherosclerosis 2009;202:557-66.
13. Moghadasian MH, McManus BM, Frohlich JJ. Homocyst(e)ine and coronary artery disease: clinical evidence and genetic and metabolic background. Arch Intern Med 1997;157:2299-308.
14. Schalinske KL, Smazal AL. Homocysteine imbalance: a pathological metabolic marker. Adv Nutr 2012;3:755-62.
15. Skovierova H, Vidomanova E, Mahmood S, Sopkova J, Drgova A, Cervenova T, et al. The molecular and cellular effect of homocysteine metabolism imbalance on human health. Int J Mol Sci 2016;17:1733.
16. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med 1998;338:1042-50.
17. Handy DE, Loscalzo J. Homocysteine and atherothrombosis: diagnosis and treatment. Curr Atheroscler Rep 2003;5:276-83.
18. Rennenberg RJ, Kessels AG, Schurgers LJ, van Engelshoven JM, de Leeuw PW, Kroon AA. Vascular calcifications as a marker of increased cardiovascular risk: a meta-analysis. Vasc Health Risk Manag 2009;5:185-97.
19. Detrano R, Guerci AD, Carr JJ, Bild DE, Burke G, Folsom AR, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. N Engl J Med 2008;358:1336-45.
20. Shioi A, Ikari Y. Plaque calcification during atherosclerosis progression and regression. J Atheroscler Thromb 2018;25:294-303.
21. Karger AB, Steffen BT, Nomura SO, Guan W, Garg PK, Szklo M, et al. Association between homocysteine and vascular calcification incidence, prevalence, and progression in the MESA cohort. J Am Heart Assoc 2020;9:e013934.
22. Roijers RB, Debernardi N, Cleutjens JP, Schurgers LJ, Mutsaers PH, van der Vusse GJ. Microcalcifications in early intimal lesions of atherosclerotic human coronary arteries. Am J Pathol 2011;178:2879-87.
23. Nadra I, Mason JC, Philippidis P, Florey O, Smythe CD, McCarthy GM, et al. Proinflammatory activation of macrophages by basic calcium phosphate crystals via protein kinase C and MAP kinase pathways: a vicious cycle of inflammation and arterial calcification? Circ Res 2005;96:1248-56.
24. Otsuka F, Sakakura K, Yahagi K, Joner M, Virmani R. Has our understanding of calcification in human coronary atherosclerosis progressed? Arterioscler Thromb Vasc Biol 2014;34:724-36.
25. Wu B, Pei X, Li ZY. How does calcification influence plaque vulnerability?: insights from fatigue analysis. ScientificWorldJournal 2014;2014:417324.
26. Kelly-Arnold A, Maldonado N, Laudier D, Aikawa E, Cardoso L, Weinbaum S. Revised microcalcification hypothesis for fibrous cap rupture in human coronary arteries. Proc Natl Acad Sci U S A 2013;110:10741-6.
27. Vancheri F, Longo G, Vancheri S, Danial JS, Henein MY. Coronary artery microcalcification: imaging and clinical implications. Diagnostics (Basel) 2019;9:125.
28. Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, et al. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. JAMA 2006;295:1556-65.
29. Banach M, Serban C, Sahebkar A, Mikhailidis DP, Ursoniu S, Ray KK, et al. Impact of statin therapy on coronary plaque composition: a systematic review and meta-analysis of virtual histology intravascular ultrasound studies. BMC Med 2015;13:229.
30. Knez A, Becker A, Leber A, White C, Becker CR, Reiser MF, et al. Relation of coronary calcium scores by electron beam tomography to obstructive disease in 2,115 symptomatic patients. Am J Cardiol 2004;93:1150-2.
31. Sarwar A, Shaw LJ, Shapiro MD, Blankstein R, Hoffmann U, Cury RC, et al. Diagnostic and prognostic value of absence of coronary artery calcification. JACC Cardiovasc Imaging 2009;2:675-88.
32. Budoff MJ, Shaw LJ, Liu ST, Weinstein SR, Mosler TP, Tseng PH, et al. Long-term prognosis associated with coronary calcification: observations from a registry of 25,253 patients. J Am Coll Cardiol 2007;49:1860-70.
