BIOMARKERS AND RISK FACTORS OF ATHEROSCLEROSIS AMONG MIDDLE-AGED MEN IN AN INTERNATIONAL POPULATION-BASED STUDY

by

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ABSTRACT

This manuscript examines three separate but inter-related research questions. After reviewing scientifically relevant literature in the first chapter, the second chapter compares the prevalence of carotid plaque among the three major race-ethnic groups in the Electron-beam computed tomography, Risk factor Assessment among Japanese and U.S. Men in the Post-World War II birth cohort (ERA JUMP Study). This study shows that prevalence of carotid plaque, a biomarker of subclinical atherosclerosis, is significantly lower among men in Japan and South Korea than in the US. This difference is independent of traditional risk factors of coronary heart disease (CHD). Further, it shows that only age, hypertension and diabetes are cross-sectionally associated with the prevalence of carotid plaque.

The third chapter examines the association between brachial-ankle pulse wave velocity (*ba*PWV) and coronary artery calcification (CAC), an established biomarker of coronary atherosclerosis and a strong predictor of future CHD risk. *ba*PWV is a non-invasive and convenient measure of arterial stiffness and is clinically used in eastern Asia as a tool for assessing future cardiovascular risk. This study found that higher *ba*PWV is cross-sectionally associated with

higher prevalence of CAC among middle-aged men in the ERA JUMP study, including White men in the US.

The final and fourth chapter of the manuscript examines the association of serum levels of soy isoflavones and equol, with CAC among Japanese men in Japan. Japanese consume soy and soy products regularly. Isoflavones are a component in soy and are known to have anti-atherosclerotic properties. Equol is a potent isoflavone produced from the dietary isoflavone daidzein by action of intestinal bacteria. This study shows that individuals who have bacteria to convert daidzein to equol, i.e. equol producers, have lower CAC than equol non-producers.

These three studies, individually and as a whole, contribute significantly to public health knowledge. Japan has significantly lower atherosclerosis than the US in spite of a similar level of risk factors. Several lessons, including change in diet, may be learned from Japan in an effort to reduce CHD mortality in the US. *ba*PWV may potentially provide similar information to clinicians as CAC without exposing the patient to radiation.

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PREFACE

Coronary heart disease (CHD), although declining, still remains the leading cause of mortality in the US. Although risk scores exist for predicting future CHD among individuals based on their risk factor profile, these risk scores fail to identify many individuals at low to intermediate risk of CHD and who would be candidates for targeted interventions. Thus, several subclinical disease biomarkers have received attention in the past two decades; these biomarkers can further identify individuals who have high risk of CHD and who may benefit most from these preventive interventions. Examples of such biomarkers are – coronary artery calcification (CAC), carotid intima-media thickness, carotid plaque, pulse wave velocity, aortic calcification and ankle brachial index.

Although CAC has emerged with highest potential for translating into clinical practice, it exposes the patient to harmful radiations. In contrast, brachial-ankle pulse wave velocity (*ba*PWV) is a non-invasive and convenient measure of arterial stiffness and is widely used by clinically in eastern Asia as a predictor of CHD. *ba*PWV carries the potential to be accepted clinically in the US, and deserves thorough examination.

In contrast to the US, CHD rates are low in Japan – this is paradoxical given similar or higher burden of some CHD risk factors exist in Japan as in US e.g. smoking and hypertension prevalence is higher in Japan. A comparison of prevalence of carotid plaque between US and Japan may provide an estimate of their relative atherosclerotic burden. Further, reasons for paradoxically low rates of CHD in Japan are not well-understood. The lower rate of CHD may be due to a

lifestyle factor such as the Japanese diet that is rich in marine derived n-3 fatty acids and soy isoflavones, both known to have anti-atherosclerotic properties.

The following research questions were examined in this manuscript –

1. What is the prevalence and risk factors of carotid plaque among Whites in the US, Japanese in Japan and Koreans in S Korea?
2. Is *ba*PWV significantly associated with coronary atherosclerosis assessed by CAC? and
3. Are serum levels of equol, a soy isoflavones product, associated with coronary calcification among Japanese men in Japan?

This work is dedicated to my wife, Parul, who has been supportive of this work as well as my decision to pursue a doctoral degree. Thank you Parul for always being there and listening to all the rants that I have had over my graduate studies. Thank you for the care that you took of me while taking time out of your own studies. I hope I'll be as good a spouse once I get back there and you start to work on your dissertation. This work is also dedicated to my friend and my guru, Sameer Sharma, who is the source of inspiration for everything good that I try to do. I treasure you being a part of my life. Whenever I have had a doubt, I tried to imagine what you would have done in that situation, and tried to follow your path. I hope I have not disappointed you as a disciple.

I am grateful to my advisor Dr Akira Sekikawa for all I have got from him over the past three years. Akira, you have been a wonderful person and I'm sure I'll appreciate all that I've learned from you over the coming years. Thank you for requesting and meticulously going over multiple revisions of my drafts – I write much better now than I ever did. I look forward to keep working together in the future.

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I also want to pay regards to my other committee members Dr Emma Barinas-Mitchell, Dr Rhobert W Evans, and Dr Matthew F Muldoon, for their support in this endeavor. Emma, I have learned a lot from you over my time here – I still remember my first class in PhD when you introduced these very subclinical biomarkers that I am researching today.

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In addition, I am grateful to all the participants of the ERA JUMP study who have volunteered to provide these measurements in the spirit of scientific progress. I am a participant for an epidemiological research study, and know it requires a significant effort to go through all that we are asked to do as a volunteer. I also want to thank the National Institutes of Health and the respective institutions in Japan and South Korea for their support for this study. I will be indebted to Rona in the Heinz lab for her help in the processing of serum samples for measuring soy isoflavones and equol. I am also indebted to Belinda at the Ultrasound Research lab who

tirelessly and selflessly worked with me to do quality control for the carotid plaque data to be included in this work.

Last, but not the least, I want to say a special thanks to my parents, my brother and Khushboo for their understanding. I promise I'll try to be a better son and a better brother in the future.

1.0 INTRODUCTION

1.1 EPIDEMIOLOGY OF CORONARY HEART DISEASE

Even after continuous and significant decline in Coronary Heart Disease (CHD) mortality achieved in the past five decades, CHD remains the leading cause of mortality in the United States.[1] The deaths attributable to cardiovascular diseases (CVD) declined by 31% between 2000 and 2010, however, CVD still accounts for 1 of every 3 deaths in the US, out of which every other death was due to CHD. One American gets a potentially lethal CHD event every 34 seconds, and one dies of a CHD event every 1 minute and 23 seconds.[1] Thus, reducing the burden of CHD still remains a public health priority.

With the significant decline in CHD achieved over the decades, the American Heart Association (AHA) has started to shift the focus from cardiovascular disease to cardiovascular health to continue this declining trend. In this regard, the AHA has identified 7 components of ideal cardiovascular health as targets for intervention: smoking, body weight, physical activity, healthy diet, total cholesterol, blood pressure, and fasting plasma glucose.[2] (**Table 1-1**) Together, these are attributable risk factors for a large majority of the cardiovascular disease events in the US.[1]

Table 1-1 Components of Ideal Cardiovascular health among adults

Smoking	Never or quit > 12 months ago
BMI	<25 kg/m^2
Physical activity	≥150 min/week moderate intensity or ≥75 min/week vigorous intensity or combination
Healthy diet score**	4-5 components
Total cholesterol	<200 mg/dl
Blood pressure	<120/<80 mmHg
Fasting plasma glucose	<100 mg/dl

*Adapted from Lloyd-Jones, 2010[2]
** Based on 2006 nutritional guidelines[3]

A majority of the deaths among patients with acute coronary event occur before reaching the hospital. Clearly, therapeutic advancements in clinical care of CHD cannot be relied upon to reduce mortality among those who die before reaching the hospital. Thus, in addition to improving the medical care for patients with CHD, it is prudent to identify individuals who have high-risk for future CHD, and to provide aggressive prevention strategies to reduce the CHD risk. One common strategy to identify such individuals is to screen them for traditional risk factors of CHD. Many guidelines and scores exist that take into account risk factors such as gender, smoking, and cholesterol have been established to predict individual's future risk of CHD – most commonly used among those is the Framingham Risk Score which predicts individual's 10-year risk of CHD/CVD.[4] Although such guidelines and risk scores have helped clinicians identify patients with high CHD risk, their prediction can be further improved among individuals at intermediate risk of CHD.[5] This can be especially important given the changing demographics in the US as well with the sustained decline in the total cholesterol levels in the past few decades – both of these being important determinants of the calculated CHD risk. To prevent any reversal of the declining trend

of CHD in US, it is important to further improve strategy to identify and prevent CHD in individuals at risk of CHD. An important strategy to identify such individuals is to examine for the presence of subclinical cardiovascular disease.

In the past two decades, many subclinical CVD markers have been examined for their validity in prediction of future CVD. In general, these subclinical CVD markers identify structural or functional changes that are said to be a result of atherosclerosis or one of the pathophysiological processes associated with atherosclerosis.

1.2 ATHEROSCLEROSIS AND ITS ASSESSMENT

1.2.1 Introduction

Atherosclerosis is the complex pathological process that leads to the development of lesions in the arterial wall. The symptoms and consequences of an atherosclerotic lesion depends on the extent, location, and stability of the lesion, with large unstable lesions in the coronary arteries accounting for a majority of CHD deaths. Because atherosclerosis is the major underlying cause of CHD, a significant effort has been made to understand and characterize the steps that lead to atherosclerosis, to assess the presence of atherosclerosis in the arteries, and to find treatment options to prevent and delay atherosclerosis and its complications.

1.2.2 Pathophysiology of Atherosclerosis

Atherosclerosis is a systemic disease process that begins early, usually by the second decade of life. Even though it is a systemic process, certain regions of the arterial tree are known to be more susceptible to the development of atherosclerosis such as the proximal portion of the left anterior descending artery where it progresses to become one of the most common cause of death in the world, and the bifurcation of the carotid artery i.e. the region around the carotid bulb. The pathogenesis of atherosclerosis involves multiple steps that have been studied in detail[6,7] – (1) deposition of low-density lipoprotein cholesterol (LDL-c) particles in the arterial wall with subsequent oxidation of these particles, (2) recruitment of leucocytes, particularly monocytes and lymphocytes, and the formation of early atherosclerotic lesions, (3) accumulation of LDL-c in the monocyte-derived macrophage and formation of lipid-laden foam cell, and (4) evolution of atheroma with death of macrophage, fibrosis and smooth muscle proliferation. As these atherosclerotic lesions advance, they also accumulate calcium with a process that is similar to bone formation (Figure 1-1)

Along with the process of lesion formation as briefed above, arterial remodeling takes place for much of lesion growth. E.g. until at a late stage of atherosclerosis, the lesion grows outwards with increasing diameter of the arterial trunk, without much compromise of the arterial lumen.[8,9]

The most catastrophic event in relation to atherosclerosis occurs when the fibrous cap of the atherosclerotic lesion ruptures, allowing the blood to come in contact with highly thrombogenic contents of the coronary plaque, and the formation of a thrombus. If the thrombus formation that

ensues is enough to occlude the victim artery, blood flow to the downstream myocardium may completely occlude, thus causing myocardial infarction.[10]

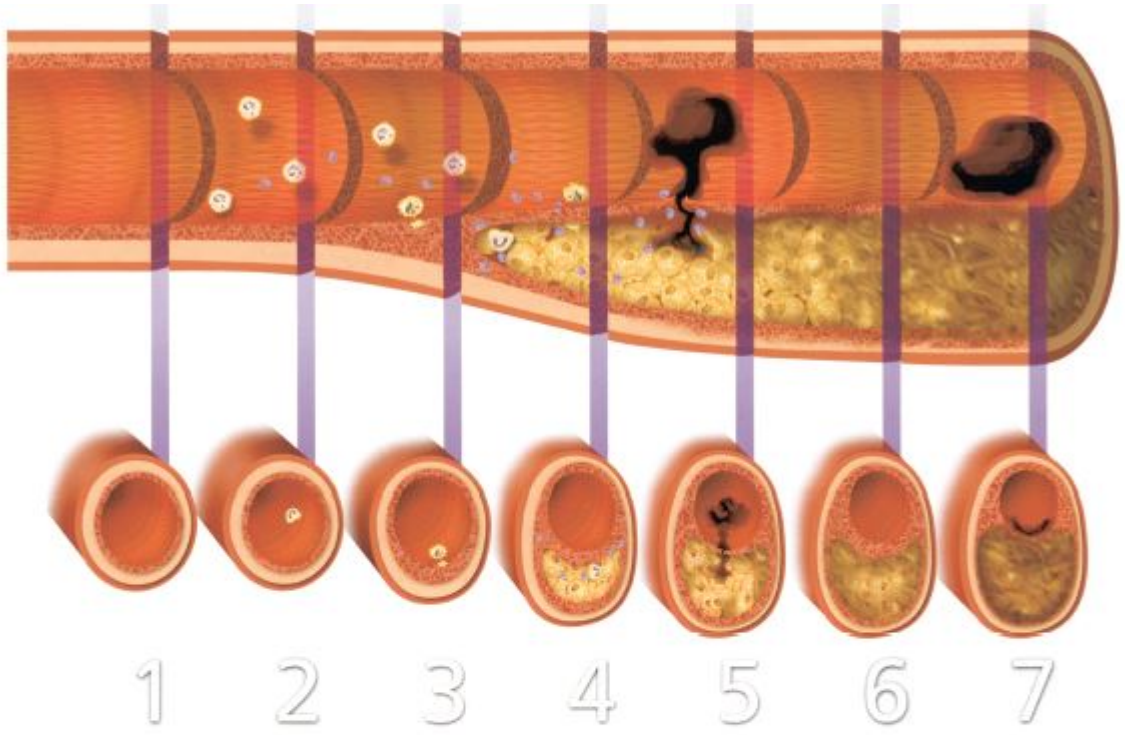

Figure 1. Initiation, progression, and complication of human coronary atherosclerotic plaque. Top, Longitudinal section of artery depicting "timeline" of human atherogenesis from normal artery (1) to atheroma that caused clinical manifestations by thrombosis or stenosis (5, 6, 7). Bottom, Cross sections of artery during various stages of atheroma evolution. 1, Normal artery. Note that in human arteries, the intimal layer is much better developed than in most other species. The intima of human arteries contains resident smooth muscle cells often as early as first year of life. 2, Lesion initiation occurs when endothelial cells, activated by risk factors such as hyperlipoproteinemia, express adhesion and chemoattractant molecules that recruit inflammatory leukocytes such as monocytes and T lymphocytes. Extracellular lipid begins to accumulate in intima at this stage. 3, Evolution to fibrofatty stage. Monocytes recruited to artery wall become macrophages and express scavenger receptors that bind modified lipoproteins. Macrophages become lipid-laden foam cells by engulfing modified lipoproteins. Leukocytes and resident vascular wall cells can secrete inflammatory cytokines and growth factors that amplify leukocyte recruitment and cause smooth muscle cell migration and proliferation. 4, As lesion progresses, inflammatory mediators cause expression of tissue factor, a potent procoagulant, and of matrix-degrading proteinases that weaken fibrous cap of plaque. 5, If fibrous cap ruptures at point of weakening, coagulation factors in blood can gain access to thrombogenic, tissue factor–containing lipid core, causing thrombosis on nonocclusive atherosclerotic plaque. If balance between prothrombotic and fibrinolytic mechanisms prevailing at that particular region and at that particular time is unfavorable, occlusive thrombus causing acute coronary syndromes may result. 6, When thrombus resorbs, products associated with thrombosis such as thrombin and mediators released from degranulating platelets, including platelet-derived growth factor and transforming growth factor-β, can cause healing response, leading to increased collagen accumulation and smooth muscle cell growth. In this manner, the fibrofatty lesion can evolve into advanced fibrous and often calcified plaque, one that may cause significant stenosis, and produce symptoms of stable angina pectoris. 7, In some cases, occlusive thrombi arise not from fracture of fibrous cap but from superficial erosion of endothelial layer. Resulting mural thrombus, again dependent on local prothrombotic and fibrinolytic balance, can cause acute myocardial infarction. Superficial erosions often complicate advanced and stenotic lesions, as shown here. However, superficial erosions do not necessarily occur after fibrous cap rupture, as depicted in this idealized diagram.

