

B. CORONARY ARTERY DISEASE (CAD) IN MEN

1. Pathophysiology of coronary artery disease and vascular calcification

a. Pathophysiology of coronary artery disease

Despite extensive intervention efforts to change lifestyle and use of new treatments to lower plasma cholesterol and reduce cardiovascular risk factors, cardiovascular disease is still major cause of death in the United States in both men and women (Breslow et al., 1997; Brauwald et al., 1997). The following review for the cellular and molecular mechanisms in the development of atherosclerotic lesions was mostly summarized from renowned papers of Scientific Statements (American Heart Association's Committee on Vascular Lesions, Stary et al., 1994; Stary et al., 1995; Stary, 2000), and Russell (1999, 1995)

1) Vascular biology

i) Endothelium of arterial wall

Atherosclerosis starts in the endothelium due to the damage occurred innermost layer of the artery. Endothelial cells normally form a continuous flattened layer, oriented in the direction of arterial blood flow. The four major properties of the endothelium are: 1) permeability, 2) thromboresistance, 3) mediation of vascular tone, and 4) inflammatory and immune response.

On the luminal surface, endothelial cells are coated with free polysaccharides and glycosaminoglycans, plus glycoprotein and glycolipid side chains from the plasma membrane. Receptors for LDL-c, lipoprotein lipase, insulin, and histamine, various organelles, and filaments are present. The endothelium is permeable to all plasma

proteins, and its permeability rates differ by arterial location, age, blood pressure, vascular tone, and protein concentration, size, and charge. Therefore, final protein concentrations in the intima are dependent on trapping, degradation, and efflux by endothelium.

Normal endothelium resists platelet adherence. Thrombomodulin in the membrane binds thrombin, leading protein-C to complex with protein S, with inactivation of coagulation factor Va. Endothelial cells also metabolize the platelet aggregating factors adenosine diphosphate, serotonin, angiotensin and prostaglandin F₁, and synthesize and secrete plasminogen activator and prostacyclin. Prostacycline, which is secreted by both endothelial and smooth muscle cells, prevents platelet aggregation and stimulates cyclic adenosine monophosphate (cAMP) production, resulting in vasorelaxation. Endothelium-derived relaxing factor (EDRF), secreted by endothelial cells, stimulates the relaxation through a process involving nitric oxide and smooth muscle cell induced phosphorylation of proteins. Normal endothelium does not support adherence of large numbers of leukocytes. However, cytokines such as interleukin-1, tumor necrosis factor α , or oxidized LDL-c stimulate the expression of leukocyte adhesion molecules. Minimally modified LDL-c is taken up by LDL receptors; highly oxidized LDL-c is taken up by scavenger receptors. Endothelial cells can express leukocyte chemotactic proteins, growth factors, hematopoietic factors and major histocompatibility antigens. Platelet derived growth factor stimulates the migration, growth and division of smooth muscle cells in media and fibroblasts.

ii) Three layers of arterial wall

The normal arterial wall is composed of three histologic layers: intima, media, and adventitia. The inner layer of intima, closest to lumen, is the proteoglycan layer containing large amount of nonfibrous connective tissue, such as proteoglycan ground substance, elastic fibers, few macrophages, and scattered smooth muscle cells. The musculoelastic intimal layer, adjacent to the media, contains abundant smooth muscle cells and elastic fibers. Extracellular matrix is the main component of the normal intima,

especially in areas of thickening. The matrix contains products from the relatively unchanging number of endothelial cells and the variable number of smooth muscle cells. Large extracellular proteoglycans function in ion exchange and transport. Small extracellular proteoglycans may regulate collagen fibrillogenesis and bind to LDL-c. Fibronectin is a glycoprotein involved in cell-to-cell and cell-to-substrate binding. Laminin is a major glycoprotein in the basement membrane. Collagen serves to bind endothelial cells to the subendothelial matrix. With aging, the ratio of collagen type changes, and elastin content decreases in relation to collagen

Media is mainly composed of smooth muscle cells. Extensive golgi apparatus, endoplasmic reticulum, collagen, elastin, and proteoglycans are present. The function of smooth muscle cells in media is to maintain vascular tone, and to regulate local blood flow dependent on metabolic requirement. Smooth muscle cells support the contractility and structural integrity of the artery. Contractility is modulated through the content of proteins and smooth muscle cell response to epinephrine and angiotension II (vasoconstriction) and prostacyclin and endothelium-derived relaxing factor (vasodilation). Structural roles involve smooth muscle cell migration, proliferation, and growth factor and connective tissue secretion. Smooth muscle cells secrete several types of collagen, elastic fiber proteins, and several proteoglycans. Smooth muscle cells may contribute to intimal thickening by migration or proliferation. Although the contractile type of smooth muscle cell is relatively resistant to platelet-derived growth factors in large amounts, platelet-derived growth factors induce the change from contractile to synthetic type of smooth muscle cell, and increase expression of PDGF receptors. Smooth muscle cells also play a role in cholesterol synthesis through phospholipid metabolism, leading to prostaglandin synthesis. Lipoproteins remove by phagocytosis, expression of LDL receptors, or both. LDL-c and VLDL are taken up preferentially.

Macrophages are derived from circulating monocytes. They are present in normal human coronary arteries from the first week of life, and the number increases with age. The function of macrophages depends upon their environment. They may

participate in remodeling of the intima, inflammatory or immune response, and scavenging. Macrophage inflammatory response involves phagocytosis, binding and presentation of antigens, antibody-dependent cell lysis, activation of T, B and natural killer cells, and cytokine and growth factor production. Scavenger functions include lipid metabolism through secretion of apolipoprotein E and lipoprotein lipase, and removal of dead cells, immune complexes, mural thrombi, and plasma proteins. The loose connective tissue matrix, the adventitia, are composed of collagen, and proteoglycans. It contains fibroblasts and smooth muscle cells

2) Atherogenesis

Atherosclerosis is the initial component (stage) of CHD and stroke. It is not only a disease by itself, but a process connecting to the pathogenesis of myocardial infarction, stroke, gangrene, peripheral arterial disease of the lower extremities, and abdominal aortic aneurysm. Atherogenesis may begin in childhood, and adolescence, and continues with cumulative effect with lifetime risk. Autopsy studies of young adults who had died of mainly accident and trauma reveal that as the number of CAD risk factors increases, the degree of asymptomatic atherosclerosis also increases (McGill et al., 2000).

Atherosclerosis is regarded as chronic inflammatory condition of the vascular wall that is converted to an acute clinical event by the induction of plaque rupture, which in turn leads to thrombosis (Berliner et al. 1995). Several etiologic factors such as hypercholesterolemia, modified lipid, homocysteine, infection and hypertension have related to inflammatory characteristics of atherosclerosis (Ross, 1999). The lesions of atherosclerosis have been categorized into three general groups: 1) the fatty streak, 2) the fibrous plaque, 3) advanced lesions with complications (Ross, 1995). The features of vulnerable plaques leading to disruption or episode of thrombosis are: 1) a large lipid core occupying at least 50% of the overall plaque volume, 2) a high density of

macrophages, 3) a low density of smooth muscle cells in the cap, 4) a high tissue factor content, and 5) a thin plaque cap with disorganized collagen structure (Davies, 1996).

Atherosclerosis is characterized by the focal accumulation in the intima of complex lipids, proteins, carbohydrates, smooth muscle cells, macrophages, lymphocytes, blood and blood constituents. The thickness of the intima varies in response to hemodynamic forces. Diffuse intimal thickening is present in arterial section. Eccentric intimal thickening is present at regions of altered mechanical stress. For instance, the intima is thin on the side of a bifurcation and outer wall of a curvature, but is thick on the wall opposite a branch and inner wall of a curvature. Intimal thickening of arteries has been related to the migration of medial smooth muscle cells into the intima (Sims FH, 2000). Recent studies suggest that the initiation of intimal thickening is a repair process for loss of internal elastic lamina leading to blood leakage into the arterial wall, and medial smooth muscle cells proliferation.

The anatomical distribution of eccentric intimal thickening and advanced atherosclerotic lesions is similar. Severely hyper-cholesterolemic patients develop atherosclerosis in all regions of the intimal wall; the thickened areas develop atherosclerotic lesions earlier and more rapidly. Intimal thickening of the coronary arteries increases with age. It has been suggested that ethnic and sex differences in CHD mortality could be related to early differences in the coronary artery intima.

i) Lesion initiation and stages

Atherosclerotic lesion first appears in the locations of adaptive intimal thickening. The Committee on Vascular Lesions of the Council of arteriosclerosis, AHA, classified six types (I-VI) of atherosclerotic plaque incorporating the descriptive stages, such as fatty streak, or fibrous plaque (Stary et al., 1994, 1995). Type I to III is categorized as early lesions due to their appearance in earlier stage of atherogenesis, and to the ability for development of advanced lesions. "Advanced" lesions (type IV-VI) may or may not

narrow the lumen detected on angiography with related symptoms. They are defined histologically by accumulation of lipids, cells, and matrix materials with structural disorganization, repair, intimal thickening, and deformity of the arterial wall.

Infiltration of monocyte and macrophages and T-cells into the intima of the artery is an early hallmark of atherogenesis (Ross 1993). The fatty streak is the earliest visible lesion and is most likely the precursor to the latter events that lead to clinically significant disease. The fatty streak consists mainly of an intimal deposit of lipid-filled, monocyte-derived macrophages with a various number of T-lymphocytes, and smooth muscle cell. The accumulation of lipid-filled macrophages, or foam cells, forms the bulk of lesions. These macrophages give the lesions a yellow discoloration. Normally, the fatty streaks are distributed randomly throughout the arterial tree, but early lesions are commonly found at branches, bifurcations, and curves in the system, which are sites of low shear stress where changes in blood flow such as decrease of flow, back currents, or eddy currents occur. A hallmark of the conversion of fatty streaks to more advanced lesions is the formation of a fibrous cap. This process involves an increase in the number of smooth muscle cells within intima and probably required the increase of migration and proliferation, as well as stimulation of connective tissue synthesis.

Lipid plays major role of induction of atherosclerotic lesions. Two different types of oxidized LDL interact with arterial endothelial cell, and smooth muscle cell during the development of lesions. The first is minimally oxidized LDL (MM-LDL) which is with little change in composition of apolipoprotein B. Minimally modified (oxidized) LDL (MM-LDL) into cell cultures produce the monocyte colony stimulating factor (M-CSF), and monocyte chemoattractant protein 1 (MCP-1), which is a potent inducer for osteoclastic differentiation. MM-LDL also induces the expression of genes related to inflammation. The some of the characteristics of modified LDL are following; 1) regulate the expression of genes for macrophage colony stimulating factor (M-CSF) and monocyte chemotactic protein, 2) injure the endothelium, 3) form cholesteryl ester rich environment by the uptake of macrophages, 4) induce the expression of inflammatory cytokines, e.g. interleukin-1, or interleukin-6. The second type of LDL-c is found while

monocytes are recruited and change into macrophages. At this stage, LDL lipids are highly oxidized and lose the affinity to LDL receptor. Oxidized LDL-c is captured by macrophages, primarily through the scavenger receptor pathway.

ii) Type I lesions

Type I lesions are the first steps of detectable lipid deposits and endothelial cell reaction. Histologically, small, but isolated groups of macrophages and lipid core can be found at this stage. Foam cell in the intima contains small, isolated macrophages filled with lipid droplets. These foam cells are cellular markers of pathological accumulations of lipoproteins. In the coronary arteries, type I lesions preferentially appear in areas of adaptive intimal thickening, and in which type II lesions later are found. Lipoprotein transport are dependent on 1) concentration dependent process, 2) no requirement of receptor-mediated endocytosis, 3) trapped in a three-dimensional space of fibers and fibrils by cells, 4) association of LDL with extracellular matrix mainly by apoB (major protein of LDL) high in the artery wall than in the plasma.

iii) Type II lesions (Fatty streaks)

The earliest visible lesion in course of atherogenesis, the fatty streaks (yellowish streak) are composed of intimal collection of lipid-filled, monocyte-derived macrophages with a number of smooth muscle cells and T-lymphocytes. Normally, the fatty streaks are distributed randomly throughout the arterial tree, but early lesions are commonly found at branches, bifurcations, and curves in the system, which are sites of low shear stress where changes in blood flow such as decrease of flow, back currents, or eddy currents occur.

Type II lesions can be detected even in children at the similar prevalence until they reaches 20 years. Among children of age 2 to 15, 99% of fatty streaks are present

in the aorta. This suggests that atherosclerosis risk factors accelerate fatty streak progression rather than promote fatty streak formation. Alternatively, some type II atherosclerotic plaques may arise independently of type I lesions (McGill et al., 2000; Stary 2000). In the coronary arteries, type II lesions generally first appear at puberty. Plasma cholesterol and LDL-c levels are correlated with the presence and extent of type II lesions in laboratory animals and humans. Puberty is associated with increases in VLDL levels, and especially in boys, decreases in total cholesterol, LDL-c and HDL-c under big changes of hormonal milieu. Most lipid droplets in type II lesions are intracellular within macrophage foam cells. A less extent of lipid accumulates within an intimal smooth muscle cells. It consists of cholesterol esters (77%), cholesterol, and phospholipids. In fatty streaks, lipids exist either as liquid or liquid crystalline. Crystalline and liquid crystalline forms of lipid have lower rates of turnover and may limit enzyme accessibility. The development of the fatty streak occur through the following steps 1) lipoprotein transport, 2) lipoprotein retention, 3) lipoprotein modification, 4) monocyte adherence, 5) monocyte migration (chemotaxis), 6) monocyte differentiation, 7) lipoprotein uptake, and 8) foam cell formation.

Macrophage foam cells are primarily organized in layers, rather than isolated. Sometimes, dead or decomposed macrophage foam cells are detected. At this stage, dead or altered foam cells are not involved with the emergence of calcium (Stary, 2000). Macrophage foam cells are probably precursors of fatty streaks, and they accumulate at the bottom of the proteoglycan layer. Smooth muscle cells of the intima may also contain lipid droplets. Some T lymphocytes and mast cells may be present. In experimentally produced fatty streaks, the turnover of macrophage foam cells, endothelial cells, and smooth muscle cells is increased.

Type II lesions that are prone to progress to type III (type IIa), are found in areas of adaptive intimal thickening. Progression is largely determined by mechanical forces. Low shear stress increases the time of interaction of blood-carrying particles such as LDL-c with the arterial wall, increasing the diffusion rate across the endothelium. The majority of type II lesions (Type IIb) occur in areas where the arteries are thin, and

progress either slowly or not at all. However, in hypercholesterolemic persons, type II lesions rapidly progress to advanced lesions even outside the progression-prone regions. Progression-prone type IIa lesions differ from progression-resistant IIb lesions by having smooth muscle cells, greater accumulation of lipoproteins and macrophages, and deeper intimal location of foam cells and extracellular lipids.