33. Mitchell JD, Paisley R, Moon P, Novak E, Villines TC. Coronary artery calcium and long-term risk of death, myocardial infarction, and stroke: the Walter Reed Cohort Study. JACC Cardiovasc Imaging 2018;11:1799-806.
34. Criqui MH, Denenberg JO, Ix JH, McClelland RL, Wassel CL, Rifkin DE, et al. Calcium density of coronary artery plaque and risk of incident cardiovascular events. JAMA 2014;311:271-8.
35. Alfrey AC, Ibels LS. Role of phosphate and pyrophosphate in soft tissue calcification. Adv Exp Med Biol 1978;103:187-93.
36. Bostrom K, Watson KE, Horn S, Wortham C, Herman IM, Demer LL. Bone morphogenetic protein expression in human atherosclerotic lesions. J Clin Invest 1993;91:1800-9.
37. McCullough PA, Chinnaiyan KM, Agrawal V, Danielewicz E, Abela GS. Amplification of atherosclerotic calcification and Monckeberg's sclerosis: a spectrum of the same disease process. Adv Chronic Kidney Dis 2008;15:396-412.
38. Schoppet M, Al-Fakhri N, Franke FE, Katz N, Barth PJ, Maisch B, et al. Localization of osteoprotegerin, tumor necrosis factor-related apoptosis-inducing ligand, and receptor activator of nuclear factor-kappaB ligand in Monckeberg's sclerosis and atherosclerosis. J Clin Endocrinol Metab 2004;89:4104-12.
39. Tanimura A, McGregor DH, Anderson HC. Calcification in atherosclerosis. I. Human studies. J Exp Pathol 1986;2:261-73.
40. Tanimura A, McGregor DH, Anderson HC. Calcification in atherosclerosis. II. Animal studies. J Exp Pathol 1986;2:275-97.
41. Proudfoot D, Skepper JN, Hegyi L, Bennett MR, Shanahan CM, Weissberg PL. Apoptosis regulates human vascular calcification in

vitro: evidence for initiation of vascular calcification by apoptotic bodies. Circ Res 2000;87:1055-62.
42. Kim KM. Calcification of matrix vesicles in human aortic valve and aortic media. Fed Proc 1976;35:156-62.
43. Golub EE. Role of matrix vesicles in biomineralization. Biochim Biophys Acta 2009;1790:1592-8.
44. New SE, Aikawa E. Role of extracellular vesicles in de novo mineralization: an additional novel mechanism of cardiovascular calcification. Arterioscler Thromb Vasc Biol 2013;33:1753-8.
45. Balcerzak M, Hamade E, Zhang L, Pikula S, Azzar G, Radisson J, et al. The roles of annexins and alkaline phosphatase in mineralization process. Acta Biochim Pol 2003;50:1019-38.
46. Anderson HC. Molecular biology of matrix vesicles. Clin Orthop Relat Res 1995;(314):266-80.
47. Parhami F, Morrow AD, Balucan J, Leitinger N, Watson AD, Tintut Y, et al. Lipid oxidation products have opposite effects on calcifying vascular cell and bone cell differentiation: a possible explanation for the paradox of arterial calcification in osteoporotic patients. Arterioscler Thromb Vasc Biol 1997;17:680-7.
48. Raggi P, Kleerekoper M. Contribution of bone and mineral abnormalities to cardiovascular disease in patients with chronic kidney disease. Clin J Am Soc Nephrol 2008;3:836-43.
49. Escobar Guzman LF, Escobar Guzman CA, Lopes NH. Pathophysiological and genetic aspects of vascular calcification. Cardiol Res Pract 2020;2020:5169069.
50. Reynolds JL, Joannides AJ, Skepper JN, McNair R, Schurgers LJ, Proudfoot D, et al. Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD. J Am Soc Nephrol 2004;15:2857-67.
51. Proudfoot D, Skepper JN, Shanahan CM, Weissberg PL. Calcification of human vascular cells in vitro is correlated with high levels of matrix Gla protein and low levels of osteopontin expression. Arterioscler Thromb Vasc Biol 1998;18:379-88.
52. Shanahan CM, Weissberg PL, Metcalfe JC. Isolation of gene markers of differentiated and proliferating vascular smooth muscle cells. Circ Res 1993;73:193-204.