Figure 1-1 Pathogenesis of Atherosclerosis

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1.2.3 Biomarkers of Atherosclerosis as Predictors of CHD

A biomarker or a biological marker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[11]

While atherosclerosis can be seen in many arterial beds, it is the presence of atherosclerosis in the coronary and carotid artery that is known to have disastrous consequences, given the relative importance of the organs to which they supply blood. Thus, significant efforts have been made in the last few decades to examine atherosclerosis in the coronary and carotid arteries at the subclinical stage i.e. before the occurrence of a CVD event. This strategy allows identification of individuals who would be candidates for intervention in the form of primary and secondary prevention of the CVD event.

1.2.3.1 Coronary Artery Calcification

Of all the subclinical measures of CVD that have been examined in different research studies for their prediction of CVD event, Coronary Artery Calcification (CAC) appears to be the most promising as a clinical tool for identification of asymptomatic patients at high risk for CVD.[12,13] The American College of Cardiology Foundation (ACCF)/AHA task force concluded in 2010 that there was some evidence that benefits from use of CAC outweigh the risk it poses among patients with intermediate (10-20%) and low-intermediate (6-10%) 10-year risk of CVD, but not among individuals with low (<6%) 10-year risk.[14] The United States Preventive Services Task Force concluded in 2009 that there is fair evidence that use of CAC predict CVD outcome.[15] These reports also point the limitations with the use of CAC – (1) Exposure to radiation, (2) validity

of repeated measurements of CAC as evidence for progression, and whether it adds anything to the risk-factor based Framingham Risk Score prediction model for CVD.[16-18] Table 1-1 Components of Ideal Cardiovascular health among adults reports important longitudinal studies examining CAC as a predictor of cardiovascular event. In the Multi-ethnic study of Atherosclerosis, participants with significant (AU≥100) and intermediate coronary atherosclerosis (0<AU≤100) respectively had 7 to 10 times and 4 times higher risk of experiencing a coronary event as compared to participants with no atherosclerosis.[19] Other studies, such as Rotterdam study[20] and Heinz Nixdorf Recall Study,[21] have also shown a similar association of CAC with future coronary event. In contrast, asymptomatic individuals with no CAC are shown to have extremely low risk for occurrence of a coronary event.[22]

Table 1-2 Important Prospective Studies reporting CAC as a predictor of cardiovascular disease event

Author-Year-Location	Population	Follow-Up	CAC definition/categories	Outcome	Results
Greenland 2004 USA [23]	Population-based, predominantly men, >45 years (mean age ~ 65 years), South Bay Heart Watch study	FU: 8.5 years	0, 1-100, 101-300, ≥301 AU	Non-fatal MI and CHD death	Across categories of FRS, CAC score improved risk prediction among patients at intermediate and high risk (>10% 10-year risk), but not at low risk (<10% 10-year risk).
Vliegenthart 2005 Netherlands [20]	Population-based, 42.5% male, 71.1±5.7 years, N=1795. The Rotterdam Study	FU: 3.3 years	0 – 100, 101 – 400, 401 – 1000, >1000	PTCA, CABG, MI, Stroke, CHD mortality, CVD mortality	CAC further improves CHD prediction from the FRS model, even in the elderly
Taylor 2005 USA [24]	Healthy army personnel, 82% male, ~43 years, N=1983. The Prospective Army Coronary Calcium Project (PACC)	FU: 3 years	0, 1 – 9, 10 – 44, ≥45; Tertiles of CAC	CHD	Presence of CAC associated with 11.8 fold higher risk of CHD. Risk increased with tertiles of CAC, among those with CAC
Becker 2008 Germany [25]	Preventive cardiology clinic patients, 59% male, 57.7 ± 13.3 years, N=1726	FU: 40.3 months	Log-transformed CAC; 0, >0 to 75th percentile, >75th percentile of CAC	Non-fatal MI or CHD death	Higher CAC score predicted higher MI and CHD mortality, no cardiac events observed among those with CAC=0
Detrano 2008 USA [19]	Population-based, 47.2% male, 62.2±10.2 years, N=6722. The MESA Study	FU: 3.8 years	0, 1 – 100, 101 – 300, >300	CHD including MI, angina followed by revascularization, definite angina not followed by revascularization, and probable angina followed by revascularization	Doubling of calcium score increased the risk of major coronary event by 15 to 35%.

1.2.3.2 Intima-Media Thickness

Intima-media thickness (IMT), commonly measured in the carotid artery, has been extensively examined as the predictor of CVD and as a measure of atherosclerosis in clinical trials.[26-28] IMT is measured using B-mode ultrasound scan of the carotid artery – the technique is reproducible, sensitive, and non-invasive. The ACCF/AHA guidelines recommends against routine use of carotid IMT testing among patients at risk of first CVD event.[29] This is because conflicting reports have emerged regarding the usefulness of IMT as a predictor of CVD, with recent meta-analysis published in the Journal of American Medical Association reporting no significant improvement in risk prediction with the use of carotid IMT.[27,28,30]

1.2.3.3 Carotid Plaque

Similar to carotid IMT, B-mode ultrasound scan can be used to examine the presence of atherosclerotic plaque in the carotid arteries. Presence of carotid plaque is also associated with incident CHD. [27] In the carotid arteries, presence of plaque is most common around the site of bifurcation of the carotid artery i.e. the carotid bulb and the proximal internal carotid artery, while plaque is rare in the common carotid artery. In the MESA study, plaque (when defined as any stenosis of the carotid artery) increased the hazards of CVD by 45% over a 8 year follow-up period.[31] Other large epidemiological studies have also reported association of carotid plaque with increased rates of cardiovascular disease. [32-34]

Many recent epidemiological studies in the US and Europe have examined carotid arteries for the presence of plaque.[32-36] In general, age, sex, hypertension, diabetes, and smoking are said to be strongest predictors of plaque. The prevalence of carotid plaque increases with age as well as with increases in the traditional CVD risk factors among all populations. (Table A-1)

Use of different methodologies to examine and define carotid plaque renders it difficult to compare the prevalence of plaque across studies. While many recent epidemiological studies have defined carotid plaque in relation to the thickness of the nearby IMT,[35,37,38] several others have defined carotid plaque with an absolute value of the carotid IMT e.g. >1.0 mm or >1.2 mm. (Table A-1 and Table B-1)

While recent meta-analyses have raised questions on the utility of IMT as a predictor of CHD, carotid plaque has emerged as a strong CHD predictor.[27] In this meta-analysis comprising of 11 population-based and 27 diagnostic-cohort studies were included. Using a meta-regression approach, the authors found that both in population-based as well as diagnostic cohort studies, the relative diagnostic odds ratio for carotid plaque was 35% higher than that for carotid IMT ($p=0.046$).[27]

1.2.3.4 Other Biomarkers of Atherosclerosis

Ankle-Brachial Index

Ankle-Brachial Index (ABI) is a non-invasive technique for screening patients for peripheral arterial disease. ABI is the ratio of the systolic blood pressure measured in the ankle

region to the systolic blood pressure measured in the arm over the brachial artery. In a healthy patient, ABI values in the range of 0.9 to 1.3 are considered normal. ABI value less than 0.9 indicate presence of peripheral arterial disease, and are strongly associated with all-cause and cardiovascular mortality.[39,40] The prevalence of PAD, and thus abnormal ABI, increases with age and is associated with traditional CVD risk factors. In contrast, a high ABI (>1.3) indicates incompressible arteries in the leg, which is also associated with worse cardiovascular outcomes. Several epidemiological studies have reported that an abnormal ABI in asymptomatic individuals predicts worse cardiovascular outcomes.[41-44] A recent review of the evidence by the United States Preventive Services Task Force found insufficient evidence to recommend screening the patients with ABI. However, the review noted that ABI and peripheral arterial disease is an active field of research, and that recommendations are likely to be updated again soon.[45]

Flow-mediated Dilatation

Brachial flow-mediated dilatation (FMD) is a non-invasive measure of endothelial dysfunction. In this measurement, first the brachial artery is compressed for sustained 4-5 minutes by inflating pressure cuff and then the vascular occlusion is released. In a healthy artery, increased flow of blood causes dilatation of the arterial lumen by release of nitric oxide. Using ultrasound of the brachial artery, the dilatation of the artery can be examined. A healthy arterial endothelium releases more nitric oxide and thus leads to a greater dilatation of the arterial lumen. Although this measure predicts future CVD, it requires high sonographer skill, and may not have high reproducibility even in the same participant.

Many epidemiological studies have documented the relationship between FMD, traditional CVD risk factors,[46] and CVD events.[47-49] However, the American College of Cardiology

Foundation (ACCF)/AHA task force (2010) gave class III recommendation (i.e. no benefit) to FMD as a tool for assessing future CVD risk, noting that the evidence for its utility in insufficient and there remained challenges in technical standardization of the measurement.

1.3 ARTERIAL STIFFNESS AS PREDICTOR OF CVD

1.3.1 Introduction

Arterial stiffness is a hallmark of aging, with contributions from co-morbidities such as diabetes, hypertension, atherosclerosis, and chronic renal disease.[50] It is considered as one of the earliest manifestation of structural and functional changes within the vessel wall.[51] With increasing age, there is an imbalance between the two core structural components of the arterial wall – elastin and collagen, with elastin progressively replaced by abnormal collagen, eventually leading to arterial stiffness.[50] The 2007 European Society of Hypertension guidelines recommend using arterial stiffness among patients with high blood pressure. Arterial stiffness is generally assessed in epidemiological studies with the measurement of Pulse Wave Velocity (PWV) which, as the name implies, is the velocity at which the pulse transits between two points on the arterial tree. Other measures for arterial stiffness, esp. the large artery stiffness such as tissue Doppler and strain imaging employ advanced echocardiography techniques; however, these are not commonly used in epidemiological studies. [52,53]

1.3.2 Pathophysiology of Arterial Stiffness

The vascular wall is held stable, resilient, and compliant through a slow, dynamic process of production and degradation of two major scaffolding proteins: collagen and elastin. A dysregulation of process with a subsequent overproduction of collagen and underproduction of elastin leads to vascular stiffness. On histological examination of the intima, a stiffened vascular wall would reveal disarrayed collagen, broken elastin molecules, abnormal endothelial cells, vascular smooth muscle cells, macrophages and mononuclear cells. In addition, increased amounts of matrix metalloproteinases, transforming growth factor (TGF)-β, intracellular cell adhesion molecules, and cytokines are present.

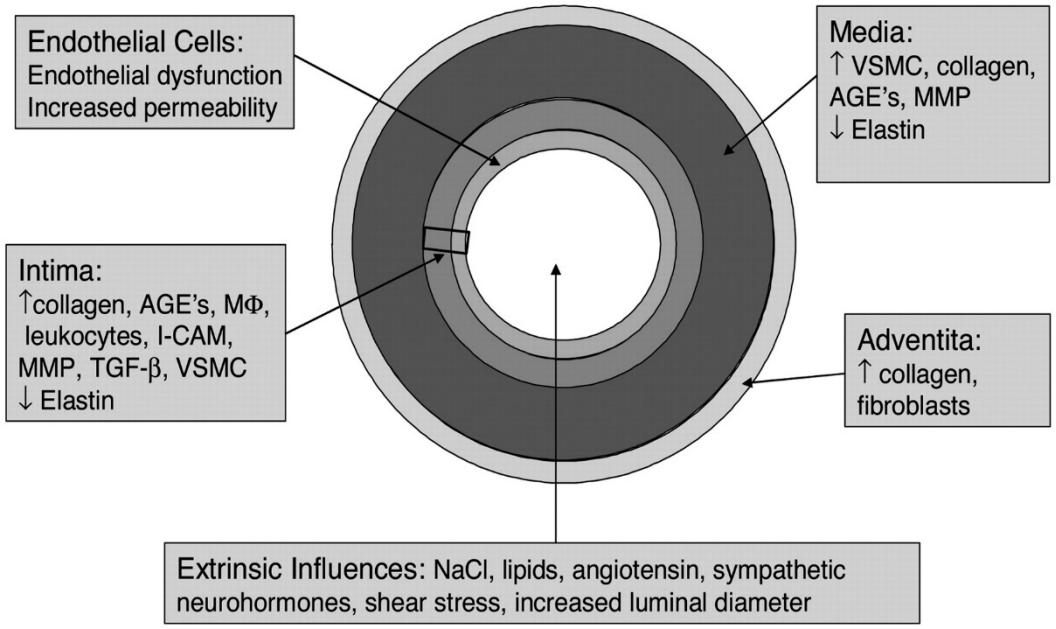

Figure 1-2 Summary of multiple causes and locations of arterial stiffness

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Collagen molecules are rendered insoluble to hydrolytic enzymes by cross-linking; thus, collagen provides tensile strength to the vessel wall. Further, collagen is susceptible to glycation cross-linking which renders it structurally inadequate. [50,54,55] Similarly, cross-links between elastin molecules are also disrupted in the process of arterial stiffness, thus rendering it weak. The weak and dysfunctional elastin is susceptible to mineralization by calcium and phosphorus. These structural changes, altered molecular repair mechanisms, together with the catabolic activities of matrix metalloproteinases (MMP's) lead to arterial stiffness.[50]

Endothelial cells as well as vascular smooth muscle cells play an active role in the pathogenesis of arterial stiffness. Vascular smooth muscle tone is regulated by cell stretch and calcium signaling, as well as by paracrine effects of the endothelium i.e. by secretion of mediators such as angiotensin II,[56] endothelin,[57] oxidative stress,[58] and nitric oxide. A positive feedback loop is suggested to exist between arterial stiffness and endothelial dysfunction. Other factors that are said to play a significant role in arterial stiffness are – angiotensin II, [56] dietary salt,[59] chronic hyperglycemia, chronic hyperinsulinemia, [60,61] and chronic renal disease.[62]

1.3.3 Biomarkers of Arterial Stiffness

While multiple markers of arterial stiffness are available, it is important to make a distinction between their ability to assess stiffness in central vs. peripheral arteries. This is particularly important as the mechanism for arterial stiffness differs between large and small arteries. While the increase in disarrayed collagen is the primary mechanism in the central conduit arteries, the vascular smooth muscle cells and endothelial function play a more prominent role in

the medium-sized muscular arteries. In contrast, stiffness in the microcirculation is critically dependent on the smooth muscle tone.[63]

1.3.3.1 Pulse Wave Velocity

Many epidemiological studies have used Pulse Wave Velocity (PWV) measurement as a marker of arterial stiffness. Although aortic or carotid-femoral pulse wave velocity (*cf*PWV) is considered by many as the gold standard marker of arterial stiffness, PWV measures from brachial-ankle arterial segment is fast emerging as a marker of arterial stiffness and a predictor of CVD.