The fatty streak progresses to form fatty plaque, fibrous plaque, and complex plaque composed of calcification. More complex lesion showed hemorrhage, thrombosis. Platelet adherence to damaged endothelial cells leads to local thrombosis (blood clot formation), with occlusion of the artery and the signs and symptoms of impaired or obstructed blood flow.

iv) Type III lesions

The type III lesion is known as an intermediate lesion, or the transitional lesion, which is morphological and a chemical bridge between type II lesion and atheroma (type IV lesion). Histologically type III lesions have extracellular lipid droplets and component particles of dead cells, which lie below the layers of macrophage and macrophage foam cells replacing intercellular proteoglycans and fiber. The lipid pools show different characteristics compared to type II lesions. Rare type III lesions contain a few calcium foci, which are same as calcium granules found in type IV lesions.

v) Endothelial and smooth muscle cell changes in type I, II and III lesions

Endothelial cells in precursor (type I, II, or III) lesions lose their orientation with respect to blood flow. They become rounded and multinucleated, with increase stromas, stress fibers and cilia, and decrease microfilament bundles. Endothelial cell proliferation increases in atherosclerosis, as does cell death. Permeability, leukocyte adherence, tissue factor expression (relating to the anti-thrombotic capacity of the cells), and endothelium-dependent vasoconstriction increase, while endothelium-dependent

vasodilation and prostacycline release decrease. Several cytokines or adhesion molecules, including monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor- α (TNF- α), vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1) and macrophage-colony-stimulating factor (M-CSF), recruit monocytes and other inflammatory cells into the vascular (arterial) sub-endothelium.

Smooth muscle cells with rough endoplasmic reticulum-rich smooth muscle cells are more numerous in precursor lesions than in normal intima. Smooth muscle cell scavenger receptor is expressed. In both animals and humans, type I and type II collagen expression, and lipoprotein lipase are increase, as are certain cytokines, including tumor necrosis factor. Macrophage changes in type I-III lesions are related to lipid metabolism and inflammatory response. There are increases in macrophage scavenger receptor expression, free and esterified cholesterol, hydrolase, 15-lipoxygenase, lipoprotein lipase, and in lipoprotein oxidation products. Macrophage is proliferating in type I-III lesions, expressing growth and chemotactic factors, CD antigens, tumor-necrosis factors, and tissue factor.

vi) Type IV lesion (Atheromas)

Type IV lesion appears in the late adolescence, and contained calcium granules within smooth muscle cells. Specific characteristic of Type IV lesions is dense extracellular lipid, known as the lipid core. Lipid core is thought to be originated from the dead foam cells and its' accumulations. The lipid core formation, which cause severe intimal disorganization, accompany with an increase in fibrous tissue. The intima above the lipid core is nearly normal. It contains macrophages and smooth muscle cells with and without lipid droplet inclusion, plus lymphocytes and mast cells. These are frequently concentrated at eh edges of the lesion, making them vulnerable to rupture. Absence of collagen and smooth muscle cells may cause the lesion to fissuring.

Calcium may be found in lipid core regions, and smooth muscle cells. Calcium particles were found in the cytoplasm of smooth muscle cells, which progressed to dead cells. Apoptotic cells, and matrix vesicles generated from smooth muscle cells may serve as 'nidi' for calcification. The possible etiologic mechanisms will be discussed in later section.

vii) Type V lesions

Formation of new fibrous connective tissue defines the type V lesion. The collagen proliferation is an attempt to repair the intima. Smooth muscle cell derived foam cells usually predominant over macrophage foam cells. Raised white plaque narrow the arterial lumen, and lesions develop fissures, hematoma, and / or thrombi. These lesions may be silent or cause over symptoms, depending on the degree of obstruction. Type Va (fibroatheroma, type VI) contains a lipid core. Additional cores may be created in new planes via luminal changes, which modify hemodynamic forces, or by repeated disruptions and repairs. Each core is separated by layers of connective tissue. In type Vb (calcific lesion, or type VII lesion), the core is calcified. In type Vc (fibrotic, or type VIII lesion), lipid is minimal and the core is absent. These could result from one or more processes including organization of thrombi, extension of fibroatheromas, resorption of lipid cores, increased wall shear stress, and effects of smoking. Smooth muscle cell in the media adjacent to intima may be decreased or disarranged. Media and adventitia may contain large numbers of lymphocytes, macrophages, and macrophage foam cells.

viii) Type VI, VII and VIII lesions

Additional features such as surface defect (surface disruption), hematoma (hemorrhage) and thrombosis are added to Type VI lesions. Surface defect or surface disruptions vary from loss of part of the endothelium to exposure of a lipid core. Type VII

lesions are predominantly calcified lesions, and Type VIII lesions are mainly consisting of reparative fibrous connective tissues. Calcium granules increase in size by encrustation, and fusion to adjacent calcium granules. Regardless of size, small and large calcium deposits share same chemical composition; 71% calcium apatite, 9% calcium carbonate, and 15% protein (Schmid et al., 1980).

The stability of atherosclerotic plaque depends on the structural integrity of the fibrous cap. Major components of fibrous cap are extracellular matrix protein (collagen) (Libby et al., 1996). The balance between proteolytic enzymes and its inhibitor maintain the stability of plaque (Fabunmi et al., 1998). Plaque rupture risk may increase by unbalanced release of toxins and proteolytic enzymes (e.g. macrophages metalloproteinase, MMP) from macrophages with lesions, coronary spasm, structural weakness of lesion, and shear stress. Subsequently, plaque rupture exposes the plaque lipid core, which contains thrombogenic tissue factor into lumen where the coagulation factors and platelets are circulating (Libby et al., 1996). The flow pattern associated with plaque formation is most evident during systole. The hemodynamic environment responsible for atherogenesis is one of low shear stress and reversing blood flow, providing increase residence time for blood components, and facilitating time-dependent interaction with endothelial cells. In thickened regions of the intima, cell turnover is increase and higher concentrations of low density lipoproteins (LDLs) may be found. Focal intimal inclusion of LDL-c, small dense LDL-c (LDL-B), and Lp (a) is enhanced. Within the intima, many plasma lipoproteins are immobilized through binding to proteoglycans.

b. Pathophysiology of Vascular Calcification

1) Overview

Cellular calcification is divided into two major types: physiologic calcification such as bone tissue, and pathological calcification (dystrophic calcification) such as vascular

calcification. Dystrophic calcification and physiologic calcification (bone) are similar in terms of tissue mineralization. However, the dystrophic calcification is abnormal calcification of soft tissues even in the presence of normal serum calcium levels (Anderson et al., 1983). Both the physiologic or dystrophic (ectopic) calcification involves in various factor and cellular conditions: 1) calcium and phosphate homeostasis, 2) the systemic and local factors, 3) templates for heterogeneous calcification, 4) activators and inhibitors of calcification (Jahnen-Dechent et al., 2001).

Vascular calcification occurs in two different sites and follows distinct processes (Shanahan et al., 1998). Differences between medial and intimal calcification was summarized (Table 2). Medial calcification occurs independent of inflammatory cells, and lipids. It forms continuous linear deposits, and found in diabetics, or in the end stage of renal disease, and in healthy elderly patients (Monckeberg's sclerosis). However, medial calcification is also related to increased risk of cardiovascular mortality in diabetic with neuropathy (Lehto et al, 1996).

The other form of calcification is intimal calcification that develops at the atherosclerotic plaque as early as the fatty streak stage (Stary et al., 1999). Intimal calcification, similar to bone mineralization, forms small scattered (focal) deposits of calcium hydroxyapatite inside matrix vesicles. Inflammatory cells, lipid, and vascular smooth muscle cells are involved. Intimal calcification is related to myocardial infarction, impaired vascular tone, dissection in angioplasty, and poor surgical outcome caused by the loss of aortic recoil (Demer et al., 1995; Farb et al., 1996). Calcification may also contribute to occurrence of plaque rupture and thrombosis leading to sudden death (Farb et al., 1996).

The etiology of vascular calcification is increasingly recognized as an active process. Several factors are likely playing a role in the calcification of the vessel wall, including 1) calcification of thrombus, 2) calcification of degenerated smooth muscle cells and macrophages, 3) local synthesis of mineralization-related proteins (e.g. matrix Gla Protein, and osteopontin), 4) local bone forming mechanism (hydroxyapatite crystal) (Table 3).

Table 2. Characteristics of intimal versus medial wall calcification.

	Intimal	Medial
Morphology	Focal, scattered deposits	Continuous linear plaque
Histological association	Associated with mast cells and macrophages, vascular smooth muscle cell, lipid	Associated with smooth muscle cells and elastin
	Occurs in lipid-rich milieu, associated with atheromatous plaque	Occurs in absence of atheroma formation
Disease	Atherosclerosis	Monckeberg's sclerosis Diabetes Mellitus Transgenic mouse models.

Adapted from Christian and Fitzpatrick. 1999. *Curr Opin Nephrol Hypertens* 8:443-448 / Farzaneh et al. 2001. *Heart* 85:13-17

Epple and Lanzer (2001) summarized basic four mechanisms related to arterial wall calcification. The first mechanism is the loss of inhibitory action of related proteins such as matrix Gla protein, and it leads to the precipitation of calcium phosphate. Second possible mechanism is that precipitation of calcium phosphate is induced by apoptotic cells such as dead foam cell. Thirdly, cholesterol crystallization will be enhanced auto-catalytically by the formation of antibodies against cholesterol crystals. The fourth mechanism is that cellular and molecular components of bone such as osteoblast-like cells, and non-structural matrix proteins will leads to the formation and remodeling of bone-like structures in the wall of arteries. The arterial calcification likely includes one, or it will include well coordinated several mechanisms.

2) Sequel of vascular calcification

Calcium deposition in atherosclerotic lesions begins immediately with the formation of lipid core (extracellur lipids at Type III lesion) (Wexler et al., 1996).

Calcification in atherosclerosis begins as early as the second decade, after fatty streak formation. Small quantities of calcium crystals are found in lipid cores. They are absent from normal (non obstructed) arterial vessels. However, the etiology of calcification has not been clearly understood. In this section, we will review the possible, but prominent mechanisms leading to vascular calcification from numerous literatures. Many of in Vitro researches on mechanisms were yielded from the studies on isolated osteoblast-like calcifying vascular cells (CVC) (Bostrom et al.1993; Watson et al., 1994), or matrix vesicle/ apoptic bodies (Abs) from vascular smooth muscle cells (Farzaneh-Far et al., 2001; Proudfoot et al., 1998; Christian and Fitzpatrick, 1999).

i) Loss of Inhibitory action – Double defense system

Evidences suggest that matrix Gla protein and osteopontin play important inhibitory role in the prevention of arterial calcification (Luo et al., 1997). An interesting hypothesis regarding MGP and osteopontin by Giachelli and others (Jono et al., 2000) is loss of inhibitory action of these two proteins. In normal tissue, the balance between inhibition and induction of calcification is well controlled because surveillance molecules (e.g. MGP) constitutively express to inhibit abnormal (ectopic) calcification. However, injured or diseased tissues are switched to favor to induce the ectopic calcification. In damaged/injured cell, damage control protein such as osteopontin induced by injury, or inflammation try to reduce the stimulation of calcification in harmony of surveillance molecule.

There are a few evidences that carboxylation of MGP and post-translational phosphorylation of osteopontin may be key regulatory mechanisms (Jono et al., 2000b). Osteopontin lacking phosphorylation was not effective to bind to growing face of apatite as well as to inhibit calcification. MGP binds avidly to bone-morphogenic protein-2 (BMP-2), a ligand also known to be present in calcifying plaques and recognized to induce Cbfa-1 expression and initiate osteoblast differentiation in mesenchymal cells. BMP-2 complexed with MGP is unable to induce osteoblast differentiation.

ii) Initiation of calcification: Calcium phosphate, lipid, and apoptosis

Initiation of calcification is still a poorly understood process. Smooth muscle cells containing calcium particles are mainly isolated ones among extracellular particles. Those cells are presumably damaged or dead and it progress to apoptotic bodies (Stary, 2000). Most smooth muscle cell, and macrophage foam cells remnants with lipid can calcify based on environment (Anderson, 1995). This includes matrix-vesicles, cell membranes, and membrane-droplet complexes (e.g. lysosome complex) (Anderson, 1995). Calcium and apatite binding properties may serve a regulation of apatite crystal nucleation and growth. MGP are expressed constitutively by normal medial VSMCS, where they serve to prevent apatite accumulation in the event of physiological or pathological cell death. The accumulation of oxidized lipids induces differentiation and mineralization of vascular cells. A dose-dependent increase in alkaline phosphatase activity was observed due to the minimally oxidized LDL in vascular cell culture.

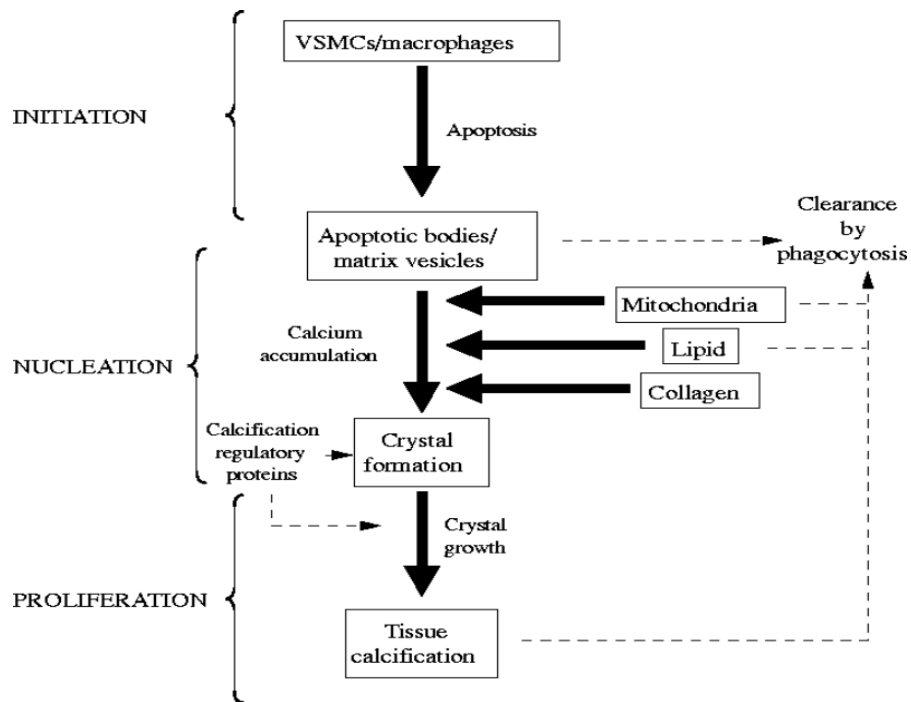


Figure 2. A model for the initiation of calcification by apoptosis, and potential regulatory influences. (Farzaneh Far: Heart, Volume 85(1).January 1, 2001.13-17)

Minimally modified oxidized lipid (MM-LDL) activates the adenylate cyclase pathway, and MM-LDL increases in cAMP levels in calcifying vascular cells (Parhami et al., 2001)

Calcium phosphate (hydroxyapatite) with fibrous tissue (collagen) and lipid deposits, is dominant crystalline forms in calcium deposits of coronary arteries (Proudfoot et al., 1998). Fitzpatrick and colleagues (1994) stained coronary artery obtained at autopsy, and found mural mineralization diffused in all atherosclerotic plaques. Hydroxyapatite was not found in normal coronary artery section by traditional light microscopy. During vascular intimal calcification, lipids may play a role in nucleating of calcium hydroxyapatite. Similarly to bone metabolism, Gla (gamma carboxyglutamate) containing proteins, having high affinity to hydroxyapatite, are important in the binding calcium to vascular wall cells. In the same manner of calcium metabolism in bone, non-hepatic bone Gla protein (osteocalcin), and matrix Gla protein (MGP) may precipitate the calcium deposits (Doherty et al., 1994). Studies identified other related proteins in atherosclerotic plaques: mRNA for a cell attachment protein (osteopontin), a protein associated with calcification (osteonectin), and Gla protein regulating mineralization (osteocalcin). Table 3 describes the localization of non-collagenous proteins in the atherosclerotic lesions.