53. Giachelli CM, Bae N, Almeida M, Denhardt DT, Alpers CE, Schwartz SM. Osteopontin is elevated during neointima formation in rat arteries and is a novel component of human atherosclerotic plaques. J Clin Invest 1993;92:1686-96.
54. Ikeda T, Shirasawa T, Esaki Y, Yoshiki S, Hirokawa K. Osteopontin mRNA is expressed by smooth muscle-derived foam cells in human atherosclerotic lesions of the aorta. J Clin Invest 1993;92:2814-20.
55. Shanahan CM, Cary NR, Metcalfe JC, Weissberg PL. High expression of genes for calcification-regulating proteins in human atherosclerotic plaques. J Clin Invest 1994;93:2393-402.
56. Dhore CR, Cleutjens JP, Lutgens E, Cleutjens KB, Geusens PP, Kitslaar PJ, et al. Differential expression of bone matrix regulatory proteins in human atherosclerotic plaques. Arterioscler Thromb Vasc Biol 2001;21:1998-2003.
57. Byon CH, Chen Y. Molecular mechanisms of vascular calcification in chronic kidney disease: the link between bone and the vasculature. Curr Osteoporos Rep 2015;13:206-15.
58. Xiao G, Jiang D, Ge C, Zhao Z, Lai Y, Boules H, et al. Cooperative interactions between activating transcription factor 4 and Runx2/Cbfa1 stimulate osteoblast-specific osteocalcin gene expression. J Biol Chem 2005;280:30689-96.
59. Kim KM. Apoptosis and calcification. Scanning Microsc 1995;9:1137-78.
60. Jakubowski H. Metabolism of homocysteine thiolactone in human cell cultures: possible mechanism for pathological consequences of elevated homocysteine levels. J Biol Chem 1997;272:1935-42.
61. Mercie P, Garnier O, Lascoste L, Renard M, Closse C, Durrieu F, et al. Homocysteine-thiolactone induces caspase-independent vascular endothelial cell death with apoptotic features. Apoptosis 2000;5:403-11.
62. Cheng Z, Yang X, Wang H. Hyperhomocysteinemia and endothelial dysfunction. Curr Hypertens Rev 2009;5:158-65.
63. Naseem KM. The role of nitric oxide in cardiovascular diseases. Mol Aspects Med 2005;26:33-65.
64. Leeuwenburgh C, Hardy MM, Hazen SL, Wagner P, Oh-ishi S, Steinbrecher UP, et al. Reactive nitrogen intermediates promote low density lipoprotein oxidation in human atherosclerotic intima. J Biol Chem 1997;272:1433-6.
65. Bian K, Doursout MF, Murad F. Vascular system: role of nitric oxide in cardiovascular diseases. J Clin Hypertens (Greenwich) 2008;10:304-10.
66. Yang Q, He GW. Imbalance of homocysteine and H2S: significance, mechanisms, and therapeutic promise in vascular injury. Oxid Med Cell Longev 2019;2019:7629673.
67. Pushpakumar S, Kundu S, Sen U. Endothelial dysfunction: the link between homocysteine and hydrogen sulfide. Curr Med Chem 2014;21:3662-72.
68. Kanagy NL, Szabo C, Papapetropoulos A. Vascular biology of hydrogen sulfide. Am J Physiol Cell Physiol 2017;312:C537-49.
69. Li JJ, Li Q, Du HP, Wang YL, You SJ, Wang F, et al. Homocysteine Triggers inflammatory responses in macrophages through inhibiting CSE-H2S signaling via DNA hypermethylation of CSE promoter. Int J Mol Sci 2015;16:12560-77.
70. Li MH, Tang JP, Zhang P, Li X, Wang CY, Wei HJ, et al. Disturbance of endogenous hydrogen sulfide generation and endoplasmic reticulum stress in hippocampus are involved in homocysteine-induced defect in learning and memory of rats. Behav Brain Res 2014;262:35-41.
71. Tang XQ, Shen XT, Huang YE, Chen RQ, Ren YK, Fang HR, et al. Inhibition of endogenous hydrogen sulfide generation is associated with homocysteine-induced neurotoxicity: role of ERK1/2 activation. J Mol Neurosci 2011;45:60-7.