1.3.3.2 Carotid-femoral pulse wave velocity (cfPWV)

*cf*PWV primarily assesses the stiffness in the large aortic trunk and, thus, is also referred to as a measure of aortic stiffness or central arterial stiffness. *cf*PWV has been reported as a predictor of future cardiovascular events and all-cause mortality.[64] European Society of Hypertension has recommended the use of *cf*PWV in assessing target end-organ damage among hypertensive patients, especially in patients in whom the target organ damage is not discovered by routine investigations. [65] However, *cf*PWV measurement suffers from limitations – (1) exposure of the inguinal region, and (2) difficulty in recording femoral waveform in patients with metabolic syndrome, obesity, diabetes, and peripheral arterial disease.[66] In contrast to *cf*PWV, another combined measure of central and peripheral arterial stiffness i.e. brachial-ankle pulse wave velocity (*ba*PWV) is fast emerging as a marker of arterial stiffness.

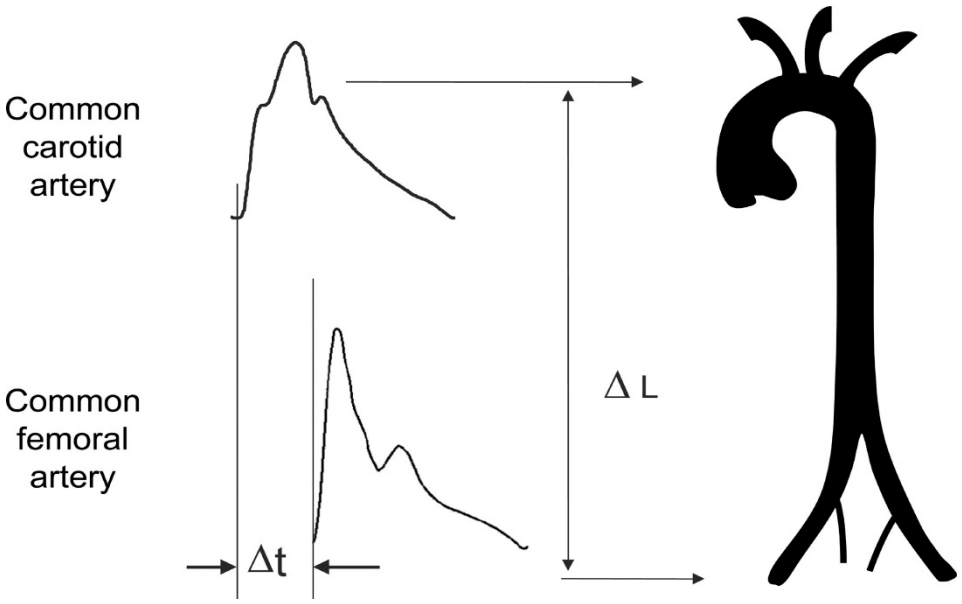

Figure 1-3 Measurement of carotid-femoral PWV with the foot to foot method

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1.3.3.3 Brachial-ankle Pulse Wave Velocity (*ba*PWV)

*ba*PWV is an index of arterial stiffness assessed by measuring the difference between the time at which the pulse wave reaches the brachial artery and the posterior tibial artery. The arterial distance between the two sites (derived automatically by the machine, based on height of the participant) is then divided by this difference to obtain the *ba*PWV. In contrast to the *cf*PWV that requires direct interrogation of the carotid and femoral arteries, *ba*PWV is conveniently measured by placing blood pressure cuffs over arms and ankles and thus has less potential for human error

in measurement. *ba*PWV is a popular clinical tool used in the east Asian countries such as Japan and South Korea to assess risk of CVD among patients. Most of the research studies examining *ba*PWV as a tool for CVD prediction also originate from these countries.

A meta-analysis was conducted to examine the prediction of cardiovascular disease events and all-cause mortality with *ba*PWV (referred to as brachial-ankle Elasticity Index, baEI in the paper). A total of 18 longitudinal studies were included in the meta-analysis, reporting on a total of 8169 participants. Out of these, 15 studies reported results on CVD events (5544 participants), 7 reported on CVD mortality (2274 participants), and 9 reported on all-cause mortality (5097 participants). The risk for CV event was significantly higher among individuals with high *ba*PWV than among individuals with low *ba*PWV (relative risk 2.89, 95% CI: 1.99, 4.20) Additionally, the relative risk for total CVD events was 1.12 (95% CI: 1.05, 1.19) with every 100 cm/s increase in *ba*PWV. (Figure 1-4) Out of 18, only 1 study was conducted in the Europe while the rest were conducted in Asia.[67]

Studies reporting *ba*PWV as a predictor of CVD events after the publication of this meta-analysis are presented in Table D-1.

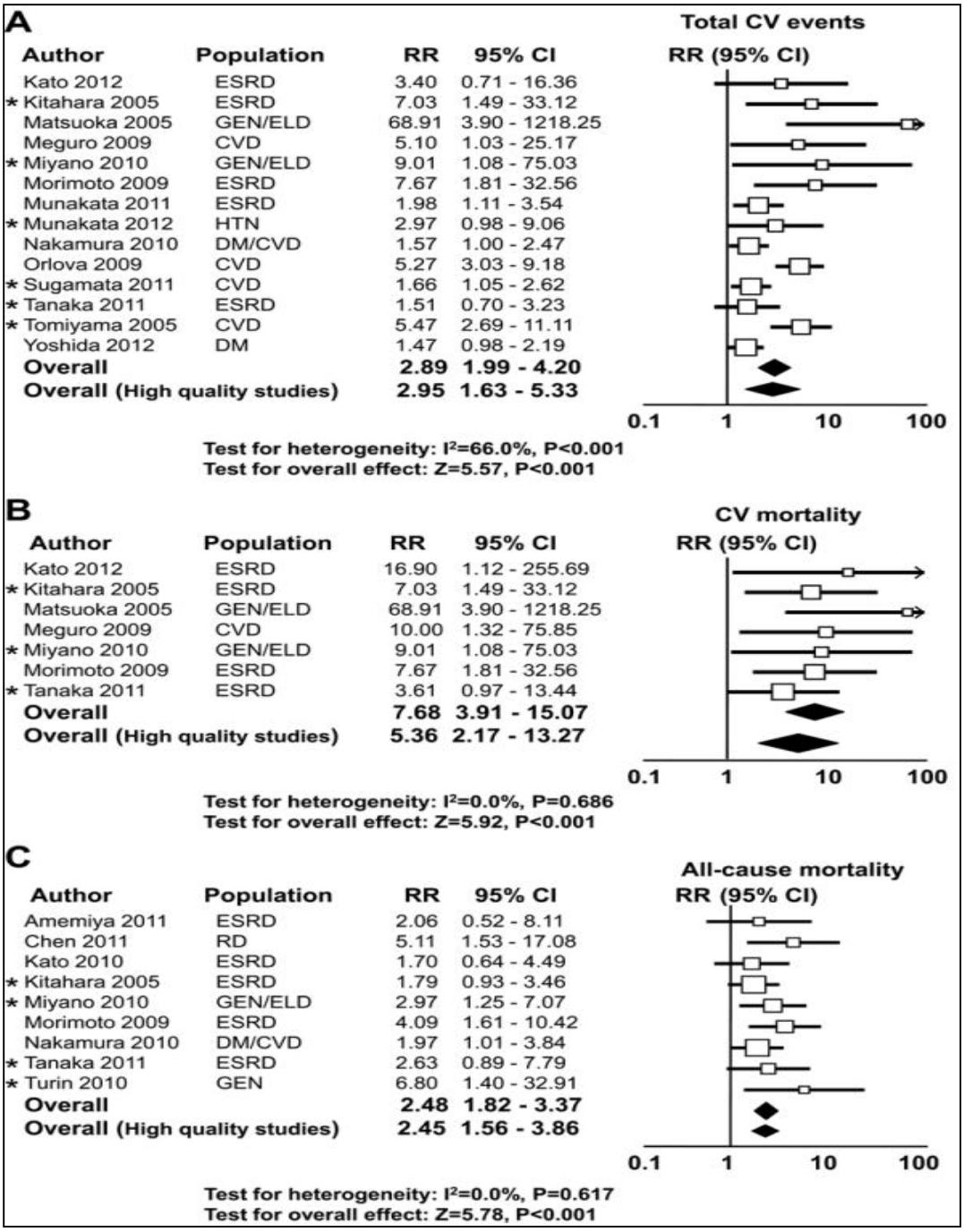


Figure 1-4 Results of the meta-analysis reporting prediction of CVD events with *ba*PWV

1.3.3.4 Other Regional Pulse Wave Velocity measurements

The pulse wave velocity can also be measured regionally, i.e. on any given arterial segment. The nomenclature of these measures is also derived from the segment of the arterial that it covers for measurement of PWV, e.g. heart-femoral PWV (*hf*PWV) measures velocity of the pulse wave when one sensor is placed over the heart, and the other is placed over the femoral artery. Techniques are available to simultaneously measure the PWVs at many sites by placing multiple sensors over the body – the machine then analyzes data from various sensors and provides estimates of the different measurements PWVs.[68]

Depending on the segment of the arterial tree they measure the PWV on, some of these measurements may be regarded as true measures of arterial stiffness, depending on the direction of travel of the pulse wave.

heart-femoral PWV(hfPWV)

Several studies, including ours, have measured *hf*PWV as a marker of arterial stiffness.[69-73] It has been shown to have stronger association with CVD than other measures of arterial stiffness than other markers of regional arterial stiffness such as femoral-ankle pulse wave velocity or heart-carotid pulse wave velocity.[74] Some regard this as a more precise measure of central arterial stiffness as the pulse travels only in one direction between the heart and the femoral artery.

heart-brachial PWV (hbPWV)

Similar to *hf*PWV, this also measures the velocity of pulse wave as it moves in one direction, i.e. from the heart to the brachial artery. Few studies have measured and reported *hb*PWV and its association with smoking,[75] FMD,[76] and advanced glycation end products.[77]

femoral-ankle PWV (faPWV)

The *fa*PWV measures PWV in the leg, i.e. between the femoral and the posterior tibial artery at the ankle. Similar to *hb*PWV, this segment represents the muscular portion of the arterial tree that is more susceptible to the process of atherosclerosis than the aortic trunk which in contrast is more susceptible to arteriosclerosis.[78] The *fa*PWV value reported in the ERA JUMP study is higher than *cf*PWV but lower than *ba*PWV.[73] Further, *fa*PWV was associated with target end-organ damage among patients with essential hypertension.[79]

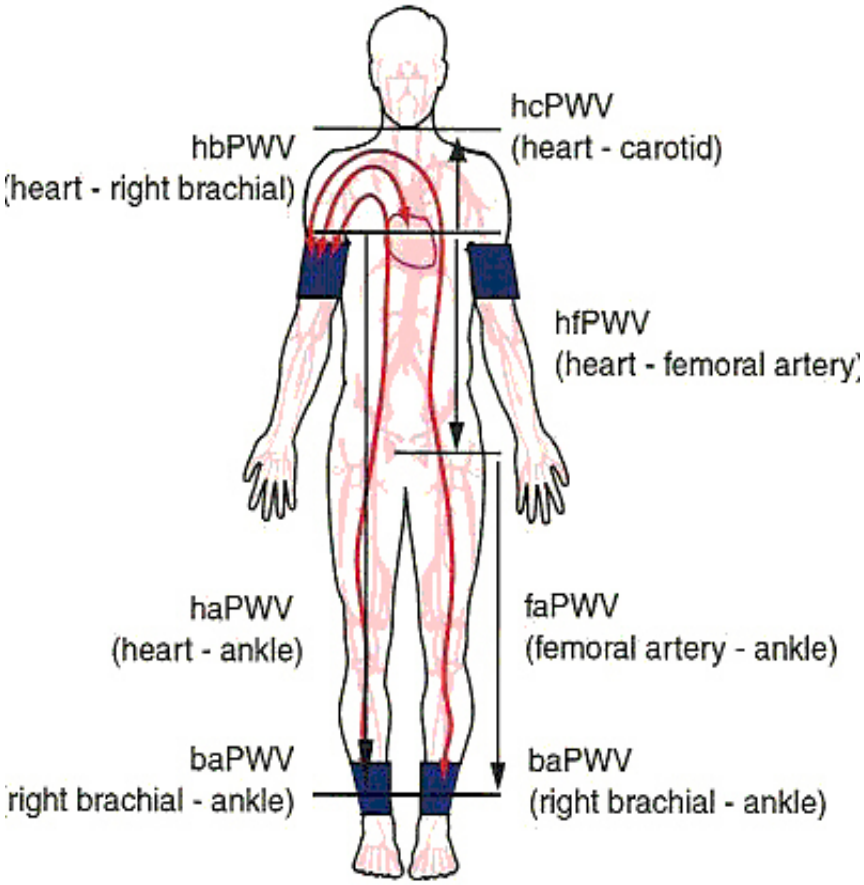


Figure 1-5 Various arterial segments for measurement of PWVs

1.3.3.5 Other Measures of Arterial Stiffness

Pulse Wave Contour Analysis

The structure and function of the arteries influence the shape of the pressure wave as it travels from the heart to the peripheral arterial tree. As it originates from the heart, the shape of the pulse wave is influenced by the mechanics of ventricular systole and the ejection of blood from the left ventricle. The shape changes as the pulse moves from the heart to the peripheral arterial

tree. A well-recognized change that occurs as the pulse move from the heart is the augmentation of the systolic pressure wave and decay of the diastolic wave. [80,81] One of the effects of ageing and vascular disease is the augmentation of the systolic central pressure. This augmentation, if evaluated clinically using pulse wave contour analysis, can provide a measure of arterial stiffness.[63]