Table 3. Non-collagenous bone matrix proteins and atherosclerotic plaques

Tissue	Proteins
Atherosclerotic plaques	Plaque Gla Protein Matrix Gla Protein Vitronectin
Vascular smooth muscle cell	OP, BSP, ON, OC, decorin, biglycan
Foam Cell	OP
Macrophage	OP, ON
Endothelial cells	OP, ON

BSP : bone sialoprotein, OC, osteocalcin, ON, osteonectin; OP, osteopontin

Giachelli and co-workers suggested the important role of intracellular phosphate activation. Loss of sodium-dependent phosphate cotransporter (NPC) in smooth muscle cell completely block the culture mineralization and smooth muscle cell calcification (Jono et al., 2000). Increased smooth muscle cell phosphate by sodium-dependent phosphate cotransporter may initiate the signaling pathways leading to differentiation of osteoblast-like cells. Then, this cascade may 1) increase osteoblast-like gene expression (e.g. core binding factor α -1) and osteopontin and osteocalcin, 2) decrease smooth muscle specific gene expression, and 3) stimulate secretion of mineral nucleating protein (e.g. alkaline phosphatase, calcium binding protein), and matrix vesicles (Giachelli et al., 2001).

First, apatite accumulation occurs on membrane of extracellular matrix vesicle. It is not regular process but is a complex process requiring calcium by organic molecules and energy (solution to solid crystal) or presence of nuclei of solid particles (substrate for heterogeneous nucleation). Phasic change occurs in an environment of organic components such as matrix vesicles, collagen fibrils, and bone regulatory proteins. Intimal calcification has been described in association with matrix vesicles, cholesterol crystals, and mitochondria like structures in intimal vascular smooth muscle cells (VSMCs). It has been proposed that apoptosis of VSMC in the media, and macrophages in the intima might initiate to form matrix vesicle and nucleate apatite (Kim, 1995; Hegyi et al., 1996). Apoptotic bodies derived from human VSMCs are rich in accumulated calcium and might initiate calcification. Apoptosis is a crucial initiation event to vascular calcification, once an intimal nucleation site has been established apatite crystal growth can occur.

iii) Nucleation of calcium

Once the nucleation is formed, the proteins found in bone are expressed. Molecular signals that regulate the phenotypic changes are not clear yet but VSMCs and osteoblasts share a common embryonic mesenchymal derivation several of bone

morphogenetic proteins and their receptors are expressed in the vascular wall. Origin of bone-forming cell in vascular system (CVCs or BVSMCs) are either from the migration of osteoblasts through or development of VSMCs in the vessel wall in situ. After focal calcification occurs, it seems to accumulate larger and visible with collection of more cholesterol debris, inflammatory cells (e.g. macrophages), or fibrotic tissue.

iv) Proliferation of calcification

The presence of skeletal matrix proteins including osteopontin, Matrix Gla protein, bone morphogenetic protein-2 from atherosclerotic plaques may help to identify the underlying mechanism. Biomineralization and demineralization is a basic mechanism of atherogenesis and bone metabolism. Molecular biological studies have shown expression of bone-associated proteins in the vascular wall and in atherosclerotic lesions. Vitamin K-dependent bone protein (Jie et al., 1996), osteopontin (O'Brien et al., 1994), matrix Gla protein (MGP) (Shanahan et al., 1994), bone morphogenetic protein-2a (BMP-2a) (Bostrom et al., 1993), osteophytes (Reid IR et al., 1991) had been investigated as contributing biochemical mediators in mineralization (calcification) of both atherosclerosis and osteoporosis.

Calcification in vascular structure is involved with hydroxyapatite, matrix vesicles, type I collagen, and non-collagenous bone associated proteins. The origin of calcifying cell follows two possibilities: 1) de-differentiation of arterial wall cells into mesenchymal cells (osteoblast, the presence of cells with osteoblastic potential within the vessel wall) or 2) migration of osteoblastic precursors from the periphery.

During bone mineralization, the final stages of osteoblast differentiation are defined by the secretion and organization of the bone extracellular matrix (ECM), matrix vesicles, and osteoid. Osteoid contains collagen type I, osteocalcin, osteopontin, bone sialoprotein and others. Despite normal ionic condition, the osteoid mineralization occurs with the help of bone matrix proteins, and matrix vesicles (Boskey et al., 1997)

approximately 10 days after its synthesis. Bone mineralization in a specialized extracellular matrix results in calcium apatite deposition. This process requires initiation of apatite accumulation (nucleation) and growth, elaboration of a permissive matrix, and production of regulatory proteins. During the osteoblast proliferation, type I collagen is maximally expressed. Following matrix maturation, alkaline phosphatase (ALP) and MGP are produced in large amounts. Then, the increased expression of osteopontin and osteocalcin, and bone sialoprotein are following during matrix mineralization (Lian et al., 1999).

In vitro study of vascular calcification, calcifying vascular cells seems to follow similar steps under the control of a cyclic AMP (cAMP) pathway. Treatment of calcifying vascular cells with a protein kinase A-specific inhibitor inhibited alkaline phosphatase activity and mineralization during spontaneous calcifying vascular cells differentiation (Reference). These result supports that the cAMP pathway promotes in vitro vascular calcification by enhancing osteoblast-like differentiation of vascular cells (Tintut et al., 1998; Tintut et al., 1999; Tintut et al., 2000).

Atherosclerotic lesions present with all the major components of bone formation; matrix vesicles, BMP-2, osteopontin, collagen type I α , MGP, osteonectin, biglycan, and osteocalcin (Bostrom et al., 1993; Watson et al, 1994; Anderson et al., 1983; Bini et al., 1999; Giachelli et al., 1995; Watson et al., 1998; Shanahan et al., 1999; Mori et al., 1998; Shanahan et al., 1994). By Bostrom et al. (1993), a similar model of osteoblasts in arterial wall has been identified from bovine aortic media (Bostrom et al, 1993). Calcifying vascular cells in artery produce extracellular matrix in the vascular wall like extracellular matrix (ECM) during the osteoblast differentiation in bone. Watson et al. (Watson et al., 1998) investigated the role of extracellular matrix in vascular calcification, and they found that fibronectin and collagen type I promote vascular calcification while type IV collagen inhibit it.

Calcifying vascular cells (CVCs) retain the features/ exhibit several osteoblastic marker including type I collagen, alkaline phosphatase, osteopontin, and osteocalcin.

Estradiol (E2) promotes CVCs differentiation. Matrix vesicles are phospholipid-enriched, membrane-bound organelle, and found in the extracellular matrix of calcified tissues. From early study done by Anderson et al. (1983), matrix vesicles, the nucleation sites for formation of hydroxyapatite, initiate of hydroxyapatite deposition associated with matrix vesicle, especially by membrane-bound lipids and lipids from apoptotic cell membrane debris.

A recent study demonstrated of expression of core binding factor $\alpha 1$ (Cbfa-1) in human neo-intimal smooth muscle cells and macrophages in early plaques (Engelse et al., 2001). Cbfa-1 has been shown to be a key factor in osteoblast differentiation in vivo and in regulation of bone deposition by mature osteoblasts (Ducy, 2000). Osteocalcin, bone sialoprotein, osteopontin, and collagen type I α have putative Cbfa-1 binding sites (Ducy, 2000). Expression of Cbfa-1 is closely related to the expression of osteopontin (Giachelli et al., 1993) and matrix Gla protein (Shanahan et al., 1994) in smooth muscle cells and macrophages. Therefore, Brindle (2001) suggest that expression of a key regulator, Cbfa-I in atherosclerotic plaque may induce the process of trans-differentiation of vascular smooth muscle cells in to osteoblast-cell types. Table 4 shows the potential regulatory factors in vascular calcification.

Table 4. Related regulatory factors for calcification from in Vitro studies

Stimulators of calcification		Inhibitors of calcification	
CAMP	Tintut, 1998	Autocrin/paracrine PTHrP Jono, 1997	
Inorganic phosphate	Giachelli, 2001	Collagen IV	Watson, 1998
Alkaline phosphatase	Giachelli, 2001	Osteopontin	Wada, 1998
Core-binding factor α	Giachelli, 2001	Matrix Gla protein	Farzaneh-Far, 2001
Vitamin D3	Jono et al., 1998		
Estrogens	Balica, 1997		
Oxidized lipids (MM-LDL)	Parhami, 1997		
Transforming growth factor β 1	Watson, 1994		
Tumor necrosis factor- α	Tintut, 2000		
Leptin	Parhami, 2001		
Fibronectin	Watson, 1998		
Collagen I	Watson, 1998		
Bone morphogenic proteins	Bostrom, 1993		

3) Clinical significance of vascular calcification

There are cumulating evidences that the extent of calcification predisposes to coronary events. The possible direct relationship proposed for the presence in lesions of juxtaposed regions of calcification and plaque disruption. It has been also speculated that risk of plaque rupture is enhanced early in calcification, but decreased later, when the calcification is thicker and more extensive. Arterial calcification may represent an attempt to stabilize the plaque. Heavily calcified cap is five times stiffer than non-calcified lesion or the normal arterial wall, and is very resistant to rupture. (Richardson et al., 1989; Lee et al.1991) However, the increases stress at the junction of the calcified cap and adjacent intima may lead to rupture. Focal calcification is the major reason for dissection after ballon angioplasty (Hodgson, et al. 1993, Fitzgerald et al.,1992).

Calcification could increase the risk of MI by loss of distensibility, vasomotion, or compensatory enlargement (Hodgson, 1993)

Coronary calcification and coronary stenosis has been inconsistently correlated. From previous studies, calcification is poorly correlated with stenosis (Clarkson, et al. 1994; Sangiorgi, et al. 1995) but closely associated with plaque volume (Sangiorgi, et al. 1995). Conversely, Margolis et al. (1980), and others (Kajinami et al., 1994) had shown that calcification in coronary arteries are related to CAD seen on angiography, and the survival rates at 6 months to 5 years was poorer in patient with calcification compared to without calcification (5 years survival rate, 58% vs 87%). The presence of calcification implies the association of lipid rich and possibly unstable plaque elsewhere. This would make calcification a predictor of rupture somewhere in the arterial wall, but not rupture of the calcified lesion. Intimal calcification may cause long-term structural changes of the arterial wall that may be related to prognosis (Glagov et al., 1987). For instance, arterial wall stiffness has been independently correlated to aortic calcification. Increased arterial stiffness leads to an increase in pulse pressure (highly significant predictor of myocardial infarction and cardiovascular death).

In summary, molecular and epidemiological evidences have shown increased importance of calcification in atherosclerosis. Moreover, investigations on the etiologic mechanisms of vascular calcification (atherosclerosis) and osteoporosis has markedly increased.

2. Epidemiology of Coronary Artery Disease in Men

a. Overview

Cardiovascular disease (CVD, ICD/9 390-459) is a chronic disease comprised of coronary heart disease (CHD, ICD/9 410-414), cerebrovascular disease (stroke, ICD/9 430-438), hypertensive disease (ICD/9 401-404), rheumatic fever/rheumatic heart disease (ICD/9 390-398), peripheral vascular disease and other cardiovascular disease (Reference). CVD is the nation's leading causes of mortality and morbidity. CVD accounts for more than 900,000 deaths annually compared to other leading causes of death: cancer (> 500,000) and accidents (> 95,000) in the United State (Hoyert et al., 1997). Among the various cardiovascular diseases, coronary heart disease (CHD, ICD/9 410-414) is the single leading killer accounting for nearly 500,000 deaths in men and women (National Center for Chronic Disease Prevention and Health, 1999) . CHD or CAD contributed to 48% of CVD deaths in 1998 U.S. statistics Atherosclerosis (ICD/9 440) is a disease process that leads to CVD characterized by a thickening of artery walls. Atherosclerosis is also a leading cause of many lives from myocardial infarction and stroke.

Mortality from CAD increases with age. Death rate is twice higher among people older than 75 years than among people older than 65 years (Heart and Stroke Statistical Update, 2001). Morbidity of CVD varies by populations. In the U.S., American Heart Association reported that there are 13.5 million people to have a history of MI or angina pectoris, and another 13.6 million more likely to have asymptomatic CAD (Ref..). Based on data from the Atherosclerotic Risk in Communities (ARIC), it was estimated that 1,100,000 Americans will have a new or recurrent myocardial infarction (MI) or fatal CHD each year (Rosamond et al., 2001). The lifetime risk of having MI, angina pectoris, coronary insufficiency or coronary death at age 50 years was calculated as 43% (95% CI: 39.5-45.8) for men and 29% (95%CI : 26.3-31.6) for women (Lloyd-Jones et al.,

1999). In addition, the lifetime risk of hard coronary events was 41% for men and 25% for women at age 50 years.

There have been substantial declines in both CHD and stroke mortality. Age-adjusted CHD mortality rates between 1970 and 1990 decreased more than 3 %, but it has been slowed at a rate of 2.7% (Cooper et al., 2000). Age-adjusted CHD mortality has decreased by 3.2 % (95% CI: 2.0-4.3) among men and 3.8% (95% CI: 1.9-5.6) among women each year between 1987 and 1996 (Rosamond et al., 2001). However, there have been inconsistent reports regarding morbidity of CAD. Several studies found modest increase, stable or decline in the incidence and recurrence rate of MIs (McGovern et al., 1996; Tunstall-Pedoe et al., 1999; Rosamond et al., 1998; Goldberg et al., 1999). An estimated \$260 billion was used to manage cardiovascular disease and most of costs were allocated to CAD in 1997.