72. Chen S, Dong Z, Zhao Y, Sai N, Wang X, Liu H, et al. Homocysteine induces mitochondrial dysfunction involving the crosstalk between oxidative stress and mitochondrial pSTAT3 in rat ischemic brain. Sci Rep 2017;7:6932.
73. Li M, Zhu Y, Jaiswal SK, Liu NF. Mitochondria homeostasis and vascular medial calcification. Calcif Tissue Int 2021;109:113-20.
74. Zhang J, Wang X, Vikash V, Ye Q, Wu D, Liu Y, et al. ROS and ROS-mediated cellular signaling. Oxid Med Cell Longev 2016;2016:4350965.
75. Fleury C, Mignotte B, Vayssiere JL. Mitochondrial reactive oxygen species in cell death signaling. Biochimie 2002;84:131-41.

76. Yu EP, Bennett MR. The role of mitochondrial DNA damage in the development of atherosclerosis. Free Radic Biol Med 2016;100:223-30.
77. Slater AF, Stefan C, Nobel I, van den Dobbelsteen DJ, Orrenius S. Signalling mechanisms and oxidative stress in apoptosis. Toxicol Lett 1995;82-83:149-53.
78. Austin RC, Sood SK, Dorward AM, Singh G, Shaughnessy SG, Pamidi S, et al. Homocysteine-dependent alterations in mitochondrial gene expression, function and structure: homocysteine and H2O2 act synergistically to enhance mitochondrial damage. J Biol Chem 1998;273:30808-17.
79. Byon CH, Javed A, Dai Q, Kappes JC, Clemens TL, Darley-Usmar VM, et al. Oxidative stress induces vascular calcification through modulation of the osteogenic transcription factor Runx2 by AKT signaling. J Biol Chem 2008;283:15319-27.
80. Kurozumi A, Nakano K, Yamagata K, Okada Y, Nakayamada S, Tanaka Y. IL-6 and sIL-6R induces STAT3-dependent differentiation of human VSMCs into osteoblast-like cells through JMJD2B-mediated histone demethylation of RUNX2. Bone 2019;124:53-61.
81. Henaut L, Sanchez-Nino MD, Aldamiz-Echevarria Castillo G, Sanz AB, Ortiz A. Targeting local vascular and systemic consequences of inflammation on vascular and cardiac valve calcification. Expert Opin Ther Targets 2016;20:89-105.
82. Wen C, Yang X, Yan Z, Zhao M, Yue X, Cheng X, et al. Nalp3 inflammasome is activated and required for vascular smooth muscle cell calcification. Int J Cardiol 2013;168:2242-7.
83. Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. Nat Rev Immunol 2013;13:709-21.
84. Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya M. Contribution of macrophage polarization to metabolic diseases. J Atheroscler Thromb 2016;23:10-7.
85. Puri R, Nicholls SJ, Shao M, Kataoka Y, Uno K, Kapadia SR, et al. Impact of statins on serial coronary calcification during atheroma progression and regression. J Am Coll Cardiol 2015;65:1273-82.
86. Raber L, Taniwaki M, Zaugg S, Kelbaek H, Roffi M, Holmvang L, et al. Effect of high-intensity statin therapy on atherosclerosis in non-infarct-related coronary arteries (IBIS-4): a serial intravascular ultrasonography study. Eur Heart J 2015;36:490-500.
87. Sun Y, Byon CH, Yuan K, Chen J, Mao X, Heath JM, et al. Smooth muscle cell-specific runx2 deficiency inhibits vascular calcification. Circ Res 2012;111:543-52.
88. Savinov AY, Salehi M, Yadav MC, Radichev I, Millan JL, Savinova OV. Transgenic overexpression of tissue-nonspecific alkaline phosphatase (TNAP) in vascular endothelium results in generalized arterial calcification. J Am Heart Assoc 2015;4:e002499.
89. Bouletreau PJ, Warren SM, Spector JA, Peled ZM, Gerrets RP, Greenwald JA, et al. Hypoxia and VEGF up-regulate BMP-2 mRNA and protein expression in microvascular endothelial cells: implications for fracture healing. Plast Reconstr Surg 2002;109:2384-97.