Pulse Pressure

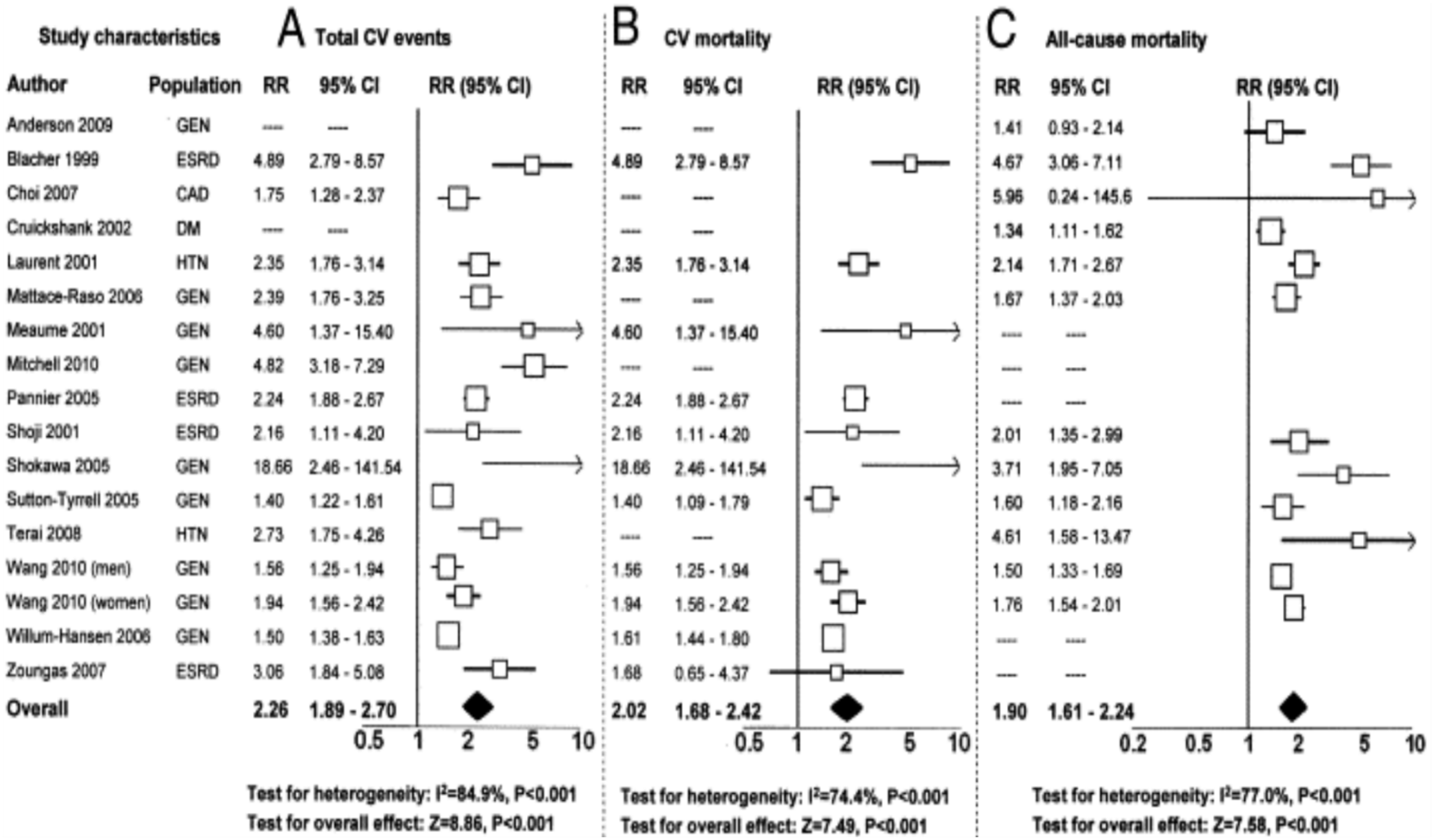
The pulse pressure i.e. the systolic-diastolic blood pressure difference provides a crude estimate of the large artery stiffness.[82,83] Elevated pulse pressure is reported to be associated with worse cardiovascular outcomes, such as heart failure,[84,85] and myocardial infarction.[84,86] Pulse pressure has also been used as a surrogate end-point for evaluating therapies for hypertension and CVD.[85]

1.3.3.6 Comparison between PWV measurements as a tool for measuring arterial stiffness

While *cf*PWV is established as a marker of arterial stiffness, *ba*PWV is a new measure of arterial stiffness that covers both central as well as peripheral arterial stiffness. While *cf*PWV spans mostly over the aortic truck and, only some portion of the peripheral arterial tree i.e. carotid artery, the *ba*PWV spans significant length of the peripheral arterial tree. As peripheral arterial wall has more smooth muscle component as compared to the aortic trunk, it is possible that the mechanism of stiffness may differ between the two types of arterial walls. It is likely that peripheral arterial wall is more affected by atherosclerosis than the aortic trunk.[78] Although the process of arterial

stiffness in the aorta has been studied in detail, similar scrutiny has not been done for the peripheral arterial tree.

In a meta-analysis of longitudinal studies examining prediction of CVD events and all-cause mortality with aortic stiffness or *cf*PWV, it was found that relative risk of clinical events increased with increasing tertiles of arterial stiffness.[64] All of the 15 studies that reported total CVD events reported a significant increase in total CVD events with PWV, and all except one studies reported a significant increase in CVD mortality and all-cause mortality with higher PWV. Many of the studies were conducted in the Europe [87-89] and the US. [90,91] Thus, similar to *ba*PWV, *cf*PWV also predicts CVD events.



Relative risk (RR) and 95% confidence interval (CI) for high aortic pulse wave velocity (PWV) and total cardiovascular (CV) events (A), CV mortality (B), and all-cause mortality (C). (Vlachopoulos, 2010, *JACC*; 55:1318-1327) © American College of Cardiology Foundation

Figure 1-6 RR and 95% CI for High Aortic PWV and Clinical Events

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1.3.3.7 Pulse Wave Velocity measurements: Advantages and Challenges

While c*f*PWV is considered the gold-standard measure of arterial stiffness, and predicts future CVD, certain methodological factors limit its wider application. *cf*PWV measurement requires some expertise from the technician. The skin sensor for the femoral artery has to be placed over the femoral artery in the inguinal region, thus requiring exposure to the inguinal region. Although this may not be an issue among symptomatic patients, asymptomatic patients and participants in epidemiological studies may be reluctant to undergo this procedure. The operator dependence and inguinal exposure may limit its utility in a busy physician's office.

In comparison, *ba*PWV measurement faces few such challenges. It is easy to perform, requires minimal operator or technical expertise, and requires only placement of cuffs over the arms and the ankles. Also, *ba*PWV is shown to predict future CVD events.

However, *ba*PWV measurement itself faces certain challenges before it can gain wider acceptability in epidemiological and clinical research as well in a physician's office.

a. Standardization and validation of the path length of *ba*PWV,

b. Measurement and validation data among non-Asian populations,

c. Establishing normal and disease reference values for the healthy as well as clinical population, and

d. Improved understanding of the disease pathogenesis, esp. in the peripheral arteries.

1.4 EPIDEMIOLOGY OF CHD – COMPARISON BETWEEN THE US AND JAPAN

1.4.1 Introduction

Coronary heart disease (CHD) remains the leading cause of mortality in the US, even after witnessing decades of declining trend in its age-adjusted mortality.[92,93] About 50% of all CHD deaths are estimated to be out of hospital and sudden.[94-98] Moreover, during the next 20 years, prevalence of CHD will rise by 16% just because of demographic changes and the costs attributable to CHD will triple from $35.7 to $106.4 billion.[99] Thus, prevention of CHD, especially its primary prevention, is of critical importance to bring down healthcare and economic burden of CHD in the US.

In contrast to the US and much of the western world, Japan has much lower rates of CHD, and has much higher longevity. This is in spite of having worse risk profile for many cardiovascular disease risk factors as compared to the United States. For example, hypertension and smoking rates are much higher in Japan as compared to the US, while total cholesterol levels are similar. However, average BMI is much lower in Japan than the US. Thus, comparison between the US and Japan might provide us with new insights into lower CHD in the US.

1.4.2 Epidemiological Studies in the 20th Century

The Seven Countries Study

The Seven Countries Study is an epidemiological study that systematically examined the effect of lifestyle and diet on CHD and stroke in different populations. A total of 12,763 men, aged 40-59 years, were recruited in USA, Europe, and Japan, and followed for more than 50 years. This was the first international study to report that Japanese men had much lower rates of CHD than others population groups.[100]

The Seven Countries Study in the 1960's revealed that CHD mortality in Japan is uniquely low, which was attributed to low serum levels of total cholesterol in Japan, i.e., 165 vs. 240 mg/dl in the US.[100] Because studies of Japanese migrants to the US show a dramatic rise in CHD,[101] CHD mortality in Japan was expected to increase as Japanese adopt more Westernized lifestyle: In fact, levels of total cholesterol in Japan have continuously increased from 165 mg/dL in the1960's to 210 mg/dl in 2000.[102] Very paradoxically, however, CHD mortality in Japan has constantly declined since the 1970's similar to the other developed countries where population levels of total cholesterol have been continuously declining, e.g., from 240 to 200 mg/dl in the US between the 1960's and 2008.[103,104] These epidemiological observations indicate some protective factors against CHD in Japanese in Japan.

Ni-Hon-San Study

The Ni-Hon-San (Nippon (Japan), Honolulu, and San Francisco) study was started in 1965 as a large epidemiological study to examine environmental factors and their association with ischemic heart disease and stroke. Only male residents of Japanese descent were recruited from the population in the US (Honolulu and San Francisco). In Japan, participants were recruited from the cities of Hiroshima and Nagasaki. Age at recruitment was 41 to 70 years in Japan, 46 to 65 years in Honolulu, and 30 to 69 years in San Francisco. The study allowed for examination of environment and lifestyle as a risk factor for CVD. A total of about 11,900 men were recruited. In addition to detailed self-administered questionnaire, participant examination was done including anthropometry, BP, vital capacity, and detailed dietary assessment. Blood measures included glucose, hematocrit, cholesterol, and uric acid analysis. The study found an increasing trend of CHD from Japan to Honolulu to San Francisco, while there was an increasing trend for stroke in the opposite direction i.e. Japan > Honolulu > San Francisco.[101,105-109]

Honolulu Heart Program

The Honolulu Heart Program was started in 1965 to examine differences in cardiovascular disease mortality between Japanese living in Japan and individuals of Japanese origin living in Hawaii, US. It was started in Oahu, Hawaii and included 8006 men of Japanese descent who were born between 1900 and 1919. The study found that Japanese in Japan had much lower CHD mortality than those in Hawaii. Age, SBP, serum cholesterol, serum glucose, cigarette smoking, and alcohol consumption were predictors of CHD incidence. The initial examination was performed from 1965 to 1968. Follow-ups were done 2 and 6 years later. Questionnaire,

anthropometric data, lifestyle, dietary, and risk factor information were collected. Twelve-lead electrocardiogram were recorded. The Honolulu Heart Program found higher rates of cerebrovascular disease among Japanese Americans living in the US than Japanese living in Japan. In addition, the study found higher rates of type 2 diabetes among Japanese Americans than Japanese in Japan. Also, there was an increase in CHD and its risk factors with acculturation among Japanese Americans.[110,111]

INTERMAP Study

The International study of Macronutrients and Blood Pressure (INTERMAP) study was a large population-based epidemiological study to specifically examine dietary factors that are responsible for high blood pressure in US, United Kingdom, China and Japan. Between 1995 and 1999, a total of 4,680 men and women, aged 40-59 years gave 24-hour dietary history and 24-hour urine samples, which were examined in a standardized manner. Various nutrients were examined esp. with respect to energy intake, minerals, vitamins, cholesterol, dietary fiber, and caffeine.[112,113] This study reported significant differences in intake of dietary factors that partly explain differences in prevalence of cardiovascular diseases between these world regions. A significant finding from this study was the inverse association between omega-3 fatty acid intake, [114] and vegetable protein intake and BP,[115] and a positive association between cholesterol and BP. [116]

Among the Japanese participants in Japan and Japanese Americans in Hawaii (The INTERLIPID study), it was found that serum omega-3 fatty acids were positively associated with HDL-cholesterol among men. Also, traditional CHD risk factors were lower among Japanese in Japan than Japanese Americans in Hawaii.[117]

1.4.3 The ERA JUMP Study

The Electron-beam computed tomography, Risk factor Assessment among Japanese and U.S. Men in the Post-World War II birth cohort (ERA JUMP Study) was started as an investigator-initiated study sponsored by the National Institute of Health (HL68200), to examine and compare the extent of atherosclerosis among 300 White and 100 Black men in Pittsburgh, US, 300 Japanese American men in Honolulu, US who were offspring of members of the Honolulu Heart Program, and 300 Japanese men in Kusatsu, Japan. Men, 40-49 year old and free of CVD or other severe diseases, were recruited from the population between 2002 and 2006. Using a similar protocol, about 300 South Korean men were also recruited from the population in Ansan, South Korea. Along with participant questionnaires and measurement of traditional cardiovascular risk factors, serum samples were collected, and subclinical atherosclerosis in the form of Carotid IMT and CAC was examined in a standardized fashion.[118]

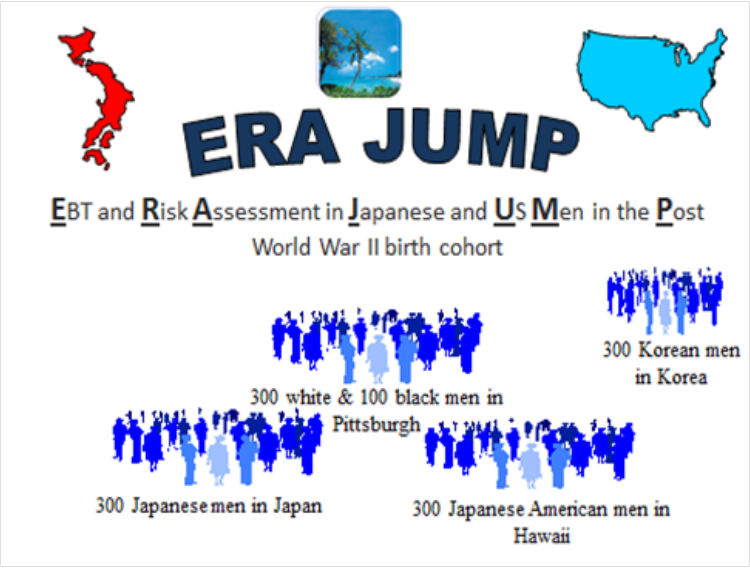

Figure 1-7 The ERA JUMP Study logo

1.4.3.1 N-3 fatty acids and subclinical atherosclerosis in the ERA JUMP study

The ERA JUMP study reported higher subclinical atherosclerosis among White men in the US than Japanese men in Japan.[118,119] Also, consistent with the findings previously reported from the Honolulu Heart Program and other epidemiological studies from the 20th century, it reported higher atherosclerosis i.e. higher carotid IMT and higher prevalence of CAC (≥10 AU) among Japanese Americans men than Japanese men in Japan.