CAD is preventable disease. With improved risk prediction methods, proven efficacious pharmacological therapies or development of new measurement technologies have resulted in a decrease in mortality. The importance of preventive strategies in the care of “at risk” populations or individuals with CAD have been emphasized. From many observational and intervention studies, prominent progresses have been established distinguishing cardiovascular risk factors and its’ relationship to the development of CAD. Nine well-known non-modifiable or modifiable risk factors were established: male gender, heredity, advancing age, smoking, high blood pressure, diabetes, obesity, physical inactivity, and abnormal blood cholesterol (Anderson et al., 1991). In addition, there are a number of new risk factors such as inflammation markers, or new measurements of subclinical disease, which may be useful for differentiating and monitoring underdiagnosed CAD among populations. In current literature review, we will discuss the effects of cardiovascular risk factors.

b. Non-modifiable risk factors of CAD

1) Age and gender

Age, gender, race, and family history of CHD are the major non-modifiable risk factors for CHD. Prospective studies such as the Framingham study have shown that CHD risk increases continuously from age 30-60, while national data show that CHD mortality increases exponentially between age of 25 and 85. About four out of five people who die of coronary heart disease are age 65 or older. At older ages, women who have heart attacks are more likely to die than men within a few weeks.

CHD is more prevalent among men than women at all ages. The divergent rates of heart disease between genders have been explained by two dominant hypotheses: 1) sex steroid hormone theory that estrogen is cardio-protective, 2) unfavorable heart disease risk factors that men possess more adverse, or unhealthy behaviors than women (Barrett-Connor, 1997). There is marked decline of the male prevalence to female relative risk after menopause, and the sex differences of mortality is converted from men to women. The Framingham study found a male to female ratio of 2.8 for the incidence of any CHD between ages 45-54. This ratio decreased to 1.9 in 55-64 and 65-74, and to 1.5 for ages 75-84. Even after menopause, when women's death rate from heart disease increases, it's not as great as men's. However, relative to males, females had more-extensive fatty streaks but a similar extent of raised lesions in the aorta and had a similar extent of fatty streaks but fewer raised lesions in the coronary artery, at all ages. Sex differences in development of atherosclerosis appeared from early lifetime.

2) Race and Family history

CHD mortality rates differ among the major ethnic populations. The mortality differences reflect the complex interaction of genetic, environmental, and social factors. African American have the highest mortality rates of CHD (186.8 per 100,000 population

in 1997), and non-Hispanic whites showed the second highest rate (182.8 per 100,000). Hispanics (124.2 per 100,000), Native American (112.7 per 100,000) and Asian (100.1 per 100,000) follow after non-Hispanic whites (Cooper et al., 2000). The mortality rates have declined more slowly in black men and women than in white men and women (Manolio et al., 1995). Atherosclerosis Risk in Communities (ARIC) study (4264 black and 11,479 white men and women, age range 45-65 years at entry) observed much higher black mortality in CHD compared to U.S National Vital Statistics data from 1968-1992 (Williams et al., 1999). The black-white mortality rate ratio increased for men and women during time period and relatively higher CHD mortality rates in blacks were observed at the younger age. For instance, the CHD mortality rate ratios of black men compared to white were ranged from 7.77 at ages 35-44 to 1.34 at ages 75-84 in 1988-92 (Williams et al., 1999). CHD mortality rates were pronouncedly greater in black women than white women at all ages. The racial differences among cardiovascular risk factors were reported (Hutchinson et al., 1997). African Americans have more severe high blood pressure, and glucose levels than whites as well as higher rates of obesity and diabetes (Hutchinson et al., 1997). Also, African American had low socioeconomic levels and more clustered risk factors than whites. Heart disease risk is also higher among Mexican Americans (Sundquist et al., 2001), American Indians (Lee et al., 1998), and some Asian Americans (Iribarren et al., 1996).

Most people with a strong family history of heart disease have one or more other risk factors. Family history of premature CHD, defined as CHD prior to age 55 in a male first-degree relative, is an important CHD risk factor. Familial Lp (a) excess, familial combined hyperlipidemia, and familial dyslipidemia are known common familial diseases associated with premature CHD (Exp Panel High Blood Chol Adults, 1993). Hunt and colleagues (1986) introduced the Family Risk Score (FRS) as a measurement of familial risk of cardiovascular disease. Some studies demonstrated that this Family Risk Score is a significant measurement to predict for future CHD risk (Higgins et al, 1996; Li et al., 2000). Family Risk Score was calculated from observed and expected CHD events using maternal, paternal, and full siblings data. In a large population based study of 3,958 African American, and 10,580 Caucasian participants aged 45-64 years

old, the hazard rate ratio of CHD related to one standard deviation increase of Family Risk score was 1.49 in black men, and 1.63 in white men with adjustment of covariates (age, carotid IMT, smoking, total triglycerides, LDL-c, HDL-C, apolipoprotein (a), fibrinogen, and hypertension) (Li et al., 2000).

c. Modifiable risk factors of CAD

1) Cholesterol and other lipids

The risk of coronary heart disease rises as blood cholesterol levels increase. When other risk factors (such as high blood pressure and tobacco smoke) are present, this risk increases even more. A person's cholesterol level is also affected by age, sex, heredity and diet. The general structure of plasma lipids (plasma lipoproteins) is combined of an outer layer of phospholipids, unesterified cholesterol, and proteins, with a core of neutral lipids, predominately cholesterol ester and triglycerides. The lipoprotein classes differ in their lipid composition, protein (apolipoprotein, Apo) composition, and protein-lipid ratio (the higher the ratio, the greater the density). Lipoproteins are commonly classified by their density and/or size; chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDLc), intermediate density lipoproteins (IDLc), and high density lipoprotein (HDLc). The two types of lipoproteins based on protein composition are those that contain apo B and those that contain apo A. With the basis of lipid composition, the lipoproteins can also divide into those that are cholesterol (C)-rich and those that are TG-rich. Chylomicrons, VLDL, IDL, and remnant chylomicrons are apo-B and TG-rich lipoproteins. Dense LDL, and lipoprotein (a) (Lp(a)) are apo-B and cholesterol ester rich lipoproteins. LDL-c containing Apo B-100 is the major transporter of cholesterol. Apo-a (I), and apo-a (II) and cholesterol ester mainly compose HDL-2, and HDL-3. Lipoprotein (a) is an LDL-like particle with (a) protein attached to apo B. A high plasma Lp (a) concentration is considered a risk factor for coronary artery disease

Many studies have demonstrated that elevated total cholesterol are associated with a higher risk of CHD. Lowering serum total cholesterol was related to reduce CAD risk ranged from 50% at age 40 to 20% at age 70 (Law et al., 1994). The benefit may actually be greater, because variation in cholesterol levels within individuals may cause systematic underestimation of the association between cholesterol and CAD in studies taking a single blood sample.

2) Hypertension

One of the most widely recognized cardiovascular disease risk factors is hypertension. The relationship between level of blood pressure and cardiovascular disease is strong, graded, linear, and etiologically significant in middle aged populations (Stamler et al., 1993). Hypertension is defined as a systolic blood pressure of at least 140 mmHg and/or a diastolic blood pressure of at least 90 mmHg. According to American Heart Association (2000), the age-adjusted prevalence of hypertension for adults age 20 and older is 25.2 % for non-Hispanic white male and 36.7 % for non-Hispanic black males. The prevalence of hypertension in blacks much higher than whites, and it attribute 1.5 times greater rate of CHD mortality risk. Individuals with high blood pressure are 3 to 4 times more likely to develop coronary heart disease than normotensive people (Adams-campbell et al., 1995). Recent observation from the Framingham heart study demonstrated that 90% of middle-aged and older individuals have a residual lifetime risk for the development of hypertension represents a major public health challenge (Vasan et al., 2002).

The prevalence of hypertension, especially systolic hypertension is dramatically increased as age increases (Sagie et al., 1993). The relation of cardiovascular diseases seems to be more significant for systolic blood pressure (SBP) than for diastolic blood pressure (DBP) (Vokonas et al., 1988; Stamler et al., 1989). For instance, Psaty et al. examined whether SBP, DBP, and pulse pressure are related to the risk of myocardial infraction, and total mortality among 1961 men and 2941 women. Following up

averaged 6.7 years, SBP was associated with total mortality, and MI. 1 SD changes (21.4 mmHg) of SBP was related to 24% of increased risk of MI (Hazard Ratio 1.24; 95%CI, 1.15-1.35) in age, sex, and other risk factor adjusted model. On the other hand, DBP was related to 13% (95% CI: 1.04-1.22) increased of MI risk per 1SD (11.2mmHg) and pulse pressure was to 21 % increased risk per 1SD (18.5mmHg).

Hypertension also increases the risk of stroke, heart attack, kidney failure and congestive heart failure (Reference). When high blood pressure coexists with other factors such as obesity, smoking, high blood cholesterol levels or diabetes, the risk of heart attack or stroke in hypertensive subjects increases several times (Kannel et al., 2000). Benefits of treatment with low-dose diuretics (water pill) and β -blockers lower the risk of cardiovascular disease in treated hypertension groups. With regard to SBP, DBP, pulse pressure, risk of MI in a population-based study was significantly larger in untreated hypertensive subjects. For instance, hazard ratio of MI per 1 SD increased of SBP was 1.31 (95%CI, 1.18-1.46) among untreated populations compared to 1.13 (95% CI, 1.00-1.28) among treated subjects (Psaty et al., 2001)

3) Diabetes

Both insulin dependent diabetes mellitus (Type I diabetes) and insulin independent diabetes mellitus (Type II diabeste) are major risk factors for CVD mortality and morbidity (Stern 1999). Even when glucose levels are under control, diabetes greatly increases the risk of heart disease and stroke. According to the Behavioral Risk Factor Surveillance System data, age-adjusted prevalence rates of diabetes from 1995 to 1998 have increased in all ethnic groups ranging from 14% to 28% (Stern 1999). Not only, is diabetes increasing in prevalence, but the CVD rates and mortality are increasing among diabetes. The cardiovascular mortality of diabetic patients is 2.3 to 5.6 times higher compared with the general population (Beckman et al. 2002). Approximately, two-thirds of all deaths among persons with diabetes attributes to coronary artery disease (Kannel et al., 1985). Also, the prevalence of diabetes with

myocardial infarction (MI) increased from 8.2 to 16.8% among men between 1970 and 1985 (Sprafka et al., 1991). Diabetes is associated with acute coronary syndrome. One-year mortality in persons hospitalized for acute anginal symptoms was almost two times higher with versus without diabetes (Fava et al., 1997).

Studies have shown that diabetes accelerates the pathological process of atherosclerosis. Autopsy studies reported that diabetes was associated with an increase in the extent of lesions in the arteries (Buchfiel et al., 1993). Furthermore, persons with diabetes are found to have a greater number of diseased coronary vessels, and greater narrowing of the left main coronary artery than those without diabetes. Diabetes is also a predictor for progression and occlusion of atherosclerotic lesions.

Diabetes may coexist hypertension, hyperlipidemia, obesity, and insulin resistance, and is related to increased risk for prothrombotic state (Aoki et al., 1996). Framingham study showed that persons with diabetes were about four times more likely to have additional cardiovascular risk factors (Brand et al., 1998) Same results from MRFIT study demonstrated that men were three times more likely to die of CAD when they had three or more cardiovascular risk factors and the presence of baseline diabetes (Stamler et al., 1993). The major cardiovascular risk factors among diabetes are elevated tryglyceride levels, low levels of HDL-c, alterations in the composition of LDL-c (small and dense LDL particles), and an increase in apoB and apoE. Among all, hypertriglyceridemia related to insulin resistance syndrome produce a vascular environment predisposed to atherogenesis.

Hyperinsulinemic patients without over diabetes are at increased risks for CVD. There are many evidences that people having prediabetic condition might have atherogenic status even before the onset of clinical diabetes. Insulin resistance syndromes or Syndrome X (low HDL-c, high triglycerides, small dense LDL, glucose intolerance, central obesity, elevated plasminogen activator inhibitor-1 (PAI-1), and fibrinogen) is tightly related to CVD. A study using intensive insulin therapy immediately following an MI reduced 1-year mortality by 30% compared to usual therapy. High

insulin concentrations may have direct effects on atherosclerosis (e.g. stimulate SMC proliferation in plaques) and indirect effects (reflecting insulin resistance) as in Syndrome X. Thus, the presence of insulin resistance syndrome may contribute to risk for CAD and diabetes.

4) Obesity and overweight

Obesity is associated with several other cardiovascular risk factors such as hypertension, increased blood viscosity, elevated triglyceride and VLDL, reduced HDL-c, denser LDL-c, and glucose intolerance. Obesity is measured in several ways, including relative weight for height, body mass index (BMI), waist hip ratio (WHR), waist circumference, percent body fat, and body fat distribution. Waist circumference and body mass index (BMI) are indirect methods to measure body composition. Waist-to-hip ratio (WHR) is another index of body fat distribution. According to National Center for Health Statistics, overweight and obesity are defined by body mass index (weight, Kg / height, m²). A BMI of 25 to 29.9 regarded as overweight, and one ≥ 30 defined as obesity. Approximately 35% of adult females and 31% men in U.S. are estimated to be overweight (Kuczmarski, 1994). Data from the National Health and Nutrition Examination Survey demonstrated that the incidence of obesity increased to 33.3% in the period of 1988 to 1991 compared to 24% in that of 1976 to 1980 (Kuczmarski et al., 1994).

The relationship of obesity to CVD is controversial; Several large prospective studies have shown the significance of obesity for developing cardiovascular disease, while others have not. The Framingham Study found that males younger than 50 with a relative weight of 130 or more had a relative risk for CHD twice that of males with relative weight of 110 or less. Excess weight was associated with sudden death and angina, but not with myocardial infarction. However, Manson and colleagues estimate that maintaining ideal body weight, compared to being 20% or more overweight, lowers

the risk of MI by 35-55%. A study in Finland found an association only in men, and it was not independent of cholesterol of blood pressure.

Not only the amount of fat mass, but the distribution of fat (central adiposity) has been related to increased risk of dyslipidemia and CVD (Larsson et al., 1984; Terry et al., 1992). In prospective studies considering on overall obesity and fat distribution, body mass index (BMI) and the waist-to-hip ratio are both important predictors of coronary heart disease. For instance, findings from the Framingham study reported that the abdominal obesity (central adiposity) was an independent risk factor on stroke, cardiac failure and cardiovascular and all-cause mortality in men (Kannel et al., 1991). Obesity and overweight is also related to metabolic syndrome, known as impaired glucose tolerance (IGT) or the insulin-resistant syndrome, has been defined as an insulin-resistant hyperinsulinemia in overweight individuals or in those with abdominal obesity. Using data from NHANES III, Ford and colleagues (2002) estimated that an age-adjusted prevalence of the metabolic syndrome is approximately 23.7%. Recent researches focuses on metabolic syndrome because persons with syndrome may have a higher risk of diabetes as well as CHD.