90. Mody N, Parhami F, Sarafian TA, Demer LL. Oxidative stress modulates osteoblastic differentiation of vascular and bone cells. Free Radic Biol Med 2001;31:509-19.
91. Henze LA, Luong TT, Boehme B, Masyout J, Schneider MP, Brachs S, et al. Impact of C-reactive protein on osteo-/chondrogenic transdifferentiation and calcification of vascular smooth muscle cells. Aging (Albany NY) 2019;11:5445-62.
92. Cola C, Almeida M, Li D, Romeo F, Mehta JL. Regulatory role of endothelium in the expression of genes affecting arterial calcification. Biochem Biophys Res Commun 2004;320:424-7.
93. Sakamoto M, Minamino T, Toko H, Kayama Y, Zou Y, Sano M, et al. Upregulation of heat shock transcription factor 1 plays a critical role in adaptive cardiac hypertrophy. Circ Res 2006;99:1411-8.
94. Dong Y, Zhang M, Wang S, Liang B, Zhao Z, Liu C, et al. Activation of AMP-activated protein kinase inhibits oxidized LDL-triggered endoplasmic reticulum stress in vivo. Diabetes 2010;59:1386-96.
95. Tabas I. The role of endoplasmic reticulum stress in the progression of atherosclerosis. Circ Res 2010;107:839-50.
96. Olas B, Kontek B. Hydrogen sulfide decreases the plasma lipid peroxidation induced by homocysteine and its thiolactone. Mol Cell Biochem 2015;404:39-43.
97. Hofmann MA, Lalla E, Lu Y, Gleason MR, Wolf BM, Tanji N, et al. Hyperhomocysteinemia enhances vascular inflammation and accelerates atherosclerosis in a murine model. J Clin Invest 2001;107:675-83.
98. Papatheodorou L, Weiss N. Vascular oxidant stress and inflammation in hyperhomocysteinemia. Antioxid Redox Signal 2007;9:1941-58.
99. Fu Y, Wang X, Kong W. Hyperhomocysteinaemia and vascular injury: advances in mechanisms and drug targets. Br J Pharmacol 2018;175:1173-89.
100. Lee SJ, Lee IK, Jeon JH. Vascular calcification-new insights into its mechanism. Int J Mol Sci 2020;21:2685.
101. Iyemere VP, Proudfoot D, Weissberg PL, Shanahan CM. Vascular smooth muscle cell phenotypic plasticity and the regulation of vascular calcification. J Intern Med 2006;260:192-210.
102. Wu JT. Circulating homocysteine is an inflammation marker and a risk factor of life-threatening inflammatory diseases. J Biomed Lab Sci 2007;19:107-11.
103. Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM, et al. Plasma homocysteine as a risk factor for vascular disease: the European Concerted Action Project. JAMA 1997;277:1775-81.
104. Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, et al. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. JAMA 1992;268:877-81.
105. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 1997;337:230-6.
106. Ubbink JB, Vermaak WJ, van der Merwe A, Becker PJ, Delport R, Potgieter HC. Vitamin requirements for the treatment of hyperhomocysteinemia in humans. J Nutr 1994;124:1927-33.
107. Landgren F, Israelsson B, Lindgren A, Hultberg B, Andersson A, Brattstrom L. Plasma homocysteine in acute myocardial infarction: homocysteine-lowering effect of folic acid. J Intern Med 1995;237:381-8.
108. Homocysteine Lowering Trialists' Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Homocysteine Lowering Trialists' Collaboration. BMJ 1998;316:894-8.
109. Malinow MR, Duell PB, Hess DL, Anderson PH, Kruger WD, Phillipson BE, et al. Reduction of plasma homocyst(e)ine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease.

N Engl J Med 1998;338:1009-15.
110. Quinlivan EP, McPartlin J, McNulty H, Ward M, Strain JJ, Weir DG, et al. Importance of both folic acid and vitamin B12 in reduction of risk of vascular disease. Lancet 2002;359:227-8.
111. Lee BJ, Huang MC, Chung LJ, Cheng CH, Lin KL, Su KH, et al. Folic acid and vitamin B12 are more effective than vitamin B6 in lowering fasting plasma homocysteine concentration in patients with coronary artery disease. Eur J Clin Nutr 2004;58:481-7.