One factor that was found to be associated with lower subclinical atherosclerosis among Japanese in Japan was serum levels of marine n-3 fatty acids. In fact, the significant difference in subclinical atherosclerosis found between Japanese men in Japan and White men became non-significant after adjusting for serum levels of n-3 fatty acids, thus highlighting the important role they may play in protection from atherosclerosis. Notably, serum levels of n-3 fatty acids were significantly higher among Japanese in Japan, consistent with their higher dietary intake.[120]

A 4 to 6 year follow-up of the ERA JUMP visit 1 has recently been completed across the study sites, and shows that incidence of CAC is inversely associated with baseline serum n-3 fatty acids,[121] thus providing further evidence that n-3 fatty acids are protective against atherosclerosis. Other prospective epidemiological studies in the US have also reported similar inverse association of higher n-3 fatty acids with atherosclerosis and CHD.[122-128]

1.4.4 Other anti-atherogenic Dietary Factors in Japan

1.4.4.1 Soy Isoflavones

One strategy to examine reasons for differences in CHD and subclinical atherosclerosis between US and Japan is to identify dietary factors having pro- or anti-atherosclerotic properties, of which consumption is strikingly different between the US and Japan, and to examine whether such nutrients have significant associations with atherosclerosis independent of traditional risk factors. In addition to n-3 fatty acids described in the earlier section, one nutrient with anti-atherosclerotic properties, of which intake is substantially higher among Japanese in Japan as compared to US, is soy isoflavones.[113,129]

In recent years, soy isoflavones, specifically its aglycone components, daidzein and genistein, have been examined for their potential protective effects on atherosclerosis,[130] CHD[131-133] and other conditions such as cognition[134], cancers,[135] osteoporosis,[136] and menopausal symptoms.[137] A randomized clinical trial in the US has reported lower intima-media thickness progression among those who has isoflavone supplementation than those in the placebo group; however, the difference was not statistically significant. Nevertheless, the difference was significant among early postmenopausal women.[130] Several laboratory and animal studies have also reported that soy isoflavones have anti-atherosclerotic properties.[138-141]

Although classified as phytoestrogens due to their structural similarity to estrogens and their estrogen receptor-binding capability, unlike estrogens, soy isoflavones preferentially bind to estrogen β receptors (ER-β) expressed in the vascular endothelium over estrogen α receptors (ER-

α) expressed in the reproductive tissue.[142,143] Thus, at concentrations present in the human, the primary effects of soy isoflavones are on the vasculature. In addition to its estrogenic properties, soy isoflavones act as antioxidants by reducing lipid peroxidation and exhibit anti-inflammatory activity by reducing nitric oxide production.[144] Mean consumption of isoflavones in Japan is 25-50 mg/day[145] whereas it is negligible in the US, i.e., <2 mg/day.[145,146]

Soy isoflavones, especially genistein, preferentially bind with ER-β but only weakly with ER-α. The binding activity of genistein on ER-β is 87% of the binding activity of estradiol, albeit at much higher concentrations. In contrast, its binding activity on ER-α is only 4% in comparison to estradiol. Daidzein, the other major isoflavone, has much lower affinity to these receptors as compared to genistein i.e. its binding activity at ER-α and ER-β is only 0.1% and 0.5% as compared to estradiol.[147]

Animal studies have reported anti-atherosclerotic effects of soy isoflavones.[138-141,148,149] In an important clinical trial of soy isoflavones among female cynomologus monkey, atherosclerosis progression was lower among the group that was fed diet rich in soy isoflavones as compared to the group that was fed control diet, although the difference was only marginally significant. The progression was lowest among the group fed conjugated equine estrogen. In this study, monkeys were fed atherogenic diet for 26 months, at which time surgical menopause was induced, and subjects were followed for another 10 months.[140] Other studies from the same research group have reported improvement in cardiovascular risk factors such as LDL-c, VLDL-c, HDL-c, and total cholesterol:HDL-cholesterol ratio,[150] and vascular reactivity[149] among monkeys.

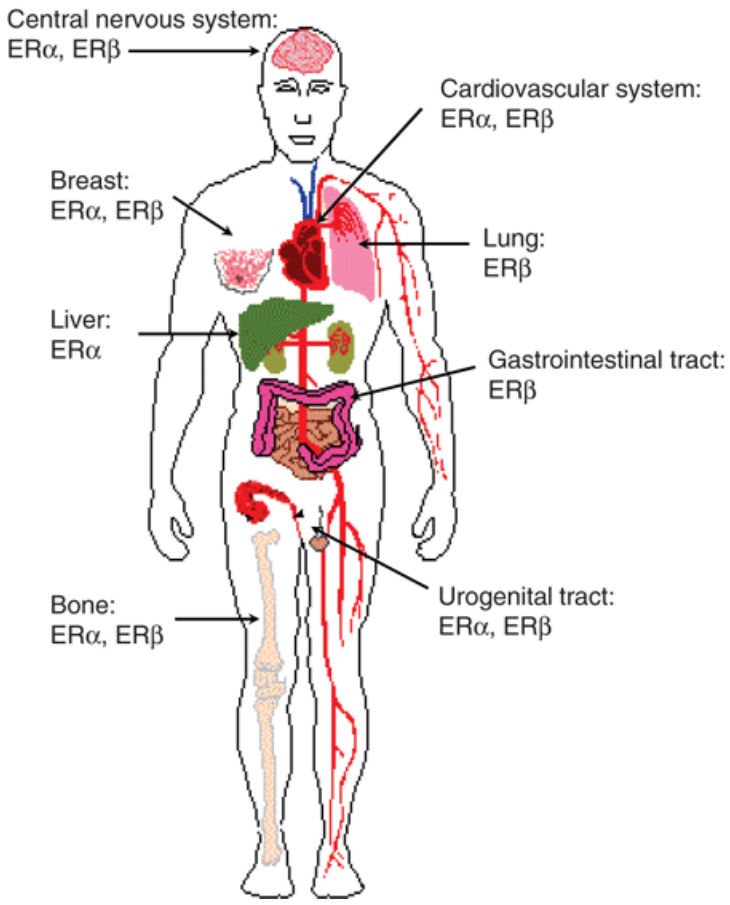

Figure 1-8 Distribution of the two subtypes of estrogen receptors

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ER-β is known to play an atheroprotective role. In a study of mice, it was found that heat-shock protein 27 (HSP27), an atheroprotective protein that acts to prevent atherogenic lesion formation, works in an estrogen-dependent manner. In vitro, HSP27 works strongly in the presence of an ER-β agonist but only modestly in the presence of an ER-α agonist.[151] In contrast, other studies have reported similar or higher atherosclerotic properties of ER-α than ER-β.[152] However, the uterotrophic and mammotrophic effects of ER-α stimulation limits the usefulness of any agonists to target these receptors. In contrast, ER-β has no such potentially harmful characteristics

and, thus, is safer to target. In spite of the continues interest in the biological roles of estrogens and its receptors, the exact pathophysiological role that these receptors play with regards to development of various disease process is not completely understood.[152]

Several epidemiological studies in the past have examined the protective effects of soy isoflavones on CHD.[131-133] Two of these three studies are from countries where consumption of

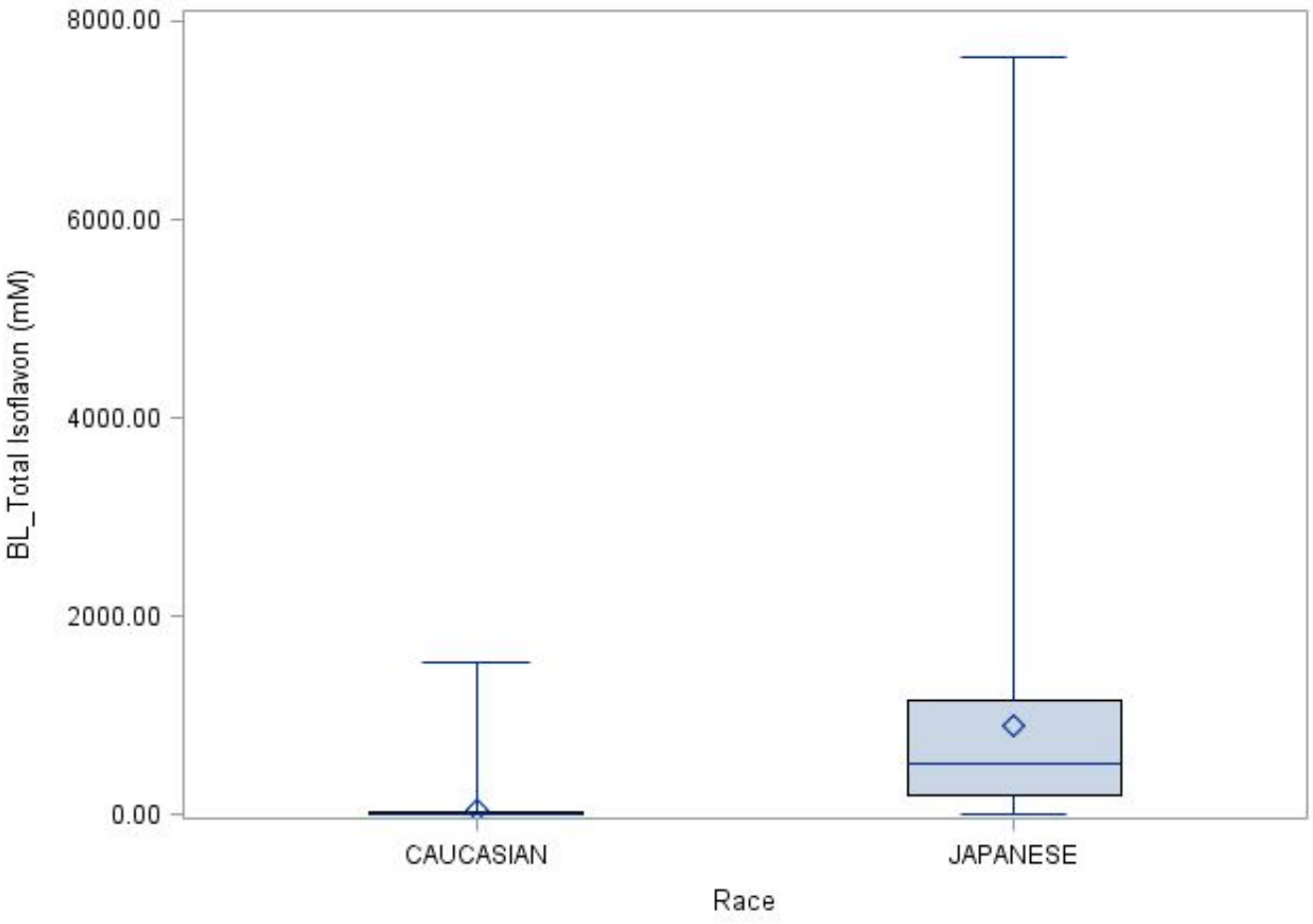

Figure 1-9 Comparison of serum levels of Soy Isoflavones between US and Japanese men in the ERA JUMP Study

soy is significantly higher than that in the US – both shown a protective effect of soy on CHD. In contrast, in Europe, where the average consumption of soy isoflavones is low, no protective effect of soy isoflavones was found.[132]

Soybeans are the almost exclusive dietary source of isoflavones. However, the average intakes of isoflavones differ markedly between the US and Japan: <2 mg/day in US vs. 25-50 mg/day in Japan. In the ERA JUMP study, median (interquartile range) levels of serum isoflavones

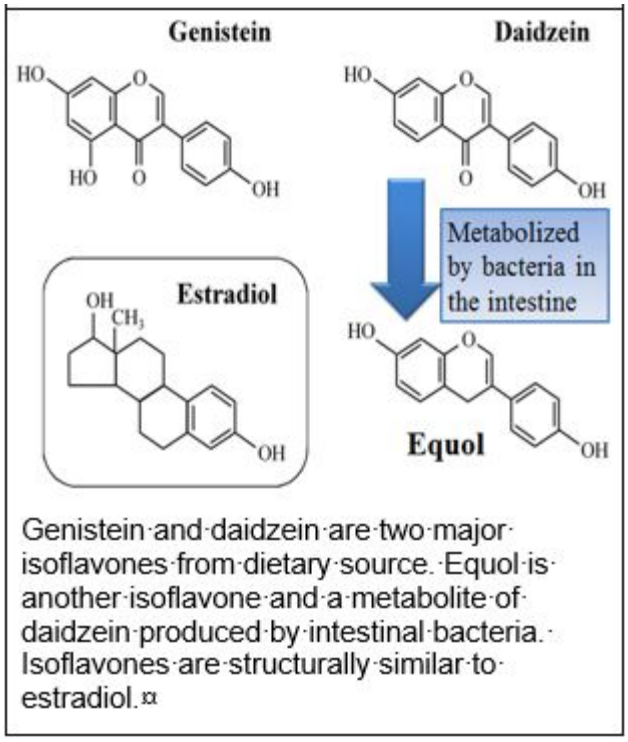

Figure 1-10 Chemical structure of Isoflavones and Equol

(nmol/L) in Japan and the US were 517 (195, 1147) and 7 (3, 58) respectively (Fig 1-7). While genistein and daidzein are the two major isoflavones in dietary sources, equol is produced from daidzein in the gut by intestinal bacteria. These isoflavones are structurally similar to estradiol (Fig 1-8). However, unlike estradiol which binds to the ER-α present in the reproductive tissue as well as with ER-β,[141,153] isoflavones bind preferentially to the ER-β expressed in the vasculature.[141] As their serum concentration is 1000-fold higher than estradiol,[154] isoflavones are able to exert anti-atherosclerotic effects through estrogenic properties on ER-β in spite of their relatively minuscule potency.[153] Observational studies from Japan[131] and China,[133] where intake

of isoflavones is much higher than the US, support the hypothesis of anti-atherosclerotic effects of isoflavones.