5) Smoking

Smoking is an independent and preventable risk factor for the development of cardiovascular disease. Researches reported that cigarette smoking related to putative mechanisms leading to CVD. Smoking is related to endothelial injury, vasoconstrictor, cardiovascular stressor (increased blood pressure), increased platelet adhesiveness and aggregation, raised fibrinogen, other inflammatory markers, reduced HDL concentration, and insulin resistance. Because of the many mechanisms by which smoking can influence CVD risk, it has a synergistic effect on that risk, in the presence of diabetes, hypertension, oral contraceptive use, or lipid disturbance. Numerous studies have found smoking to be an independent predictor of CVD incidence and mortality. Smokers have almost 3.4 times higher risk of fatal CHD events compared to

never smokers in combination of quintiles for blood pressure and cholesterol (Stamler 1992). The risk of subsequent coronary event is also increased in those who have CAD, but continue to smoke (Neaton and Wentworth, 1992).

6) Alcohol consumption

Light intake of alcohol is consistently associated with lowering CVD risk. There is an established inverse relationship between the regular light consumption of alcohol (5-10 g/day) and the incidence of coronary artery disease (CAD) (van Tol and Hendriks, 2001). The relationship with cardiovascular disease and total mortality is U shaped or J shaped curves. The possible effect as antioxidants (wine) was related to antithrombotic effect. Heavy drinking (more than 3 drinks per day) has increased the risk of hypertension, silent myocardial ischaemia and angina. The relationship between alcohol and CVD is controversial and confounded by various factors, such as diet and psychosocial factor.

7) Physical inactivity

The prevalence of physical inactivity has increased with age. The degree of physical inactivity is higher in women than men, and it is the highest in black and Hispanic populations (A Report of the Surgeon General 1999). Physical inactivity has associated with incidence of coronary heart disease and total mortality (Folsom et al., 1997). Physical activity levels were also related to the risks of CHD in dose-response ways (Williams et al., 2001). The risks of CHD linearly reduced in association with increasing percentiles of physical activity. The relative risk of coronary heart disease associated with physical inactivity ranges from 1.5 to 2.4, an increase in risk comparable to that observed for high blood cholesterol, high blood pressure or cigarette smoking (JAMA 1995; 273:402-407) A meta-analysis of studies of non-occupational activity estimated that the adjusted relative risk for CHD mortality in sedentary

individuals compared to the active was 1.9 (95% CI 1.6-2.2). In a summarized study of investigating the interaction between physical activity and CHD, stroke, and hypertension, physical activity were inversely related to CHD or high blood pressure, but unclearly to stroke. Sedentary lifestyle was considered of similar magnitude for cardiovascular risk as smoking, high cholesterol, and hypertension.

8) Psychosocial Factors: depression

The importance of psychosocial factors in the development of coronary artery disease has been demonstrated significantly in several epidemiological studies (Williams et al., 2000; Ariyo et al., 2000; Ford et al., 1998; Barefoot et al., 1996). Some scientists have noted a relationship between coronary heart disease risk and stress in a person's life, their health behaviors and socioeconomic status. Researches were mainly emphasized on the following psychosocial factors: depression, anxiety, personality factors and character traits (e.g. hostility), social isolation, and chronic life stress (Rozanski et al., 1999). For instance, the depression symptoms were consistently demonstrated significant positively relationship with incidence of CVD events in recent epidemiological studies of healthy (Ariyo et al., 2000; Barefoot et al., 1996; Ford et al., 1998) and coronary artery disease populations (Denoillet et al., 1998; Frasure-Smith et al., 1995).

The direct pathophysiological effects of depression is involved in hypercortisolemia (elevated blood cortisol levels) which related to attenuation of the adrenocorticotropin hormone response, and failure of suppression of cortisol secretion feedback loops leading to increased blood pressure and heart rate (Rozanski et al., 1999). Also, depressed symptoms are associated with abnormal activations of the proinflammatory response system (interleukin 1 (IL-1), IL-6, Tumor necrosis factor α). This impairment results in decreased heart-rate variability (HRV) (Rozanski et al., 1999). Depressive symptoms may indirectly influence CVD through an association with

traditional risk factors such as cigarette smoking, excessive alcohol consumption, higher body mass index, and lower physical activity (Kubzansky and Ichiro, 2000).

From epidemiological studies, relative risk of developing myocardial infarction among subjects with depressive symptoms was 1.7 to 2.1 in large prospective studies even though the measurement scales were different (Barefoot et al., 1996; Ford et al., 1998). Recently, Ariyo et al. examined the depressive symptoms and the risk of coronary heart disease and mortality in 4493 elderly subjects (age range 65 to 98 years) who were free of CVD. The depression symptoms using Center for Epidemiological Studies' Depression Scale measured at baseline and annually for an average of 6 years. The unadjusted hazard ratio of development of coronary heart disease was 1.15 (p-value: 0.006) in men with every 5-unit increase of mean depression score. The risk of development of CVD increased by 40% among those who had highest cumulative mean depression scores compared to lowest mean scores. For depressed men, the rate of cardiac death was 7.0% in contrast to 2.4% of the non-depressed.

Depressive symptoms have also been related to recurrent CVD events, and cardiac mortality (Denoillet et al., 1998). In study of the relationship between post-cardiovascular events and depression, Frasure-Smith et al., studied patients who hospitalized for MI (cases) and usual care (controls) following 1 year (1999). Among patients who hospitalized for acute MI, increased Beck Diagnostic Interview (BDI) scores (greater than or equal to 10) were significantly related to cardiac mortality for both men and women. The odds ratio for mortality in 167 men with MI was 3.05 (95% CI = 1.29-7.17). Current literature supports depression as a risk factor for the development of CVD. Also, depression, and other psychosocial components as risk factors for the comorbidity of CVD are an emerging focus of multidisciplinary research.

d. Emerging Cardiovascular Risk Factors

1) Inflammation: C-reactive protein

Proinflammatory cytokines stimulate smooth muscle cells, to express the genes that are capable of weakening the fibrous cap of atherosclerotic plaque. C-reactive protein (CRP) is an acute phase reactant and a sensitive marker for underlying systemic inflammation response. CRP is a monomer having molecular weight of 21,000 and is a pentamer in blood (Tracy 1999). CRP is a plasma protein, which is unaffected by hormones or anti-inflammatory drugs. It is regulated by especially Interleukin-6.

The serum levels of CRP have been related to predict risk of myocardial infarction, ischemic stroke (Ridker et al., 1997; Koenig et al., 1999) not only in healthy men but also in postmenopausal women (Ridker et al., 1998 a). Higher levels of serum CRP are also related to increased risk of future CAD events in patients with prior MI (Ridker et al., 1998 b), stable (Haverkate et al., 1997) or unstable angina (Kennon et al., 2001) or in smokers (Kuller et al., 1996). From several clinical studies, CRP is identified as independent risk factor for coronary artery disease. In Physicians' Health study, elevated CRP is correlated to the first MI occurrence, and peripheral vascular disease. Combined with total cholesterol level, high CRP predicts greater future events. CRP was better predictor of CVD events in smokers than in nonsmokers (Tracy et al, 1997). High blood level of CRP in women is related to future events of CVD. CRP also links to promote thrombosis and increase clot formation, lipid oxidation, cell activation and proliferation.

According to Pasceri et al. (2000), CRP was related to the expression of adhesion molecules in human coronary artery endothelial cells. CRP induced the expression of vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule (ICAM-1), and E-selectin. Therefore they suggest that CRP may play a role in promoting the inflammatory component in the developing of inflammation. In the

Physicians' Health Study, Ridker and colleagues showed that CRP and serum amyloid A (SAA) had a positive correlation with recurrent risk of MI. Pasceri et al. also demonstrated that CRP could induce adhesion molecule expression in endothelial cells with effects on monocyte chemoattractant protein-1. Interleukin 1 and 6, and tumor necrosis factor α are main modulators of CRP expression.

2) Atherogenic/prothrombotic factors

Serum homocysteine is an intermediate amino acids-compound in the metabolism of methionine to cysteine. Two pathways metabolize Homocysteine: vitamin B6 dependent transsulfuration and vitamin B12 dependent remethylation. Fasting total homocysteine level in plasma of 5 to 15 mmol/L is regarded as normal status. Hyperhomocystinemia, chronic condition is elevated homocysteine levels with rare homocystinuria. Rare genetic diseases (homocystinuria) increase the risk of CVD including stroke in patients. It may be resulted by low dietary intake of folate, and deficiency in vitamin B6 and vitamin B12. Increased homocysteine in plasma is known to related to renal insufficiency, hypothyroidism, breast, or ovarian cancer. The possible etiologic mechanisms are: 1) vascular endothelial toxicity 2) proliferation of smooth muscle cells, 3) activation of thrombogenic protein, protein-C. Elevated homocysteine is related to ageing, male sex, menopausal status, and current smoking, and is investigated for causing direct toxic effect on endothelium (Mattson et al., 2002).

From various case-control studies, homocysteine and premature atherosclerosis have positive association. Even moderate levels of serum homocysteine levels were related to three fold increased risk of CHD in highest 5% of plasma homocysteine level subjects. There is strong dose-dependent association was noted between plasma homocysteine, CVD and overall mortality (Bostom et al., 1999). High plasma level greater than 15 mmol/L compared with level of 10 mmol/L showed 60% increase of adjusted mortality ratio. One meta-analysis demonstrated that 10% of CAD is attributed to homocysteine, and homocysteine is same amount of attributable risk of total

cholesterol. Yet, the relationship between homocysteine and atherosclerosis is not consistent.

However, researchers from ARIC study reported that vitamin B6 was more prominent independent risk factor than homocysteine to coronary heart disease (Folsom et al., 1998). Genetic polymorphism of methylene tetrahydrofolate reductase (MTHFR) gene is associated with increased plasma homocysteine, which is related to atherosclerosis. Homocysteine is a new emerging cardiovascular risk factor. Elevated total homocysteine level in blood is strongly related to cardiovascular risk. (Moustapha et al., 1999). In a review by Eikelboom et al. (2000), 5'-10'-methylene tetrahydrofolate reductase (MTHFR) polymorphisms (Frosst et al., 1995) was related to high homocysteine level, and strong prediction of hyperhomocysteinemia, but not to cardiovascular disease. The point mutation causing alanine (A) to valine (V) substitution was related to thermolabile activity of MTHFR. Cross section studies showed significant relationship between homocysteine level and atherosclerosis ($r = 0.63$) (Konecky et al., 1994) Recently, MTHFR polymorphisms also reported to related to bone mineral density in 307 postmenopausal Japanese women (Miyao et al., 2000). The mutated Valine/Valine genotypes showed significant lower BMD at total body ($p=0.03$), and lumbar spine ($p=0.016$). Women with VV genotype, lumbar spine BMD was 5.8% and total body BMD was 3.8% significantly lower than the subjects with AA or AV genotype (lumbar spine BMD 0.84 ± 0.14 versus 0.89 ± 0.17 g/cm² ($P = 0.035$), total body BMD 0.93 ± 0.09 versus 0.97 ± 0.11 g/cm² ($P = 0.023$), respectively). However, there are inconsistent results.

Thrombogenic factor promoting the growth of atherosclerosis plaque and occlusive thrombus also related to increase risk of CVD. Fibrinogen is a large glycoprotein made in the liver acts as a clotting factor that activates thrombin, and stimulates smooth muscle proliferation. Fibrinogen is a main coagulation protein in the plasma, a determinant of blood viscosity, and a cofactor for platelet aggregation (Maresca et al., 1999). Several prospective studies have shown an independent relationship between plasma fibrinogen and the incidence of CAD and stroke. Elevated

fibrinogen levels was related to increased risk of CVD. The levels higher than 350 mg/dL were significant risk factors for stroke and MI. In addition, it was related with two or three times increased risk. The serum levels of fibrinogen is also related to the inflammatory activity in plasma (Imperatore et al., 1998).

Low fibrinogen level was associated with under protection against CVD. From cross-sectional studies, and case-control studies, it is correlated with age, smoking, physical activity, obesity, and presence of diabetes, lipid profiles.

Plasminogen activator inhibitor-1 (PAI-1) is a component of the fibrinolytic system in blood. When a clot forms in blood, tissue plasminogen activator (tPA) rapidly converts plasminogen to plasmin, which degrades fibrin to dissolve the blood clot. PAI-1 forms stable complexes with tissue plasminogen activator (tPA), and it inhibit the tPA process. Thus, PAI-1 inhibits fibronolysis and promotes thrombosis. High levels of PAI-1 had been related to elevated acute phase reaction and insulin resistance syndrome. Elevated PAI-1 is reported to be with increased risk for venous thrombosis and re-infarction after history of MI in young men (Hamsten et al., 1987).

3. Non-invasive Measurement and Epidemiology of Vascular Calcification

a. Non-invasive measurement of Calcification

Calcification is detected in approximately 90% of atherosclerotic plaques. Calcium deposition is an intermediary step in the evolution of atherosclerosis, potentially accompanying the clinical manifestations of coronary plaque. As shown by autopsy and angiographical studies, the extent of coronary calcium has been correlated positively with coronary atherosclerosis (Rumberger et al., 1994; Rumberger et al., 1995; Budoff et al., 1996; Schmermund et al., 1998). It has also been shown as early as the stage of fatty streak (Stary et al., 1990). For instance, the development of coronary artery calcification was related to coronary risk factors (increased BMI, blood pressure, decreased HDLc) in the childhood, and adolescence in a study of 384 subjects (197 men, 187 women) (Mahoney et al., 1996). Several studies published that the extent of coronary atherosclerosis correlates with the frequency of future coronary events (Nallamothu et al., 2001; Wong et al., 2000; Arad et al., 2000).

The following review of measurements of coronary calcification was summarized from several papers on arterial imaging techniques (O'Rourke et al., 2000; Stanford et al., 1993; Wexler et al., 1996; Vallabhajosula and Fuster, 1997; Celermajer et al. 1998) and others. Calcium deposits in artery systems are detectable by plain radiography, coronary arteriography, fluoroscopy, magnetic resonance imaging, conventional, helical, and electron beam computerized tomography. We will discuss these techniques in this review. Calcification also is potentially detected in intravascular ultrasound, mammography, and transthoracic echocardiography.

1) Plain Chest Radiography

Even though extensive calcified plaques are detected, coronary calcification is not easily detected with chest radiography. Usefulness of chest x-ray is its readily

availability and low cost. In a study of radiography compared to fluoroscopy (also not accurate), the accuracy was only 42%. However, advanced digital radiography in 77 patients gave better detection rate of 71% in the left anterior descending artery compared to 32% detection rate using plain radiography (Kelly et al., 1983; Sakuma et al., 1988).

2) Magnetic resonance imaging (MRI)

Magnetic resonance techniques using gradient echo methods generate images of flowing blood as positive contrast within blood vessels. Magnetic resonance imaging (MRI) demonstrates plaque in the aorta and carotid arteries relatively well. MRI is also useful for studying the progression of experimental atherosclerosis in animal models, and for imaging the plaque components e.g. fibrous tissue, scar tissue, or hemorrhage (Skinner et al., 1995). MRI is capable of differentiating of lipid cores, fibrous caps, calcification, and intimal layers in vivo (Toussaint et al., 1996). MRI imaging of atherosclerosis and coronary calcification, however, has several limitations: it is not widely available; it is highly operator-dependent; it is expensive, and it is not standardized. Additionally, there are no large population studies of its prognostic value.