112. Homocysteine Lowering Trialists' Collaboration. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. Am J Clin Nutr 2005;82:806-12.
113. Hodis HN, Mack WJ, Dustin L, Mahrer PR, Azen SP, Detrano R, et al. High-dose B vitamin supplementation and progression of subclinical atherosclerosis: a randomized controlled trial. Stroke 2009;40:730-6.
114. Kim BJ, Kim BS, Kang JH. Plasma homocysteine and coronary artery calcification in Korean men. Eur J Prev Cardiol 2015;22:478-85.
115. Joo NS, Jung S, Kim YN, Choi BH. Cut-off value of serum homocysteine in relation to increase of coronary artery calcification. J Investig Med 2021;69:345-50.
116. Held C, Sumner G, Sheridan P, McQueen M, Smith S, Dagenais G, et al. Correlations between plasma homocysteine and folate concentrations and carotid atherosclerosis in high-risk individuals: baseline data from the Homocysteine and Atherosclerosis Reduction Trial (HART). Vasc Med 2008;13:245-53.
117. Jacques PF, Kalmbach R, Bagley PJ, Russo GT, Rogers G, Wilson PW, et al. The relationship between riboflavin and plasma total homocysteine in the Framingham Offspring cohort is influenced by folate status and the C677T transition in the methylenetetrahydrofolate reductase gene. J Nutr 2002;132:283-8.
118. Heese P, Linnebank M, Semmler A, Muschler MA, Heberlein A, Frieling H, et al. Alterations of homocysteine serum levels during alcohol withdrawal are influenced by folate and riboflavin: results from the German Investigation on Neurobiology in Alcoholism (GINA). Alcohol Alcohol 2012;47:497-500.
119. Wilcken DE, Wilcken B. B vitamins and homocysteine in cardiovascular disease and aging. Ann N Y Acad Sci 1998;854:361-70.
120. Olthof MR, van Vliet T, Boelsma E, Verhoef P. Low dose betaine supplementation leads to immediate and long term lowering of plasma homocysteine in healthy men and women. J Nutr 2003;133:4135-8.
121. Steenge GR, Verhoef P, Katan MB. Betaine supplementation lowers plasma homocysteine in healthy men and women. J Nutr 2003;133:1291-5.
122. Bidulescu A, Chambless LE, Siega-Riz AM, Zeisel SH, Heiss G. Usual choline and betaine dietary intake and incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. BMC Cardiovasc Disord 2007;7:20.
123. Dalmeijer GW, Olthof MR, Verhoef P, Bots ML, van der Schouw YT. Prospective study on dietary intakes of folate, betaine, and choline and cardiovascular disease risk in women. Eur J Clin Nutr 2008;62:386-94.
124. Lever M, George PM, Elmslie JL, Atkinson W, Slow S, Molyneux SL, et al. Betaine and secondary events in an acute coronary syndrome cohort. PLoS One 2012;7:e37883.
125. Amer M, Qayyum R. The relationship between 25-hydroxyvitamin D and homocysteine in asymptomatic adults. J Clin Endocrinol Metab 2014;99:633-8.
126. Al-Bayyari N, Al-Zeidaneen S, Hailat R, Hamadneh J. Vitamin D3 prevents cardiovascular diseases by lowering serum total homocysteine concentrations in overweight reproductive women: a randomized, placebo-controlled clinical trial. Nutr Res 2018;59:65-71.
127. Young KA, Snell-Bergeon JK, Naik RG, Hokanson JE, Tarullo D, Gottlieb PA, et al. Vitamin D deficiency and coronary artery calcification in subjects with type 1 diabetes. Diabetes Care 2011;34:454-8.
128. Thiele I, Linseisen J, Meisinger C, Schwab S, Huth C, Peters A, et al. Associations between calcium and vitamin D supplement use as well as their serum concentrations and subclinical cardiovascular disease phenotypes. Atherosclerosis 2015;241:743-51.
129. Mao PJ, Zhang C, Tang L, Xian YQ, Li YS, Wang WD, et al. Effect of calcium or vitamin D supplementation on vascular outcomes: a meta-analysis of randomized controlled trials. Int J Cardiol 2013;169:106-11.