1.4.4.2 Equol – a soy derivative

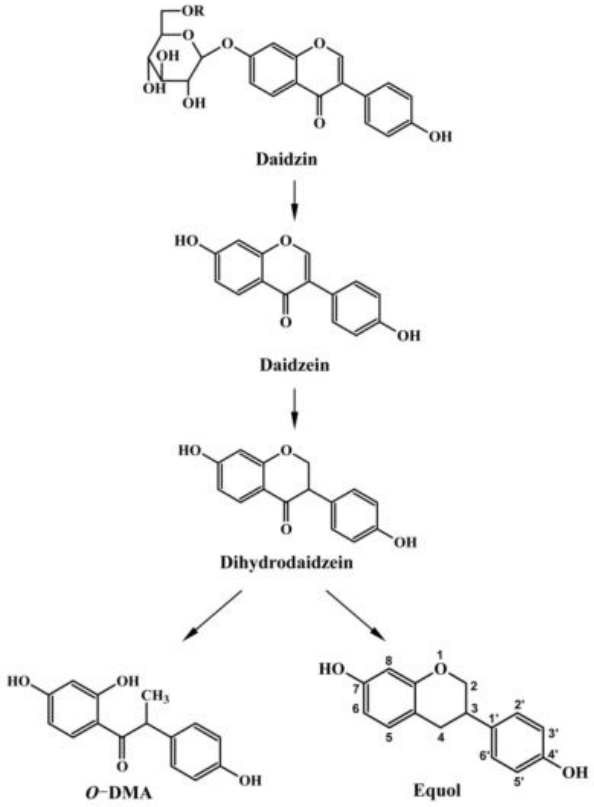

Figure 1-11 Conversion of Daidzein into Equol by intestinal bacteria

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Equol [7-hydroxy-3-(4'-hydroxyphenyl)-chroman] is an isoflavone derived from daidzein. Equol was first isolated in 1932 from pregnant mare's urine,[155] and was found in human urine in 1984.[156] Similar to genistein and daidzein, equol is classified as a non-steroidal

estrogen.[157] However, equol does not occur freely in nature - daidzein is acted upon by intestinal bacteria to produce equol (Figure 1-11).[158] Equol can now also be artificially produced from daidzein.[159] [160] It exists in two forms - an active S-(-)equol and an inactive R-(+) equol form.

Equol has several pharmacokinetic properties that differ from those of its precursor, daidzein. After being absorbed from the intestine, equol undergoes first-pass metabolism - glucuronidation and sulfur-conjugation in the alimentary tract. Once formed, it has longer half-life, and higher bioavailability than daidzein – a study examining pharmacokinetics of daidzein and equol found that while daidzein had a plasma clearance of 17.5 L/h, the clearance of equol was much slower at 6.85 L/h.[161] Equol has much higher free and unbound form (49.7%) than daidzein (18.7%) and estradiol (4.6%) in the blood.[162] Almost all of it is excreted in the urine.[163,164]

Although equol is derived from daidzein through a series of steps in the intestine, (Figure 1-11) it retains many of the important properties of daidzein. E.g. equol retains structural similarity to estradiol and has affinity for the ER-β.[157] Between genistein, daidzein and equol, daidzein has lowest affinity for estrogen receptor. While the affinity for estrogen receptor is similar between equol and genistein, equol induces more transcription than genistein through activation of the intracellular mechanisms, and thus results in more biologic effect.[165] In fact, equol has the highest biological potency of all isoflavones.[161] Equol has higher anti-oxidant properties, higher bioavailability, and slower clearance rate compared to other isoflavones.[161,166-169] Thus, equol may provide an exciting opportunity to bring down atherosclerotic burden in the US.

Equol Producer status

Given that equol is produced from daidzein by the action of intestinal bacteria, both presence of daidzein in diet as well as equol-producing bacteria in the intestine are necessary to produce and absorb equol from the intestine. It is estimated that 10-50% of Japanese [170-174] and 25-40% of Americans have intestinal bacteria to convert daidzein to equol.[158,174,175] Although 25-40% of Americans are equol producers, the proportion of the Americans consuming daidzein on a regular basis is much lower. Thus, estimation of equol producer status among Americans cannot be performed without giving a daidzein challenge to the individual. Equol producing status is reported to be stable and is determined by intestinal bacteria.[158,176,177] It is unlikely that genetics has any role to play in production of equol.[173-175,178] Given that only some individuals have the bacteria to produce equol, a terminology of "equol producer" and "equol non-producer" is frequently used to distinguish between these two groups in the scientific literature.

Setchell et al. have examined dietary factors that may be associated with equol producer status in a study comprising of Caucasians who were followed for 2 years. In their study, 30.3% of Americans, and 28.6% of Australians were equol producers [defined as $\log_{10}$(urinary equol/daidzein) > -1.75]. The study investigators found that as compared to equol-producers, equol non-producers had lower polyunsaturated fat intake as well as lower polyunsaturated fat/saturated fat intake ratio.[177] Of the 20 participants among which the equol producer status was followed, producer/non-producer status remained stable among 18 participants (90%) which were followed for 2 years. Additionally, administration of an anaerobic antibiotic (i.e. metronidazole) did not have effect on equol producer status among 4 of the 5 participants.[177]

Criteria for determining Equol producer status

Several clinical studies have been conducted to determine the prevalence of equol producing status in the population. These studies used varied definitions to determine equol producer status. The WISH trial which is the largest clinical trial to date of soy isoflavones in the US, used plasma level of ≥20 nmol/L as the cut-off point to determine equol producer status.[130] Setchell et. al. recommended use of ≥83 nmol/L in plasma as the cut-off point for determining equol producer status in 2002, while updated their recommendation and prescribed $\log_{10}$(urinary equol/urinary daidzein) > -1.75 as the criterion in 2006.[179] Following this, a majority of studies have used log-transformation formula for determining equol producer status. Although the log-transformation formula is accepted by several studies, it is recommended only when equol and daidzein levels are measured in the urine.[179] When the equol and daidzein levels are measured in the plasma, Setchell et al. have found that this formula did not correctly determine equol producer status.[179]

Although several studies have examined equol producer status by assessing equol concentration in the urine, few have measured equol in the plasma. [130,179,180] Further, these studies have used different criteria to determine equol producer status. Thus, the definition to assess equol producer status by measuring equol in the plasma is unclear from the scientific literature. Further, all the previous studies have been conducted by measuring equol after giving a challenge with soy isoflavones or soy containing food. However, none of these studies have examined equol producer status in the general population without administration of isoflavones. Although Setchell et al. updated their definition of equol producer in 2006 when measuring equol in urine, their definition

for measuring equol producer status when measuring equol in the plasma (i.e. ≥83 nmol/L) still holds.

Table 1-3 Studies using varying criteria to determine equol producer status

Author-Year, Type of Study	Study Population	Equol assessment	Soy challenge?	Equol producer definition	Prevalence of Equol producers
Setchell-1997, clinical trial, US[181]	N=25, all infants	Urine	Soy-based infant formula	Any detectable equol in plasma	57.1%
Watanabe-1998, soy pharmacokinetic study, Japan[182]	N=7, all males, Age range: 23 to 54 years	Urine	Soy challenge	Any measurable equol in urine	28.1%
Rowland-2000, crossover clinical trial, UK[183]	N=24, 79.1% female, Age range: 19-40 years	Both serum and urine	High and low isoflavones containing diet	Definition not provided	36%
Setchell-2002, soy pharmacokinetic study, US[161]	N=16, all premenopausal females	Urine	Soy food for 7 days	Any measureable equol in urine	37.5%
Wiseman-2004, clinical trial, UK[184]	N=76, 50% female, Age ~ 24 years	Urine	Soy supplements for 10 weeks	> 1000 nmol/24 hr	34%
Setchell-2006, clinical trial, US[179]	N=41, 44% female, 71% vegetarians, Age range: 26-64 years	Both serum and urine	500 ml/day of soy milk for 3 days	Log_{10}(equol/daidzein) > - 1.75	Vegetarians: 59% Non-vegetarians: 25%

Table 1-3 contd.

Vedrine-2006, clinical trial, France[180]	N=12, all white postmenopausal females	Both urine and plasma	soy supplements	Plasma: >40 nmol/L Urine: >1000 nmol/ 36 h	41.6%
Maskarinec-2007, clinical trial, US[175]	N=43, all Japanese American females, Age range: 18-78 years	Urine	Soy challenge with soymilk	Any measurable equol in urine	33%
Atkinson-2008, clinical trial, US[185]	N=200, all female, Age range: 40-45 years	Urine	3 soy bars or bag of soy nuts	Any detectable equol in urine	27.5%
Thorp-2008, crossover clinical trial, Australia[186]	N=91, 63.7% female, Age range: 18-80 years	Urine	Isoflavones containing diet	Log_{10}(equol/daidzein) > - 1.75	32.9%
Hodis-2011, clinical trial, US	N=350, all female, Age range: 45 to 92 years	Plasma	Soy protein diet	Plasma equol ≥20 nmol/L	Consistent producers: 26% Intermittent producers: 23.3%
Sen-2012, clinical trial, USA[187]	N=82, all female, Age: 39.2 ± 6.1 years	Urine	High and low soy diet	(i) daidzein > 2 nmol/g creatinine, or (ii) equol/daidzein > 0.018	52.4%

Table 1-3 contd.

Wong-2012, clinical trial, Canada[188]	N=85, 50.6% female, Age 59.9 ± 9.9 years	Urine	Soy food supplements	(i) >1000 nmol/day, or (ii) Log_{10}(equol/daidzein) > - 1.75	35.3%
Setchell-2013, clinical trial, US and Australia[177]	N= 159, 57.2% female, Age range: 21-61 years	Urine	480 ml/day of soy milk for 3.5 days	Log_{10}(equol/daidzein) > - 1.75	US: 30.3% Australia: 28.6%
Tseng-2013, clinical trial, US[189]	N=224, all Chinese American female, Age range: 36 to 58 years	Urine	Soy challenge	Urine: ≥30 nmol/L	30%
Van der Velpen-2014, crossover clinical trial, Netherlands[190]	N=58, all female, Age ~ 63 years	Urine	Isoflavones supplement	Log_{10}(equol/daidzein) > - 1.75	27.3%
Nakatsu-2014, clinical trial, US[191]	N=17, all female, Age: 60.2±7.3 years	Urine	Soy bar	Log_{10}(equol/daidzein) > - 1.75	23.5%

Equol: possible mechanisms of action

Of all metabolites of daidzein, equol has the most potent action on the ER-β,[192] and most potent anti-oxidant effects.[161] Given these effects, it is possible that equol producers have less atherosclerosis and lower CHD than non-equol producers. Although many studies from East Asian countries have examined this hypothesis, its assessment among US populations in an observational setting is limited by the fact that consumption of daidzein is negligible in the US. Due to this limitation, it is unlikely that even those US individuals having intestinal bacteria to produce equol would have been exposed to appropriately high concentration of equol over their lifetime. In contrast to the US, soy is a part of daily diet in Japan with foods such as tofu and miso providing a bulk of the isoflavones. Thus, it is safe to assume that anyone with low equol level in the blood in Japan does not have intestinal bacteria to convert daidzein into equol.

To date, only one pilot clinical trial of equol has been conducted.[178] This 12-week randomized double-blinded crossover trial was conducted on 54 overweight or obese individuals (age: 59.4 ± 1.3 years, 70.4% female) in Japan; efficacy of daily intake of 10 mg equol supplement was tested against placebo. Equol supplementation led to significant reduction in waist circumference, HbA1c, LDL-c, and cardio-ankle vascular index, a measure of arterial stiffness.[193] Further analysis showed that findings were stronger among women than men. Also, findings were significant only among those who were determined equol non-producers at baseline with almost no effect seen among equol producers. Although this small trial resulted in significant improvement on a variety of cardiovascular risk factors, little is known about the mechanism of

action of equol in humans. Larger studies are needed to assess true potential of equol as an anti-atherosclerotic supplement.

In addition to the clinical trial of equol, several clinical trials of soy/ soy protein/ isoflavones have determined equol-producer status and reported the effects of isoflavones by equol producer status. In the Women's Isoflavones Soy Health (WISH) trial comprising of 350 postmenopausal women in the US, equol producers had non-significantly lower IMT progression than non-producers.[130] In a case-control study within the original Shanghai Men's Health and Shanghai Women's heath cohorts, increasing urinary equol quartiles were associated with lower CHD among women but not in men.[194] In a small clinical trial of soy isoflavones, those consuming high-dose isoflavones had similar reductions in LDL-c in both equol producers and non-producers, however, HDL-c and apoA-1 were preserved only among equol producers.[188] Finally, a recent 8-week clinical trial in Netherlands (n=57) examining effects of isoflavones supplementation on biopsy-derived adipose tissue showed down-regulation of energy metabolism-related genes with high-dose isoflavones supplementation but an inverse effect with low-dose isoflavones supplementation.[190]

1.5 SPECIFIC AIMS

This manuscript examines the following specific research questions

- What is the prevalence of carotid plaque among middle-aged Caucasian men in US, Japanese men in Japan and Korean men in South Korea in the ERA JUMP study? Which risk factors are significantly associated with carotid plaque in these populations?

- Is brachial-ankle pulse wave velocity associated with presence of coronary artery calcification among middle-aged men in the ERA JUMP study?

- Are serum levels of soy isoflavones and equol producer status inversely associated with presence of coronary artery calcification among Japanese men in Japan in the ERA JUMP study?

2.0 CAROTID PLAQUE: COMPARISON AMONG MIDDLE-AGED MEN LIVING IN US, JAPAN, AND KOREA

2.1 ABSTRACT

Background: Carotid plaque has emerged as a strong predictor of coronary heart disease (CHD) risk. Comparison of carotid plaque burden between race/ethnicities may provide a relative estimate of their future CHD risk.