3) Fluoroscopy

Fluoroscopy has been used to detect calcification with relatively good sensitivity. The cardiac shadow is examined in the 90° lateral-view. The image intensifier is subsequently rotated through a 90° arc resulting in anteroposterior view. Positive calcification is visualized in one or more small, dense shadows. Sensitivity in detecting coronary calcification in greater than 50% diameter narrowing lesions ranged from 40% to 79%, with specificity ranged from 52% to 95% in collected 2670 patients (Wexler et al. 1996). Loecker et al (1992) studied 613 asymptomatic men of 1466 screening subjects (age range 26 to 65) using both fluoroscopy test and coronary arteriography.

Coronary calcification measured by fluoroscopy had a 66.3% sensitivity and a 77.6 % specificity in significant angiographical stenosis (>50%). Detection rates with greater than 10% stenosis, sensitivity and specificity were 60.6% and 85.9%, respectively. One report of a 117 symptomatic male subjects reported 74 % sensitivity, and 69% specificity in fluoroscopic method compared to 85%, and 63% in electrocardiogram (Hung et al, 1985).

In a comparison study of fluoroscopy and EBT, only 52% of calcification measured by EBT was significantly detected with fluoroscopy (Agatston and Janowitz. 1992). There was mean calcium density difference in lesions detected by the two methods (EBT, +99 HU vs. Fluoroscopy + 546 HU). It indicated that fluoroscopy could detect moderate to large calcification with good sensitivity, but not in small calcified deposits. Fluoroscopy is relatively inexpensive, and readily used in clinic settings. However, it has low to moderate sensitivity, especially in small deposits, and is highly operator dependent. In addition, quantification of calcium is not possible with fluoroscopy.

4) Helical (Spiral Computed Tomography)

Newer techniques utilize helical CT acquisitions with some advantages over conventional CT. Helical CT (Single or Double) can image as fast as 0.6 second. Using double helix CT, Shemesh et al (1995) measured coronary calcification in individuals with angiographically significant obstructive CAD with a sensitivity of 91% and a specificity of 52%. The authors concluded double helical CT was useful in predicting absence of CAD. However, a recent study (Budoff et al., 2001) reported fair correlation with EBT, but a high false negative rate (26%) in spiral CT using similar protocol (Agatston score, 3 mm slices, maximal density over 130 HU). The widespread availability and relatively low cost of spiral CT is attractive as it could make coronary calcium screening more widely available test. Limitation of helical CT exists; slow to obtain clear and motionless pictures of the heart compared to EBT. Thus helical CT has

been limited and interfered by cardiac motion. It takes approximately 250-300 msec per image compared to 50 to 100 msec per scan with EBT.

5) Electron beam computed tomography (EBT)

EBT scanning involves imaging the heart in 20-40 slices and calculates the amount of calcium as a score. EBT coronary calcium score has been correlated with atherosclerotic plaque burden and future CAD events (Arad et al., 2000). Emerging data suggest increased CHD events in subjects with the highest quartiles of calcium score. Electron beam computed tomography (EBT) uses the applied radiographic technique that is electrocardiogram triggered, so that consecutive scans at each tomographic levels are obtained at the same phase of the cardiac cycle to minimize cardiac motion artifact. The high resolution imaging of coronary arteries obtained using EBT shows both the presence and the quantity of calcium in atherosclerotic plaque with great sensitivity. It is currently the only non-invasive method that we can accurately quantify calcium in vivo, expressed as a calcium score. However, EBT does not supply any information on plaque morphology.

EBT employs a stationary source-detector pair coupled to that x-rays are produced as a rotating electron beam is swept across a series of 1 to 4 semicircular tungsten targets situated beneath the subject. The imaging chain has no moving parts, and scanning through or at preset times during the cardiac cycle and from adjacent cardiac sites is possible in rapid succession. EBT is also a noninvasive means to obtain quantitative information on cardiac anatomy, function, and flow, whole body examination. On EBT, consecutive single-slice, 100 ms scans are performed with 1.5 mm to 3 mm (up to 6 mm) tomographic thin slices. 20 to 40 sequential scans of the heart with electrocardiographic triggering (80% of RR interval) during late diastole can be completed in about 20 to 40 seconds. Secci and colleagues (1997) compared the two scanning protocols (3mm thickness vs. 6 mm) in 326 high-risk individuals (average follow up 32 months), and found equal accuracy of two protocols for the prediction

future events. To obtain tomogram from the root of the aorta and the origin of the left main coronary artery through distal portions of the right coronary artery, patients need to hold two serial breaths. During scanning, epicardial coronary artery motion is frozen, and blurring of vessel borders due to motion un-sharpness is minimal.

EBT measurement offers four ideal aspects for detecting and quantifying coronary artery calcification: 1) rapid, 2) consecutive, 3) thin-slice (1.5 to 3.0 mm) tomographic scanning during a predefined phase of the cardiac cycle without any blurring by cardiac motion. It gives three-dimensional image, and there is no need for intravenous administration of contrast medium. EBT can also measure the amount of coronary calcium which is positively correlated with the amount of related atherosclerotic plaque ($r > 0.90$) (Rumberger et al., 1995) and plaque volume (Sangiorgi et al., 1998).

Agatston et al (1990) developed a calcium- scoring algorithm for EBT images now widely using in research and clinical setting. The “calcium score” is a product of the area of calcification per coronary artery segment and a factor ranked 1 through 4 dictated by maximal calcium density above 130 (measured as Hounsfield units: HU) within that segment. The calcium score calculation follows: 1= 130-199 HU, 2 = 200-299 HU, 3=300-399 HU, and 4= over 400 HU. The calcium score is the product of slicing factor for the peak density in a lesion and the lesions are in mm^2 . The score for an individual is the sum of lesion scores. A calcium score is calculated for a specific coronary artery, or for the entire epicardial coronary system (left main, left anterior descending, left circumflex, and right coronary artery) as composite score (Rumberger et al., 1996).

Limitations exist to the use of EBT as a prediction or diagnosis tool of CAD. There are wide ranges of variation in protocols for performing EBT, and it may lead to problems with its reproducibility and accuracy. In a few prospective studies indicated that EBT could not detect small lesions or culprit lesions. Furthermore, EBT do not provides physiologic or functional data (e.g. no information about left ventricular

function). Finally, the prevalence of coronary calcification depends on age and gender. Janowitz et al. (1993) demonstrated that the coronary artery calcium increased with aging regardless of gender. Asymptomatic women showed lower prevalence of calcium than men until approximately 70 years old. Thus the application of EBT measurement on different age and gender should be cautious.

6) EBT: Relationship to other techniques

Coronary calcium scores measured by EBT are highly correlated with a number of noninvasive or invasive quantification methods (angiography, $r=0.85$ (Guerci et al., 1997); intravascular ultrasound, $r=0.75$ to 0.90 (Chou et al., 1996); autopsy, $r=0.93$ (Rumberger et al., 1995). (Table 5)

Histopathological studies confirm that EBT-qualified calcium directly correlates with the extent of atherosclerotic disease regardless of age or gender. The quantification of calcium was found to be comparable to coronary angiography for prediction of coronary artery disease with the effect of established risk factors in study of 211 patients using both angiography and EBT (Guerci et al., 1997). In addition, EBT was found to offer improved discrimination over conventional risk factors in identifying individuals with angiographic CAD in a study of 368 symptomatic patients (mean age 54 ± 12 years, 211 men and 157 women) without a previous history of CAD (Kennedy et al., 1998). Coronary calcium (p -value < 0.0001) measured by EBT as well as male sex (p -value < 0.05) stronger independent predictor of angiographic coronary disease than any other risk factors such as diabetes, hypertension, high cholesterol, and smoking. Breen JB et al (1992) reported that sensitivity of detecting any calcium was 100%, and specificity was 47% confirmed by angiographic result.

In comparison studies of pathologic and electron beam computed tomographic results, Simons et al (1992) reported a good correlation between the total atherosclerotic plaque and the area of calcification. Yet, the degree of lumen narrowing (stenosis) was significantly different even when the degree of calcification was similar.

Baumgart and colleagues (1997) compared the EBT imaging and IVUS method in 56 patients. 97% of patients having significant IVUS results have positive calcium score by EBT method. Among patients without any significant plaque by IVUS, 25% had coronary calcification while 47 % of patients having soft plaque showed significant calcium deposits.

The sensitivity of EBT for obstructive coronary artery disease is above 95%, and was 99% for multivessel disease in a large multicenter study (Budoff et al., 1996). In comparison, a study of helical CT to angiography demonstrated a 88% sensitivity for obstructive coronary disease (Broderick et al., 1996). Another study by Shemesh et al. (1995) observed the sensitivity of 91%, and the specificity of 52%. In a recent comparison study (EBT Vs. Spiral CT), Budoff et al (2001) reported better accuracy of EBT in the measurement of relatively small calcium lesions. Among 33 asymptomatic subjects (10 women, 23 men; mean age 54 ± 9 years), 9 persons had zero scores on either EBT or spiral CT. Three persons had negative EBT studies with positive SEQ results, while 6 persons had positive detected calcium by EBT but negative SEQ results (mean score difference EBT versus SEQ: 47 ± 25.7 (range 9 to 99)). These authors supported that EBT is more sensitive method than SEQ. Even the advances of faster acquisition times, the false negative rates (26%) by spiral CT were most likely the result of motion artifacts and partial voluming effect of lesions. Carotid B-mode sonography measures intimal-medial thickness (IMT) of the carotid arteries. IMT correlates with carotid arteriosclerosis, which in turn relates to major hard coronary events (MI, stroke) (O'Leary et al., 1999). Although IMT is an independent validated predictor of CHD events from studies with large cohorts and long-term follow up, it is a highly operator-dependent technique. Studies in men and women show that the highest CHD event rates occur in those with the highest IMT. Seese et al (1998) compared the ability of EBT and carotid ultrasound in predicting the coronary atherosclerosis. Moreover, IMT (range 0.81-1.21 mm) were similar across the coronary calcium quartiles in older population (Newman et al., 2000)

Table 5. Comparative studies (EBT) with other measurements

Investigators	Population	Age (year)	Measurement	Comparisons	Finding	Comments
Kajinami (1995)	JN, 174 men, 77 women	16 –86 years	EBT	Eletrocardiography, exercise testing	High sensitivity (0.77), high specificity (0.86)	No significant difference to arteriography, but significant different to stress test
Mautner et al. (1994)	50 autopsy heart specimen from men aged 30-69 with known cause of death; 4298 segments	N/A	EBT	Histomorphometric examinataion (% of stenosis)	Highly correlated (r=0.96, p<0.0001) between calcium score and % of stenosis	
Moshage et al. (1995)	24 men, 3 women	50-70 years	EBT	Angiography	Reproducible methods of coronary artery stenoses by constrast enhanced EBT	Contrast-enhanced EBT
Shield et al. (1995)	31 men, 19 women	31 – 73 years	2 times measurement of EBT		High test-retest reliability of scans (0.99)	High in LM, LAD, RCA not in CX
Rumberger et al. (1995)	13 autopsy hearts from 8 men, 5 women	17 –83 years	EBT (coronary calcium area)	Histopathology (plaque area)	High correlation in whole heart (r=0.93), coronary arteries (r=0.90)	Threshold value for plaque area to coronary calcium
Budoff (2001)	23 men, 10 women, 4 wks interval bet/w SEQ and EBT	Mean 54 ±9	EBT (3mm)	Spiral CT (SEQ: sequence scan mode)	Range of CAC with EBT (0-175) vs. with SEQ (0-253); 3 negative results(EBT) vs 6 negative results (SEQ) Sensitivty (74%), specificity (70%)	Limited overall diagnostic accuracy 73% of SEQ, limited detection of low calcium

LM: left main, LAD left anterior desending, RCA right coronary artery, CX the circumflex ; IVUS intravascular ultrasound

7) Accuracy of EBT

EBT demonstrated high reliability to detect coronary artery calcification in same subjects (Shields et al., 1995; Devries et al., 1995) Devries et al. (1995) reported that variability in subsequent scans showed different ranges dependent of total calcium score (< 10, variability 72% ; 11-100, 60 %; >100, 28%). In a study of scanner, and patient variation in EBT, in vitro and in vivo estimate of calcium deposit had a precision of 2% and 7%, respectively (Mcollough et al.,1995). According to a recent meta-analysis to estimate the accuracy of EBT in diagnosing obstructive CAD, pooled sensitivity for EBT was 92.3% (95% CI, 90.7% -94.0%) with moderate specificity of 51.2% (95%CI, 47.5%-54.9%) (Nallamothu et al., 2001). Using summary receiver operating characteristic (ROC) curve, maximum joint sensitivity and specificity was estimated as 75 % from this combined of 9 studies with 1662 subjects. Thus, investigators concluded that EBT as a diagnostic test for obstructive CAD is valid based on sensitivity and specificity rates.

8) Reproducibility of EBT

Raggi (2001) stated that EBT is the 'gold standard ' for calcium detection in coronary arteries. It has been described as repeatable and very rapid measurement. Reproducibility of EBT was characterized. Shields et al (1995) assessed the reliability of EBT in detection of calcium deposits. 50 participants underwent two different EBT measurements an average of 12 minutes apart. Among 34 subjects having coronary calcification, the reliability of total calcium score and total number of lesions was 0.99 and 0.98 respectively. EBT scoring is operator independent process. Same study investigator also evaluated the inter-rater reliability of detecting calcium deposit. The inter-rater correlation coefficient was 0.98 or higher in sample films from 50 subjects. EBT films from participants were reviewed by two raters, and was confirmed that calcium was reliably scored (Shield et al., 1996). However, there were some discrepancies in detection of very small amount of calcium in two measurements on

same subjects (Shield et al., 1995). Kaufmann et al (1994) also found negligible but disagreements in involving very small lesions on a lesion-by-lesion basis from 25 men aged 23-59 years.

There has been some disagreement of good reproducibility between different image acquisition protocols. Wang et al.(1996) compared different image acquisition protocols (20-slice, 6mm slice thickness protocol; 20-slice, 3 mm thickness protocol; 30-slice, 3 mm thickness protocol) among 324 volunteer subjects and demonstrated the better reproducibility of the 20-slice, 6mm thickness protocol than other two protocols.