130. Kheiri B, Abdalla A, Osman M, Ahmed S, Hassan M, Bachuwa G. Vitamin D deficiency and risk of cardiovascular diseases: a narrative review. Clin Hypertens 2018;24:9.
131. Barbarawi M, Kheiri B, Zayed Y, Barbarawi O, Dhillon H, Swaid B, et al. Vitamin D supplementation and cardiovascular disease risks in more than 83 000 individuals in 21 randomized clinical trials: a meta-analysis. JAMA Cardiol 2019;4:765-76.
132. Park KY, Chung PW, Kim YB, Moon HS, Suh BC, Won YS, et al. Serum vitamin D status as a predictor of prognosis in patients with acute ischemic stroke. Cerebrovasc Dis 2015;40:73-80.
133. Kriebitzsch C, Verlinden L, Eelen G, van Schoor NM, Swart K, Lips P, et al. 1,25-dihydroxyvitamin D3 influences cellular homocysteine levels in murine preosteoblastic MC3T3-E1 cells by direct regulation of cystathionine β-synthase. J Bone Miner Res 2011;26:2991-3000.
134. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature 1997;386:78-81.
135. Booth SL, Al Rajabi A. Determinants of vitamin K status in humans. Vitam Horm 2008;78:1-22.
136. Berkner KL, Runge KW. The physiology of vitamin K nutriture and vitamin K-dependent protein function in atherosclerosis. J Thromb Haemost 2004;2:2118-32.
137. Vermeer C. Gamma-carboxyglutamate-containing proteins and the vitamin K-dependent carboxylase. Biochem J 1990;266:625-36.
138. Lanham-New SA. Importance of calcium, vitamin D and vitamin K for osteoporosis prevention and treatment. Proc Nutr Soc 2008;67:163-76.
139. van Ballegooijen AJ, Beulens JW. The role of vitamin K status in cardiovascular health: evidence from observational and clinical studies. Curr Nutr Rep 2017;6:197-205.
140. Geleijnse JM, Vermeer C, Grobbee DE, Schurgers LJ, Knapen MH, van der Meer IM, et al. Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. J Nutr 2004;134:3100-5.
141. Shea MK, O'Donnell CJ, Hoffmann U, Dallal GE, Dawson-Hughes B, Ordovas JM, et al. Vitamin K supplementation and progression of coronary artery calcium in older men and women. Am J Clin Nutr 2009;89:1799-807.
142. Beulens JW, Bots ML, Atsma F, Bartelink ML, Prokop M, Geleijnse

JM, et al. High dietary menaquinone intake is associated with reduced coronary calcification. Atherosclerosis 2009;203:489-93.
143. Gast GC, de Roos NM, Sluijs I, Bots ML, Beulens JW, Geleijnse JM, et al. A high menaquinone intake reduces the incidence of coronary heart disease. Nutr Metab Cardiovasc Dis 2009;19:504-10.
144. Vossen LM, Schurgers LJ, van Varik BJ, Kietselaer BL, Vermeer C, Meeder JG, et al. Menaquinone-7 supplementation to reduce vascular calcification in patients with coronary artery disease: rationale and study protocol (VitaK-CAC Trial). Nutrients 2015;7:8905-15.
145. Schurgers LJ, Joosen IA, Laufer EM, Chatrou ML, Herfs M, Winkens MH, et al. Vitamin K-antagonists accelerate atherosclerotic calcification and induce a vulnerable plaque phenotype. PLoS One 2012;7:e43229.
146. Westenfeld R, Krueger T, Schlieper G, Cranenburg EC, Magdeleyns EJ, Heidenreich S, et al. Effect of vitamin K2 supplementation on functional vitamin K deficiency in hemodialysis patients: a randomized trial. Am J Kidney Dis 2012;59:186-95.
147. Bjorklund G, Svanberg E, Dadar M, Card DJ, Chirumbolo S, Harrington DJ, et al. The role of matrix Gla protein (MGP) in vascular calcification. Curr Med Chem 2020;27:1647-60.
148. Schurgers LJ. Vitamin K: key vitamin in controlling vascular calcification in chronic kidney disease. Kidney Int 2013;83:782-4.

https://doi.org/10.4082/kjfm.21.0227