Methods: A population-based international study was conducted among apparently healthy middle-aged men aged 40-49 years (ERA JUMP study (n=924)): 310 Whites in Pittsburgh, US, 313 Japanese in Otsu, Japan, and 301 Koreans in Ansan, S Korea. Carotid plaque and CHD risk factors were assessed using a standardized protocol across all centers. Prevalence of carotid plaque was compared between race/ethnicities after age-BMI, and after multivariable adjustment for other CHD risk factors. Cross-sectional associations of risk factors with carotid plaque were examined.

Results: Whites (22.8%) had about five-fold higher prevalence ($p<0.01$) of carotid plaque than Japanese men (4.8%) while the prevalence among Koreans was 10.6%. These differences remained significant after age, BMI as well as after multivariable adjustment – odds ratio (95% confidence-interval) for plaque prevalence was 0.18 (0.09, 0.32) for Japanese and 0.43 (0.26, 0.74) for Koreans. Further, these differences remained significant even after adjusting for carotid intima-media thickness (IMT). Age, hypertension and diabetes were significantly associated with presence of carotid plaque in the overall population.

Conclusion: Whites had significantly higher carotid plaque than men in Japan and S Korea. Lower carotid plaque burden among Japanese and Koreans was independent of traditional CHD risk factors and IMT.

2.2 INTRODUCTION

Presence of plaque in the carotid artery has emerged as a strong predictor of coronary heart disease (CHD). A recent meta-analysis reported carotid plaque as a stronger predictor of CHD than the carotid intima-media thickness (IMT)[27,195] which is commonly used as a measure of subclinical atherosclerosis. Thus, comparison of carotid plaque prevalence between race/ethnicities may provide an estimate of their relative burden of atherosclerosis, which is the major underlying cause of CHD.

CHD remains the leading cause of mortality in the United States (US), with more than three-fold higher CHD mortality rates in the US than in Japan.[1] Although being industrialized for several decades with westernization of diet and lifestyle, Japan continues to have extremely low CHD rates. The author's research group has also reported lower coronary atherosclerosis and lower IMT among Japanese men than US Whites.[118] While several studies have examined race/ethnic differences in CHD as well as subclinical disease markers such as carotid IMT,[196,197] few studies have compared prevalence of carotid plaque across various race/ethnicities.[198] Further, previous studies used varying methodology to examine and define carotid plaque,[27] thus limiting validity of any post-hoc comparison of plaque prevalence between different studies. With the emergence

of carotid plaque as a predictor of CHD, it is now important to systematically examine the differences in the prevalence of carotid plaque between the US and Japan.

Although many population-based studies have assessed the prevalence of carotid plaque,[198,199] no study has compared the prevalence of carotid plaque between the US and Japan. Thus, a standardized protocol was used to compare the prevalence of carotid plaque among Whites in the US, Japanese in Japan, and Koreans in Korea in the ERA JUMP study, an international population-based study for assessing subclinical atherosclerosis in 40-49 year-old men. The hypothesis for the present study was that the prevalence of carotid plaque would be highest among Whites in Pittsburgh. Further analysis was performed to examine which CHD risk factors were associated with the presence of carotid plaque in this sample population.

2.3 METHODS

2.3.1 Participants

During 2002–2006, a population-based sample of 925 men aged 40–49 years, with no clinical cardiovascular disease (CVD) or other severe diseases was obtained from 4 centers: 310 Whites from Pittsburgh, Pennsylvania; 313 Japanese from Kusatsu city, Shiga, Japan; and 302 Koreans from Ansan, Gyeonggi-do, South Korea as previously described.[118] The final sample for this study consisted of 924 men (310 Whites, 313 Japanese men in Japan, and 301 Koreans) with complete data. Written informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of the following institutions: the University of

Pittsburgh, Pittsburgh, Pennsylvania; Shiga University of Medical Science, Otsu, Japan; and Korea University, Seoul, South Korea.

2.3.2 B-Mode Carotid Ultrasound Scanning

Presence and extent of plaque were evaluated in each of 5 segments of the left and right carotid arteries i.e. distal and proximal common carotid artery (CCA), carotid bulb, proximal internal carotid artery (ICA) and external carotid artery, and summarized as the presence or absence of any plaque. Plaque was defined as a distinct area of the vessel wall protruding into the vessel lumen that was at least 50% thicker than the adjacent IMT. Number of distinct plaques was counted in the abovementioned carotid segments on both sides.[200]

Before the start of the study, sonographers at all centers received training for carotid scanning provided by the Ultrasound Research Laboratory at the University of Pittsburgh. Continuous-quality assessment programs developed by the Ultrasound Research Laboratory to assure scanning quality were applied across all study sites throughout the study.[200] Pittsburgh and Kusatsu sites used a Toshiba 140A scanner (Tokyo, Japan) equipped with a 7.5-MHz-linear-array imaging probe while the Ansan site used a Titan high-resolution ultrasound system with a 10.5 MHz linear array. Readers were blinded to participant's characteristics and the study centers. Under continuous-quality assessment programs, there was excellent agreement between sonographers for carotid plaque assessment (kappa statistic, κ=0.78).

For the purpose of measurement of IMT, digitized images of the carotid artery were sent to the University of Pittsburgh from other centers, and were read by a trained reader using standardized protocol.[118] The sonographer measured the average IMT across 1-cm segments of near and far walls of the common carotid arteries and the far wall of the carotid bulb and internal

carotid arteries on both sides. Average IMT was calculated by taking a mean of the IMT measurements.

2.3.3 Risk Factor Assessment

All participants underwent a physical examination, completed a lifestyle questionnaire and a laboratory assessment as described previously.[118,201,202] Body weight and height were measured while the participant was wearing light clothing without shoes. Body-mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Blood pressure was measured - after the participant emptied his bladder and sat quietly for 5 min- twice on right arm with an automated sphygmomanometer (BP-8800, Colin Medical Technology, Komaki, Japan) using an appropriate sized cuff with; the average of the two measurements was used. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg or use of anti-hypertensive medications. [203] Venipuncture was performed early in the clinic visit after a 12-hour fast. Blood samples were stored at −80°C and shipped on dry ice to the University of Pittsburgh. Serum lipids were determined using the protocol standardized by the Centers for Disease Control and Prevention. [204] Hyperlipidemia was defined as LDL-c ≥ 160 mg/dl or use of lipid-lowering medications.[205] Serum glucose was determined by using hexokinase–glucose-6-phosphate-dehydrogenase enzymatic assay. Diabetes was defined as individuals with fasting glucose ≥126 mg/dl or use of medications for diabetes. [206] Alcohol drinking was defined as intake ≥ twice/week. Ever smoking was defined as current or past cigarette smoking. Use of blood pressure-lowering, diabetes, and lipid-lowering medication was ascertained through questionnaire. Data collection procedures were standardized across all centers.

2.3.4 Statistical Methods

Descriptive characteristics, as well as segment-wise presence of carotid plaque, were assessed after stratification by race. To compare the prevalence of carotid plaque between these population groups, crude, age-BMI adjusted, and multivariable-adjusted prevalence ratios were calculated through log-binomial regression using GENMOD procedure in SAS. When any of the above models failed to converge, the prevalence ratio was calculated using log-poisson regression with robust variance estimator.[207]

To examine which CVD risk factors were associated with the presence of carotid plaque, forward-selection logistic regression analysis was performed in a combined sample of the four racial groups. Carotid plaque presence was kept as the outcome variable and following variables as the predictor variables in the regression model – age, BMI, SBP, glucose, ever smoking, alcohol drinking status, HDL-c, LDL-c, and triglycerides (log-transformed). Alpha was set at 0.05 for determining statistical significance. As a confirmatory analysis, the above analyses was performed using traditional logistic regression model forcing all the above variables in the regression equation. All analyses were performed using SAS/STAT software v9.3 of the SAS System, Cary, NC, USA.

2.4 RESULTS

2.4.1 Comparison of Risk Factors

The mean age was about 45 years in all the three population groups. Whites had significantly higher BMI than that of Japanese in Japan and Koreans, although Whites had much

lower smoking rates than Japanese in Japan and Koreans. Whites had LDL-c similar to Japanese men in Japan, while LDL-c was lowest among Korean men. Whites had higher HDL-c than Koreans but less than Japanese in Japan. Whites and Koreans had lower hypertension rates than Japanese in Japan. Whites had the lowest prevalence of diabetes in the three groups although the differences were not statistically significant. (Table 2-1)

When comparing population characteristics by presence of carotid plaque in the combined population of four groups, those with plaque (n=117) were older (46.1±2.8 years vs. 44.8±2.8 years, p=<0.01), had higher BMI (26.9±4.4 kg/m^2 vs. 25.2±3.7 kg/m^2, p=<0.01), SBP (125.5±14.6 mmHg vs. 122.7±13.9 mmHg, p=0.04), glucose (105.4±17.9 mg/dl vs. 103.2±16.4 mg/dl, p=ns), diabetes (12.8% vs. 5.2%, p=<0.01), and hypertension (29.9% vs. 17.4%, p=<0.01), while they had similar rates of alcohol drinking (50.4% vs. 52.2%, p=ns) and smoking (53.8% vs. 62.3%, p=ns). (Table 2-3)

2.4.2 Carotid Plaque Prevalence

White men (22.8%) had significantly higher prevalence of carotid plaque as compared to Japanese men in Japan (4.8%) and Koreans (10.6%). A majority of participants with prevalent carotid plaque had only one plaque in the carotid arteries. In all three race/ethnicities, more plaque was present in the carotid bulb than in common carotid or internal carotid artery. (Table 2-2)

The differences in plaque prevalence were independent of age and BMI (Model I). (Figure 2-1) The differences remained significant even after further adjusting for other risk factors such as hypertension and diabetes (Model II). As compared to Whites, the multivariable adjusted prevalence ratio for carotid plaque was 0.18 (95% CI: 0.10, 0.30) for Japanese in Japan, and 0.44 (95% CI: 0.30, 0.65) for Koreans. The differences remained significant even after further adjusting

for carotid IMT (Model III). (Figure 2-1) Similar results were seen after removing participants with hypertension, diabetes, and hyperlipidemia from the analysis.

2.4.3 Risk Factors for Carotid Plaque

In a race-adjusted logistic regression model created using forward-selection strategy (Model A), age, hypertension, diabetes, and ever smoking were significantly associated with the presence of carotid plaque. (Table 2-4) These risk factors together explained 18% of the total variance (R^2) in carotid plaque. When other cardiovascular risk factors were force-entered in the model (Model B), the results were similar, and the R^2 increased marginally to 19%. (Table 2-4)

When IMT was further included in the model, the R^2 increased from 19% to 33% (Model C). In this regression model, IMT had the strong association with the presence of carotid plaque with a β_{std} of 0.56.

2.5 DISCUSSION

In this population-based international study among middle-aged men without CVD, wide variations in the prevalence of carotid plaque were found, with Whites having about five-fold higher prevalence of carotid plaque as compared to Japanese in Japan. Further analysis showed that age, hypertension, diabetes, and smoking were independently associated with carotid plaque while other cardiovascular risk factors added little to the prediction model. This is the first population-based international study to compare the prevalence of carotid atherosclerotic plaque among different population groups living in different countries.

The prevalence of carotid plaque among Whites was 22.8%, which was significantly higher than that in Japanese and Korean men. Similar prevalence (26.6%) of carotid plaque among Whites has been reported from the Atherosclerosis Risk in Communities study among 45-49 year old men.[208] In the Multi-Ethnic Study of Atherosclerosis, 46% of the White men and women aged between 45-84 years had carotid artery plaque.[199] In Japan, previous studies among older populations have reported much higher prevalence than in the present study. In the Suita Study, i.e. among 1,694 Japanese in Japan, men (n=814) in age groups of 50-59 years, 60-69 years and 70-79 years had 79%, 91% and 98% prevalence of carotid plaque respectively. In a smaller Japanese study consisting of 100 CVD patients (age: 61±14 years, 76% male) and 132 participants with no CVD (age: 58±14 years, 67% male), prevalence of carotid plaque was 59% and 41% respectively. However, carotid plaque was defined as IMT > 1.1 mm and IMT > 1.0 mm in these Japanese studies, respectively. It is likely that the increased prevalence in previous studies was due to older sample population and use of a different definition for carotid plaque. In Korea, a population-based study reported approximately 31% prevalence of carotid plaque in a rural population free of CVD. This study included both men and women 40-91 years of age (mean ~ 61 years).[209] Another population-based study in a similar age group reported 37% prevalence of plaque among men.[210] The prevalence of plaque reported in these studies is higher likely due to the older population than that in the present study.

2.5.1 Eastern Asia vs. the US

Whites had more than four times higher prevalence of carotid plaque than Japanese in Japan although Japanese in Japan had higher rates of hypertension, diabetes and smoking, and similar levels of LDL-c. The difference remained after adjustment for BMI, smoking, and other CVD risk

factors. This author's group have also reported significantly more coronary atherosclerosis and higher IMT among Whites than Japanese in Japan.[118] The differences in the prevalence of carotid plaque were independent of the differences in carotid IMT. These differences in subclinical atherosclerosis are probably due to one or more protective lifestyle factors prevalent among Japanese in Japan but absent in the US.

Koreans had about half the prevalence of carotid plaque than Whites even after adjusting for traditional risk factors. Although Koreans share many biologic and lifestyle characteristics with Japanese in Japan, Koreans had twice their carotid plaque prevalence. This is in spite of similarities in BMI and smoking prevalence, and lower SBP, glucose, LDL-c than Japanese in Japan, although Koreans had lower HDL-c and lower prevalence of alcohol drinking. In this sample population, Koreans also had higher carotid IMT than Japanese in Japan.[211] Further, previous reports have identified CHD mortality among South Koreans that is lower than the US but higher than Japanese in Japan.[212] Thus, similar to Japan, there may be one or more protective factors in the South Korean lifestyle that leads to lower atherosclerotic burden in these populations.

Several studies have tried to assess reasons for lower CHD in Japan.[213,214] While higher fish intake is consistently shown to be protective against CHD by several observational studies[120,215,216] and also a clinical trial in Japan,[217] low-to-medium dose trials of omega-3 fatty acids have failed to show significant benefit in western populations. It is possible that the absence of significant benefit in clinical trials in the West was due to omega-3 fatty acid supplementation that was less than their average daily intake in Japan over lifetime. Further, it is possible that the lower CHD in Japan is due to some other lifestyle/dietary factors or their combination such as very high intake of soy products in eastern Asia including Japan.