One of the most useful applications of EBT to the non-invasive imaging of CAD is its employment for the follow-up of progression of CAD. However, the application of EBT in follow-up study has been challenged by limited reproducibility reflecting progression of calcium deposit (Hernigou et al., 1996; DeVries et al., 1995). EBT measurement using Agatston's calcium score had limited reproducibility and it has not been suggested for follow-up studies. To address more reliable scoring methods, Callister et al (1998) suggested a volumetric calcium scoring method using isotropic interpolation methodology. Volumetric scoring systems slice the calcified lesions (volume) lying between two imaging planes into several sections. Then, the density of each section is mathematical interpolated based on the original imaging planes. The processes are repeated at high speed in all three orthogonal directions. Volumetric calcium scoring method is capable of avoiding partial volume averaging effects, and has a reported inter-scan reproducibility ranging from 9% to 15%. According to Callister and colleagues, the reproducibility and accuracy of the volume score was consistently greater than Agatston score (39.5 % overall reduction in error). This scoring modality is preferred method for follow-up studies (Budoff et al., 2000).

b. Epidemiology of vascular calcification measured by EBT

Although coronary artery calcification is associated with hard coronary future events, the influence of calcification on biomechanical plaque stability, which may rupture and result in myocardial infarction, is still unclear (Stary et al., 1995). The stiffness of calcium plaques especially on marginal area was proposed to induce an adverse stress distribution leading to increased tendency of rupture. On the contrary, Lee and others suggest that calcification is only a marker for the extent of atherosclerosis, or process of infection not related to plaque stability (Lee et al., 2000; Huang et al., 2001)

An attempt to describe the normal distribution of coronary calcification in asymptomatic population has been done. Raggi et al (2000) reported the nomogram of calcium score in 9728 asymptomatic subjects (5433 men, 4297 women) who underwent EBT imaging at EBT Research Foundation (Nashville, Tenn) (Figure 3 and Figure 4). Men show a rapid increase in extent of coronary calcification after age 40. Women demonstrate a slower increase of calcium score and smaller average calcium than in men. This trend is similar to the result of previous study (Janowitz, et al. 1993), and women show significantly greater time lag up to 10 to 15 years than men in extent of calcium scores.

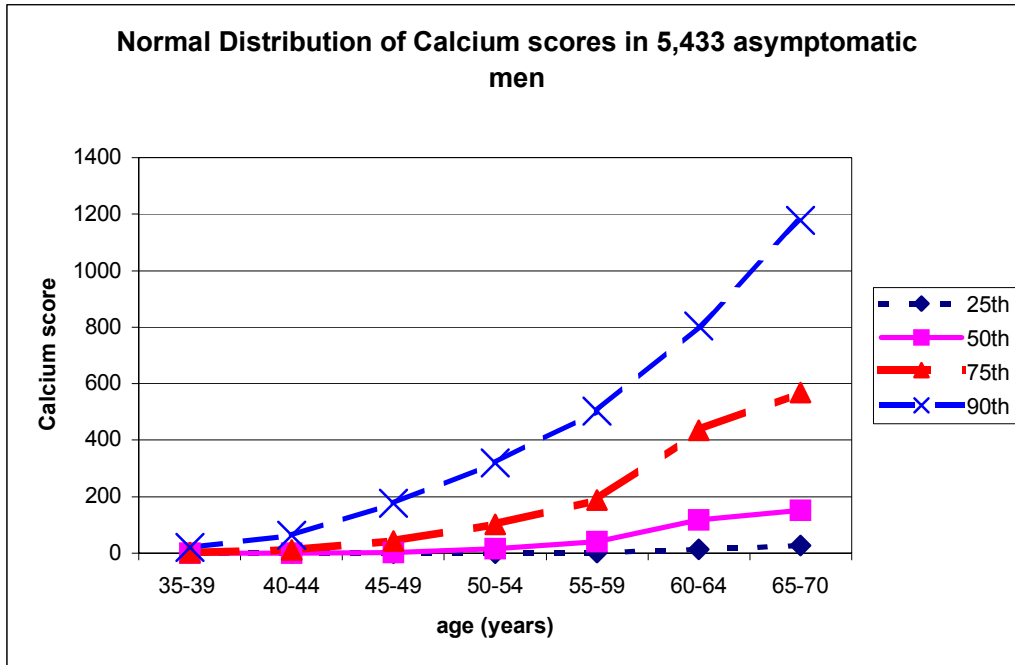


Figure 3. Normal distribution of calcium score in men (modified from Raggi et al. 2000. Circulation 101:850-855)

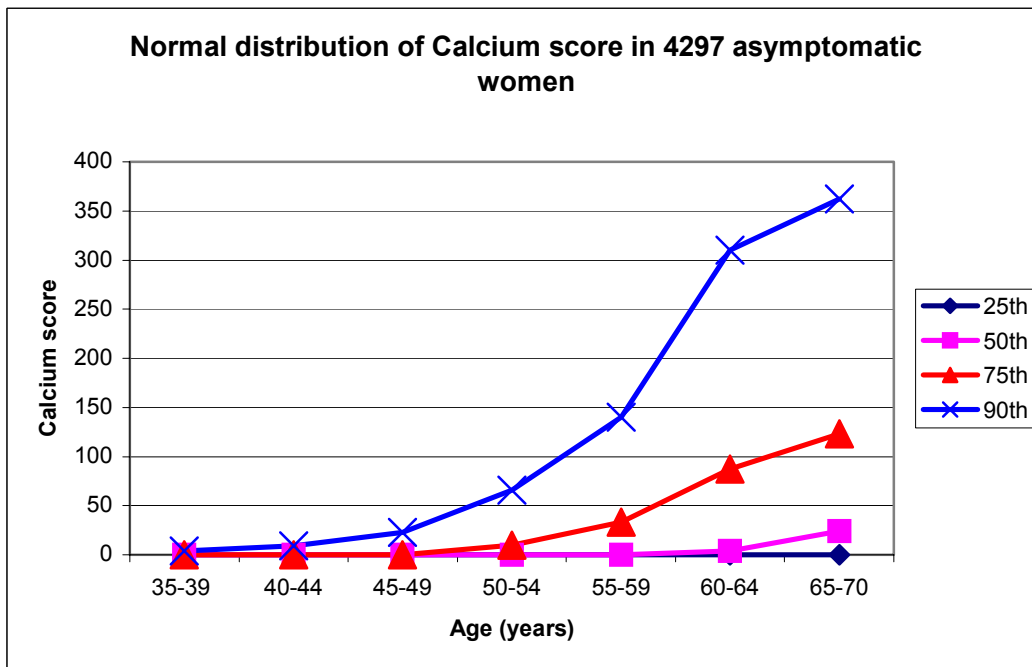


Figure 4. Normal distribution of calcium score in women (modified from Raggi et al. 2000. Circulation 101:850-855)

1) Measurement of CAC for prediction future events

Clinical studies have demonstrated both positive (Arad et al., 1996) and negative association (Detrano et al., 1999) between coronary artery calcification and acute coronary events. According to epidemiological and postmortem studies, calcium plaques are associated with a 10 to 100 times higher risk of coronary heart disease compared to for those who have non-measurable or negative calcium score. Budhoff et al. performed a multicenter EBT study of 710 patients: 95% of patients who had significant angiographic disease showed coronary calcification. A study of 1289 asymptomatic patients during 19 month demonstrated a 6.9 fold increase of myocardial infarction and cardiac death in patients with calcium score over 50 compared to those with zero or lower scores (Detrano et al., 1997).

Arad et al (2000) followed 1173 asymptomatic patients for an average of 3.6 years. A total of 39 cardiovascular events (death, MI, or stroke) were recorded. Patients with EBT score of over 160 had Patients with 'hard' events had a higher calcium score at screening visit compared to patients without events (median calcium score: 764 Vs 135, $p < 0.0001$). There were threshold effect of EBT coronary calcium, and greater than 160 of calcium score group was associated with an odds ratio (22.2) of developing events. Interestingly, none of conventional risk factors (low HDL-c, hypertension, diabetes, or family history) was significantly related to subsequent events. Raggi et al (2000) measured coronary calcification among 632 asymptomatic patients who had MI. Coronary calcification was higher in post-MI 172 patients within 60 days (average follow-up 32 ± 7). A total of 19 MI events and 8 deaths were recorded. Majority of events were occurred to individuals having 75% percentile of calcium score. Further, the event rate in the upper quartile of calcium score percentile was 20 times higher than in the lowest quartile of calcium. Interestingly, they also demonstrated the higher risk of having events in upper quartile of risk factors ($RR=0.09$ in 1st quartile of risk factors vs. $RR =1.05$ in 4th quartile). Compared to conventional risk factor assessment, EBT showed improved assessment of persons with angiographic coronary disease. Risk of CVD events was detected at least 3-fold higher in (Detrano et al., 1996).

Recent investigations have more convincingly demonstrated the usefulness of EBT as a noninvasive evaluation of the prediction of future coronary events, or the relations between traditional or emerging cardiovascular risk factors and CAD. The modification of “Framingham Global Risk Score” was introduced by Grundy (2000). Based on individual’s calcium score percentile, a weighted factor of age categories for a given subjects (different from men and women) was introduced. Grundy suggest that each percentile of calcium adjusted for age factor in Framingham algorithm (-2 in 0-24th; -1 in 25-49th; +1 in 50-74th ; + 2 in 75-89th; +3 in >90th) need to apply to generate total CAD risk assessment . It is currently the most accurate way to estimate the severity and extent of coronary arterial disease non-invasively in a population study.

Coronary artery calcification may be a good predictor of cardiovascular events. Nallamothu and coworkers estimated the pooled sensitivity and specificity of 9 published papers regarding to coronary calcium measurement and obstructive CAD (\geq 50% angiographic stenosis), and concluded that EBT measuring calcium might be an effective diagnostic test for predicting an individual’s likelihood of future CAD events (Nallamothu et al., 2001). Thus, some of discrepancy for prediction future CAD events might be related to the differences in the characteristics of the study populations (Pitt and Rubenfire, 1999)

2) Progression or regression of calcification

The extent of coronary calcium correlates closely with the burden of the underlying atherosclerotic plaque (Sangiorgi et al., 1998). Therefore, the application of EBT on monitoring the progression of CAD was appealing to the researchers and clinicians. Many studies have studied the progression or even regressions of calcium score (calcification) in relation to the medical treatments such as lipid lowering drug, HMG-CoA reductase inhibitors (Table 6).

Janowitz and colleagues reported a significant difference of 48% in 10 patients with angiographically proven CAD with 28% in 10 asymptomatic patients (1991). Budoff et al (2000) followed 299 asymptomatic individuals (227 men, 72 women) who had sequential EBT screenings up to 6.5 years. On consecutive EBT measurements, they found increased annual changes in the calcium score (Agatston score, $33.2 \pm 9.2\%$ per year) regardless of lipid lowering treatment. Interestingly, annual changes in calcium score were significantly different in statin treated group ($n=62, 15 \pm 8\%$) compared to the untreated subjects ($n=61, 39 \pm 12\%$). The net reduction of calcium volume score was only observed in treatment patients who had LDL below 120 mg/dL at first EBT scan. There was no significant gender difference. In a study of 82 consecutive participants (51 men; 37 women) in Rochester, MN, investigators reported a mean annual progression of coronary calcification of 24% in 82 healthy subjects (mean age 46 ± 7 years) following an average of 3.5 years (Maher et al., 1999). The average coronary calcium score increased from 18.4 to 44.6 ($p < 0.05$). Relative changes were lower in older subjects than in younger group, but there was no difference by gender. High correlation between calcium score over time ($r = 0.71, p = 0.001$) was observed.

Shmermund et al. (2001) followed 102 symptomatic patients (aged 59 ± 9 ; men ($n=87$) who had sequential EBT imaging, medical treatment, and other risk factors (e.g. LDLc, HDLc, or fibrinogen). At the end of an average follow-up period (mean duration 18.2 ± 12 months), the calcium area and score of progression were recorded. Among 4 patient groups based on baseline calcium plaque burden, the significant progression in calcium score was detected based on baseline calcium score. Interestingly, the lowest calcium score group at baseline showed the highest changes in calcium score (baseline calcium score (1-30), changes 57% vs. calcium score (>400), 15%; linear trend $p < 0.05$). Using volumetric score, Callister et al (1998) also assessed the progression of calcification in asymptomatic high-risk patients (men, $n=92$; mean age, 56 ± 11 years) with HMG-CoA reductase inhibitor treatments.

Table 6. Progression of coronary calcification in relationship with CAD

Authors (study)	Population	Age (years)	Measurement*	Findings	Comments
Schmermund (2001) Germany	CA, 102 symptomatic pts (80% male), average follow-up(FU) 18±15 mos	Mean 59± 9	CAC (EBT, 3mm), calcium area in 12 major coronary segments	Significant trend in absolute change (greater in highest group (> 400 CAC) ; relative changes opposite direction (sig.)	LDLc is not sig. predictor of progression of CAC
Callister (1998), Retrospective	149 (61% men) asymptomatic average FU 12 mos	Age 32 to 75 years	CAC (EBT, 3mm volume score), cholesterol (LDL), history of lipid-lowering tx (Statins)	Statin tx group had 5 ± 15% of progression compared to unTx patients (52±36%, p<0.001)	Statin Tx groups lower progression,
Maher (1999)	CA, 82 healthy subjects (51 men) at least 2.5 yr	Mean 46± 7	CAC(EBT, 3mm at 1 st & 2 nd), baseline risk factors (Chol, SBP, BMI, smoking, Hypertension)	Sequential correlation of CAC measurement (r=0.71, p<0.0001) Annual changes (24% range 18.4 to 44.6, p<0.05); Relative changes is lower in older than in younger	Independent of age, and sex
Budoff (2000)	299 asymptomatic (277 men, 72 women) FU 1-6.5 yrs	Mean 58±10 years	CAC (EBT, 3 mm), history of lipid lowering treatment, baseline risk factors (hypertension, smoking, family Hx, gender, Cholesterol)	Average annual change 33.2 ± 9.2%; Statin Tx group (15±8 %)vs. untreated patient (39±12%)	No significant interaction bet/w CAD risk factors and CAC changes
Raggi (1999)	269 asymptomatic limited FU 2.5 yr	Mean 53	CAC(EBT< 3mm), CAD events	23 events in patients having progression of CAC.	
Sutton-Tyrrell (2001) HWS study, Cohort	80 women postmenopausal women, average of 18 months apart	Mean 63 years at first scan	Serial EBT (3mm at 1 st scan, 6 mm at 2 nd visit)	Significant average changes in coronary (Δ 11, p < 0.001). '0' calcium scores tend to be '0'; extent of coronary calcium related to progression of calcium in aorta (p=0.013)	HDLc, triglycerides, smoking at 1 st scan visit significantly related to increased calcium group

*: Calcium volumetric score was indicated

The average LDL-c level was correlated with the relative change in volume score during the follow-up (range 12-15 months). The patients without treatment (n=44) had mean final volumetric calcium score of $52\pm 36\%$ with mean LDL-c level of 147 mg/dl. Contrarily, the calcium score change for all treated patients (mean LDL-c, 114 ± 23 mg/dl) was $5\pm 28\%$, and it was significantly different from untreated patients. When they looked at the rate of progression based on baseline score (large group, > 400 calcium score; moderate, 100-400; small, < 100), they found similar progression pattern in untreated patients like Shermund and others. While treatments halted progression of calcification similarly across the score group, the progression among small to moderate calcium group showed bigger changes ($63\pm 37\%$) than among the patients with large score at baseline ($29\pm 33\%$, $p < 0.03$).

Study of coronary and aortic calcification, Sutton-Tyrrell et al (2001) recently reported significant increase of coronary and aortic calcification in postmenopausal women aged 63 years over a period of 18 months. In this community-based study (n=80), significant average annual changes in coronary ($\Delta 11$, $p < 0.001$), aortic ($\Delta 112$, $p < 0.001$) calcium were detected in only women with baseline calcification. Women who had zero calcium scores at the first EBT measurement tended to maintain zero calcium score at consecutive EBT imaging. Furthermore, the extent of coronary calcium was related to progression of calcium in aorta ($p=0.013$). HDL-c ($p=0.004$), triglycerides ($p=0.005$), prevalence of smoking ($p=0.036$) at 1st scan visit significantly related to increase of calcium group (n = 27) compared to 53 no-change participants.

Even with the treatment, the patients showed continuing progression of calcification. These study evidences may be compatible with the results from animal studies. Human atherosclerotic lesions decreased with treatment, however, it is not known which of the components in the lesions (e.g. calcium volume) regressed. The series of animal studies demonstrated continuing increase in calcification with diminished extracellular lipids, and absent of macrophage and macrophage foam cells after the regression diet (Stary 2001; Clarkson et al., 1981). Therefore, the future studies will need to investigate the true effect of intervention methods, and the complete

understanding of vascular calcification mechanism in context of atherosclerotic regression.

3) Risk factors for coronary calcification (Table 7)

i) Age and gender

An increasing number of studies in coronary calcium progression with EBT measurement has assessed the relationship between traditional risk factors (Framingham Risk factors) and the extent and progression of calcium (Callister et al., 1998; Budoff et al., 2000; Schmermund et al., 2001). Age and gender are the most important influencing factors for the prevalence of coronary calcification. The presence of calcium (>0) ranges from 15 % to 93% in 675 men (< 40 – 15% positive calcium; 10 years period of age, 45%, 67%, 83%, 93%) and 27% to 75% in 190 women (Wong et al., 1994). Other studies reported a higher prevalence of calcium deposition in men than in women (Agatston et al., 1991; Janowitz 1993). Figure 5 summarized the gender difference in symptomatic men and women with or without significant coronary symptoms ($\geq 50\%$ stenosis) (Haberl et al., 2001). Furthermore, gender difference in prevalence of coronary calcification was observed even in childhood and younger population. Mahoney et al (1996) reported the coronary calcium deposition of 31% in men (191 men) and 10% in women (187 women). Maher and colleagues (1996) reported that the relative increase in calcium score was significantly higher in younger participants than in older groups (a 40-year-old man, 55% increase vs. a 50 year-old man, 24% increase). An interesting study of the influence of gender and age on the rate of progression of calcium score more EBT screening, and their mean interscan interval was 25.9 months. Men had a significantly higher rate of change of calcium score compared to women (2.7 versus 1.3). When grouped by age, only women aged of 40 to 49 year-group had a significantly different rate of progression in calcium score than men (0.2 versus 0.7, $p=0.04$). Other age groups (e.g. 50 years or older age groups) did not show any significantly gender difference in rate of progression.

Table 7. Coronary calcification in asymptomatic population: relation to conventional risk factors

Investigators	Population	Age (years)	Measurement	Findings	Comments
Wong et al (1994)	675 men , 190 women	22 to 85	Coronary calcium (EBT), Self administered cardiovascular risk factors	Significant relationship to age, hypertension, male gender, diabetes, hypercholesteolemia, obesity	Graded pattern with the number of risk factors
Taylor (2001) PACC study Prospective study	630 men and women (82% male)w/o known CAD	Mean: 42 years (39-45 yrs)	CAC (EBT, 3mm thickness), Framingham risk factors (e.g. cholesterols, smoking, hypertension)	Prevalence of 20.6 % in male, significant relationship between CAC and LDL (AUC, 0.61 ± 0.03 , $p < 0.001$), CAC in men increased across FRI quartiles (17%, 20.8%, 33.0% and 29.2%, $p = 0.033$)	Family hx, homocystein, insulin, Lp (a), fibrinogen were not related to CAC
Iribarren (2000) CARDIA study Prospective cohort study	374 men (91 W/91 B) and women (77 W/110 B)	18 to 30 at baseline	CAC (EBT, 3mm) at 10 yr exam; Cook-Medley hostility assessment at baseline and 5 yr exam	13% of sample had calcium score (> 0). 1SD difference (8.2 units in hostility scores, adj) related to any calcium score (OR 1.48, 95% 1.0-2.2); high (>20 units) vs low hostility score for having calcium score (≥ 20) OR 9.6 (95% 2.3-65.9)	Adjusted for age, sex, race, field center (alcohol, smoking, LDL, BMI, SBP) in logistic regression
Newman 2000 CHS, US, Cohort study	60 women, 46men w/o any CAD or subclinical CAD - sub-analysis	Mean 78 years	CRP, calcium score (EBT), subclinical CVD measurements (e.g. ankle-arem index)	No significant difference of CRP across quartiles of coronary calcification	
Hunt 2001 PACC project, Nested C/C	94 control, 94 control men w/o CAD symptoms: CAC presence	40 to 45 years	CRP, smoking, calcium score (EBT)	No significant difference of CRP in men w/w calcium, and w/o calcium; total cholesterol, LDL	

FRI : Framingham Risk Index, AUC: Area under receiver-operator characteristic (ROC) curve , hx : history, Lp(a) : lipoprotein(a)

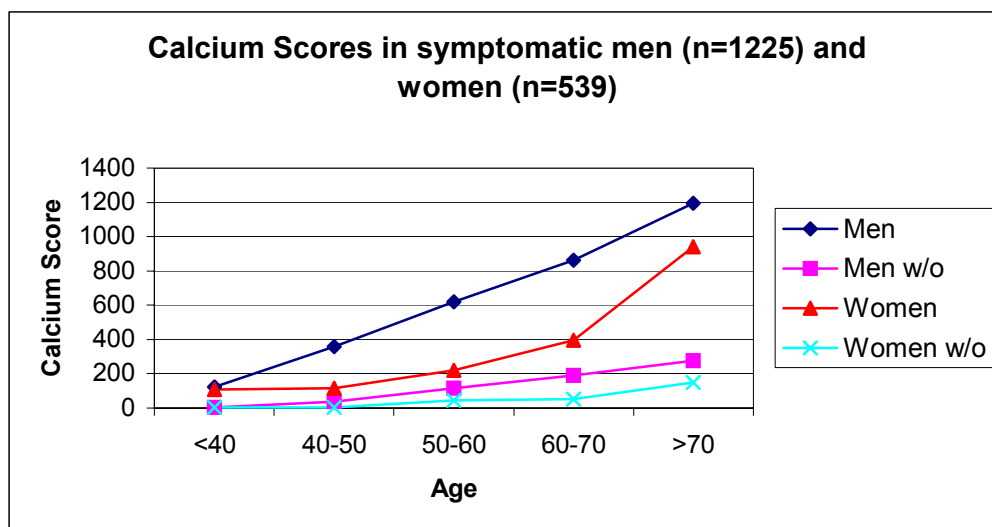


Figure 5. Calcium score in symptomatic men and women
(Modified from Haberl et al., JACC 2001;37:451-457)

Rumberger et al. (1996) pointed that coronary artery calcium detected by EBT follows a pattern similar to that of the gender difference in prevalence of CAD. There are 10-year lag between men and women in terms of quantity of coronary calcium (Janowitz et al., 1993). Women have lower coronary calcium until they approach to the age of 70 years. After this period, there is same prevalence of coronary calcium between women and men. Gender difference in coronary calcium levels support the current concept of gender gap in CAD; The prevalence of disease in women reaches that of men during the first decade after menopause or about the age of 65 years.

Considering age and gender effect on calcification, Kung and Detrano (1996) emphasized the diagnostic and prognostic significance of coronary calcium more in symptomatic, older, and high-risk population than in younger subjects.

ii) Lipids - LDL-c

Several studies have examined for lipids as risk factors associated with coronary artery calcification. Elevated plasma LDL levels have been shown to be associated with

coronary calcification in younger population, postmenopausal women or elderly men (Mahoney et al., 1996; Hoeg et al., 1994; Kuller et al., 1999). The relationship of LDLc to the extent of coronary calcification is possibly modified by smoking status, hormone replacement therapy. In a longitudinal analysis, Kuller et al (1999) demonstrated that the significant relationship of systolic blood pressure, elevated baseline triglyceride levels (69% of no calcification in group of < 100 mg/dL), and waist circumference (27% in women with < 80cm compared to 71% in women with > 88 cm, $p < 0.001$) with the extent of coronary calcification in 169 women (mean age 59 years, follow-up 11 years). Spearman correlation between ApoB, HDLc, or systolic blood pressure and coronary calcification were 0.21 ($p = 0.007$), -0.28 ($p < 0.001$), 0.17 ($p = 0.028$), respectively (Kuller et al., 1999).

iii) Smoking, and blood pressure, and obesity

Cigarette smoking, elevated blood pressure, obesity (higher BMI) also has been related to coronary calcium deposition, or progression of calcium (Mahoney et al., 1996, Maher et al., 1994; Kuller et al., 1999; Budoff et al., 2000)

iv) Inflammation marker: C-reactive Proteins (CRP)

There are numerous studies investigating the interaction between some non-lipoprotein factors such as homocysteine, fibrinogen, and C-reactive protein and coronary calcification. Redberg et al. (2000) investigated whether the CRP was positively correlated with coronary calcification in postmenopausal women. In the 172 postmenopausal women, they found no association of high-sensitivity testing for C-reactive protein (hsCRP) to calcium score by EBT. Three divided groups based on calcium score (0 to 10; > 10 to 50 ; > 50) showed no difference (0.24 ± 0.43 ; 0.33 ± 0.47 ; 0.17 ± 0.32). They demonstrated a full range of CRP levels in women without calcification.

Newman et al. (2000) reported that the levels of CRP did not differ by coronary calcification in 106 subjects (mean age 78 years) without clinical or subclinical CVD. Hypertension and smoking were significantly related to calcification. Hunt et al. also reported the non-significant relationship between serum levels of CRP and the presence of calcification in middle aged 188 men (age range 40 to 45 years) enrolled in the Prospective Army Coronary Calcium (PACC) Study.

v) Coagulation factor -- Fibrinogen

Plasma fibrinogen is a significant independent factor of coronary heart disease (Danesh et al., 1998). In the community-based Epidemiology of coronary artery calcification (ECAC) study, Bielak and colleagues (2000) demonstrated the significant association between plasma fibrinogen concentration and high amount of coronary calcium (at or above the 80th percentile of calcium at one's sex and 10 year age group). 1-SD increase in fibrinogen levels was significantly associated with an odds ratio of 1.6 (95% CI: 1.1-2.5) of having high quantity of coronary calcium in univariate model, but there was no longer significant after adjustment of multiple risk factors, such as age, BMI, systolic blood pressure (Odds Ratio, 1.3; 95%CI 0.8-2.2). In women, an increase of 1 SD in fibrinogen as well as interaction term between fibrinogen and age were significantly related with high amount of calcium without and with adjustment of other risk factors (p-value < 0.0001).

vi) Psychological factors

In studies of psychological traits and CAD, depression, anxiety, and hostility have reported to be associated with the risk of CAD. Efforts to investigate these relationship has expanded to explore the relation between psychological variables and coronary artery calcification in asymptomatic population (O'Malley et al.,2000). Among several traits, however, only somatization (the number and severity of durable physical

symptoms) is inversely related to coronary calcification ($r=-0.12$, $p=0.003$) in the total of 630 participants (mean 42 ± 2 years). Conversely, hostility at study baseline was related to the prevalence of calcification in much younger population (aged 18 to 30 years) (Iribarren et al., 2000). The significance were persistent even after adjusting for age, sex, race and concomitant unhealthful lifestyle behaviors (e.g. smoking, alcohol consumption). Those with hostility scores above 20 units had significantly greater odds of having any coronary artery calcification (OR=2.38, 95% CI 1.12-5.20) or having greater than 20 coronary calcium score (OR=9.56, 95% CI 2.29-65.9) than those in below 20 hostility scales.

vii) Treatment effects: Lipid lowering drugs

Lipid lowering drugs, especially treatment of statin, has been related to delay the progression of coronary calcification in asymptomatic individuals (Callister et al., 1998). Callister and colleagues (1998) observed that LDL cholesterol reduction treatment (HMG-CoA reductase inhibitors) led to a significant mitigation of progression of calcium score in 149 individuals. Asymptomatic patients who received treatments demonstrated stabilization of calcium volumetric score ($5 \pm 28\%$) compared to the progression of volume score ($52 \pm 36\%$, $p<0.001$) in all untreated patients. Among the treated patients, sixty-five treated subjects who maintained an LDL-c < 120 mg/dl showed the decrease ($-7\pm 23\%$) in mean overall mean calcium volume compared with treated subjects with LDL-c > 120 mg/dl (p -value <0.001). Budoff et al (2000) also followed 299 individuals (227 men and 72 women) with several CAD risk factors for a period of 1 to 6.5 years. They reported that an average annual progression was similar among untreated patients regardless of baseline risk factors (e.g. smoking, hypertension, or family history of CAD). However, calcium score were significantly lower in patients with treatments of statins (15 ± 8 % per year) compared in untreated groups ($39\pm 12\%$ per year; $p< 0.001$). Thus, they conclude that statin therapy induced a 61% reduction in the rate of coronary calcification progression.

On the contrary, the use of lipid lowering drugs did not differ between patients with higher versus lower baseline amounts of calcium (63% vs 72%) in a recent study by Schmermund (2001). Use of treatment was not different in patients with progression of calcification (80%) compared to others (62%, $p=0.3$). Interestingly, LDL-c was not significantly associated with prediction of calcium in arterial segments, but LDL-c showed a tendency of greater progression in patients having above the median level of lipids compared with patients below the median levels (142 ± 44 mg/dl vs. 130 ± 33 mg/dL, $p=0.1$) (Schmermund et al. 2001). Moreover, this study indicated that baseline calcified plaque alone is an independent predictor of progression of atherosclerotic calcification. The studies of lipid lowering drugs showed that the effects of HMG-CoA reductase inhibitors on the progression of calcification were relative to the effects of drugs on LDL-c levels. Even among treated patients, however, the progression of calcium score was continued.

The amount of calcification measured by EBT is related closely to the amount of plaque and plaque burden. EBT can detect sensitively significant luminal disease obtained at histologically, and predict the presence of angiographically significant disease in patients with symptomatic coronary disease. However disadvantage of EBT are listed as follows: the calcium score is not directly correlated degree of luminal narrowing (stenosis) and provides little advantages over traditional methods for investigating patients with definite symptomatic CAD.