2.5.2 Risk Factors for Plaque

Of all traditional CVD risk factors, only age, hypertension, and diabetes were significant predictors of carotid plaque after adjusting for race in this cross-sectional study. Even in this relatively homogenous population of men aged 40-49 years, age was the strongest predictor of carotid plaque. In the NOMAS study, i.e. an older population-based cohort in the US comprising of 60% Hispanics, age, smoking, SBP, DBP, diabetes, and blood pressure medication use were significant predictors of carotid plaque.[36] Similarly, Klein et al have reported that in a sample of 876 clinical patients (mean age: 53.4±12.0 years; 47% female), age, sex, SBP, smoking, diabetes, and lipid medication were significant predictors of carotid plaque.[35] The present study found lesser number of significant predictors than these previous studies, possibly because these studies had older populations, and included clinical patients.

In the present study, 18.16% of the variance in the prevalence of carotid plaque was explained by the four significant predictors in the model. Forcing other variables in the model did not result in an increase in the R^2. Previous studies have reported similar results – in the NOMAS study, risk factors explained 19.5% of the carotid plaque variance.[36] Similar to the present study, age, was the single largest contributor to that R^2. Klein et al reported from a clinical population the R^2 of 48.6% for total carotid plaque area, and R^2 of 13.9% for total carotid stenosis.[35]

In this study, the differences in plaque prevalence remained even after adjusting for carotid IMT. This was somewhat expected as the definition used to identify plaque had a measure of IMT in it, i.e. plaque was defined as focal protrusion at least 50% thicker than the surrounding IMT. Thus, assessment of plaque in an individual was based on his IMT. Further, while IMT is strongly associated with age and blood pressure,[218] carotid plaque are atherosclerotic in nature and have a

lipid core.[219] Thus, it is likely that IMT and carotid plaque have different pathophysiological origins. In another study in France, carotid IMT is shown to be a significant predictor of carotid plaque occurrence in a 59 to 71 year old population followed over 4 years.[220] In the present study as well, IMT was the strongest determinant of plaque among all risk factors. Thus, it seems that IMT is an important, but not the only, determinant of plaque.

Results of this study should be interpreted in light of certain limitations. Results of this study are based on analysis of cross-sectional data, thus the temporality of association is not established. Use of non-invasive assessment limits examination differences in carotid plaque characteristics such as presence of intraplaque calcification and hemorrhage. However, ultrasonographic carotid artery examination was performed given the population-based design of this study. All the participants in this study were males 40-49 years of age, thus limiting the generalizability of the findings. Assessing atherosclerosis in this middle-aged sample gives the opportunity for potential intervention to prevent the development of future clinical disease. This study cannot perform analysis to examine risk factors of carotid plaque in individual races as there are few men with carotid plaque among Japanese in Japan and Koreans. A majority of participants with prevalent carotid plaque had only one plaque in the carotid arteries; thus presence of carotid plaque was used as an indicator for carotid atherosclerotic burden. However, this is the first population-based epidemiological study to use a standardized protocol for risk factor and carotid plaque assessments across the geographic regions.

2.6 CONCLUSION

Whites have significantly higher carotid atherosclerotic plaque than Japanese in Japan and Koreans despite having more desirable profile on several CVD risk factors. These differences are not explained by traditional cardiovascular risk factors, and were independent of the carotid IMT.

2.7 TABLES AND FIGURES

Table 2-1 Prevalence of carotid plaque and other cardiovascular risk factors among 40-49 year old men in the ERA JUMP Study

Characteristic	Whites (n=310)	Japanese in Japan (n=313)	Koreans (n=301)	**Differences (p<0.05)**
	1	**2**	**3**	
Age (years)	45.0 ± 2.8	45.1 ± 2.8	44.8 ± 2.8	2=1=3
BMI (kg/m^2)	28.0 ± 4.4	23.7 ± 3.1	24.7 ± 2.7	1>3=2
Systolic Blood Pressure (mmHg)	122.6 ± 11.2	125.0 ± 16.1	121.6 ± 14.1	2>1=3
Hypertension (%)*	15.2	26.5	15.6	2>3=1
Glucose (mmol/l)	5.65 ± 0.86	5.93 ± 1.04	5.71 ± 1.0	2>3=1
Diabetes (%)†	3.6	6.1	9.6	3=2=1
Ever Smoking (%)	27.1	82.8	74.1	2>3>1
Current alcohol drinker (%)‡	44.3	67.3	44.0	2>1=3
LDL-cholesterol (mmol/l)	3.48 ± 0.86	3.42 ± 0.93	3.00 ± 0.82	1=2>3
HDL-cholesterol (mmol/l)	1.23 ± 0.33	1.40 ± 0.35	1.18 ± 0.30	2>1>3
Total cholesterol: HDL-cholesterol ratio	4.7 ± 1.3	4.2 ± 1.3	4.4 ± 1.2	1> 3=2
Triglycerides, mmol/l median (IQR) §	1.46 (1.04, 2.08)	1.55 (1.16, 2.05)	1.51 (1.08, 2.27)	2=3=1
Medications (%)				
Hypertension	8.7	5.4	4.6	1=2=3
Diabetes	1.0	1.9	0.3	2=1=3
Lipid	12.3	3.5	1.3	1>2=3
Intima-Media thickness	0.68 ± 0.10	0.61 ± 0.07	0.66 ± 0.09	1>3>2

Values are mean ± SD unless otherwise mentioned.

* - Hypertension was defined as presence of one or more of following – i) Systolic blood pressure (BP) ≥140 mmHg, ii) Diastolic BP ≥90 mmHg, or iii) use of antihypertensive medication

† - Diabetes was defined as either glucose ≥7 mmol/L or use of diabetic medication, or both

‡ - Alcohol drinking was defined as 2 or more drinks per week; § - IQR - Interquartile range

Table 2-2. Site-specific prevalence of carotid plaque in the four population groups

	White (n=310)	Japanese in Japan (n=313)	Koreans (n=301)	Difference
Plaque prevalence (%)	22.8	4.8	10.6	1>3>2
Plaque in CCA (%)	0.6	0.3	0.3	1=3=2
Plaque in bulb (%)	16.9	3.5	9.3	1>3>2
Plaque in ICA (%)	10.1	1.6	2.3	1>3=2

CCA: common carotid artery; ICA: internal carotid artery

Table 2-3 Prevalence of carotid plaque and other cardiovascular risk factors among 40-49 year old men in the ERA JUMP Study

	Plaque present (n=117)	No Plaque (n=803)	p-value
Age (years)	46.1 ± 2.8	44.8 ± 2.8	<0.01
BMI (kg/m^2)	26.9 ± 4.4	25.2 ± 3.7	<0.01
Systolic Blood Pressure (mmHg)	125.5 ± 14.6	122.7 ± 13.9	0.04
Hypertension (%)*	29.9	17.4	<0.01
Glucose (mg/dl)	105.4 ± 17.9	103.2 ± 16.4	ns
Diabetes (%)†	12.8	5.2	<0.01
Ever Smoking (%)	53.8	62.3	Ns
Current alcohol drinker (%)‡	50.4	52.2	ns
LDL-cholesterol (mg/dl)	133.7 ± 35.3	127.0 ± 34.7	0.05
HDL-cholesterol (mg/dl)	47.5 ± 13.6	49.5 ± 13.1	0.12
Total cholesterol: HDL-cholesterol ratio	4.7 ± 1.3	4.4 ± 1.3	0.02
Triglycerides, mg/dl median (IQR) §	131 (95, 201)	132 (97, 183)	ns
Medications (%)			
Hypertension	13.7	5.0	<0.01
Diabetes	3.4	0.6	<0.01
Lipid	8.6	5.4	ns
Intima-Media thickness	0.75 ± 0.12	0.63 ± 0.08	<0.01

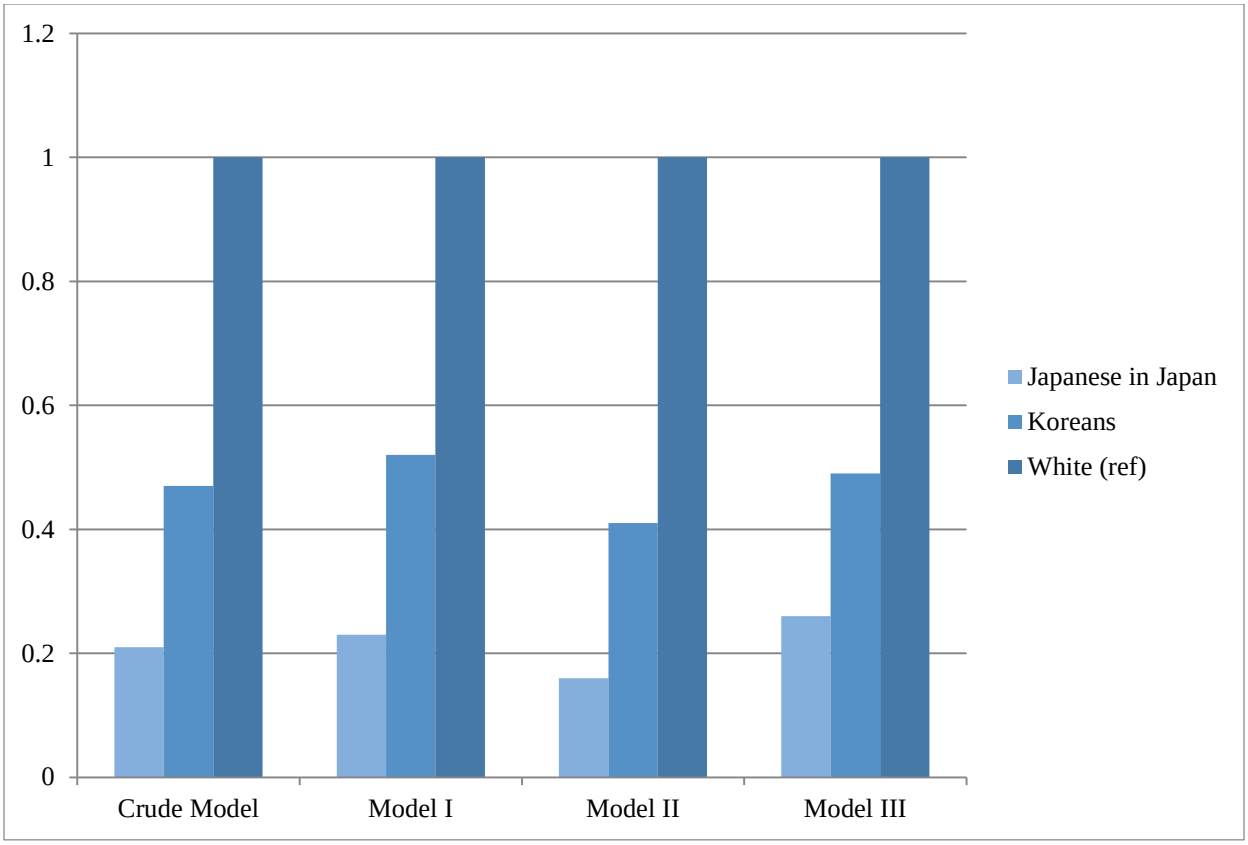
Values are mean ± SD unless otherwise mentioned.

*** - Hypertension was defined as presence of one or more of following – i) Systolic blood pressure (BP) ≥140 mmHg, ii) Diastolic BP ≥90 mmHg, or iii) use of antihypertensive medication**

† - Diabetes was defined as either glucose ≥126 mg/dl or use of diabetic medication, or both

‡ - Alcohol drinking was defined as 2 or more drinks per week

§ - IQR - Interquartile range



White was treated as the referent category in the analyses
All prevalence ratios in the analyses were significant at p-value <0.01
Crude Model: Unadjusted model
Model I: Age, BMI- adjusted
Model II: Model I + SBP, total cholesterol: HDL-cholesterol ratio, diabetes, hypertension, smoking, and alcohol drinking
Model III: Model II + carotid intima-media thickness

Figure 2-1 Prevalence Ratio of Carotid plaque among the three races in the ERA JUMP study

Table 2-4 Regression models for prediction of Plaque in the ERA JUMP study

Model	Variable	Standardized β (β_{std})	β	SEM	p-value	R^2
Model A[*,†]	Age	0.2899	0.1870	0.0403	<0.0001	18%
	Hypertension		0.3392	0.1277	0.0079	
	Diabetes		0.5040	0.1855	0.0066	
Model B[*,‡]	Age	0.2844	0.1834	0.0407	<0.0001	19%
	Hypertension		0.3313	0.1331	0.0128	
	Total cholesterol: HDL-cholesterol ratio	0.0945	0.1361	0.1046	0.1933	
	Diabetes		0.4876	0.1883	0.0096	
	BMI	0.0173	0.0082	0.0313	0.7926	
	Ever Smoking		0.1497	0.1249	0.2305	
	Drinking frequency		0.0591	0.1137	0.6034	
	Triglyceride (log)	0.0111	0.0404	0.2404	0.8665	
Model C	Age	0.2277	0.1467	0.0445	0.0010	33%
	Hypertension		0.2095	0.1440	0.1456	
	Total cholesterol: HDL-cholesterol ratio	-0.0113	-0.0163	0.1152	0.8878	
	Diabetes		0.2305	0.2088	0.2696	
	BMI	-0.0803	-0.0382	0.0347	0.2712	

Table 2-4 contd.

	Ever Smoking		0.2709	0.2686	0.3131
	Drinking frequency		0.0144	0.1230	0.9070
	Triglyceride (log)	0.0117	0.0428	0.2913	0.8832
	Carotid IMT	0.5596	10.9479	1.3497	<0.0001

*** Models were additionally adjusted for Race**
† Model A was developed using forward-selection strategy in logistic regression with p-value for significance at 0.05
‡ Model B was traditional model with risk factors forced into the logistic regression
Model C: Model B + Carotid IMT

3.0 BRACHIAL-ANKLE PULSE WAVE VELOCITY IS ASSOCIATED WITH CORONARY ATHEROSCLEROSIS AMONG 1,171 HEALTHY MIDDLE-AGED MEN

3.1 ABSTRACT

Background: Brachial-ankle pulse wave velocity (*ba*PWV) is a simple and reproducible measure of arterial stiffness, and is extensively used to assess cardiovascular disease risk in eastern Asia. This study examined whether *ba*PWV is associated with coronary atherosclerosis in an international cohort of apparently healthy middle-aged men.

Methods: A population-based sample of 1,171 men aged 40-49 years was recruited – 281 White and 83 Black men in Pittsburgh, US, 235 Japanese-Americans in Honolulu, US, 292 Japanese in Otsu, Japan, and 280 Koreans in Ansan, Korea. *ba*PWV was measured with an automated waveform analyzer (VP2000, Omron) and atherosclerosis was examined as coronary artery calcification (CAC) by computed-tomography (GE-Imatron EBT scanner). Association of the presence of CAC (defined as ≥10 Agatston unit) was examined with continuous measure as well as with increasing quartiles of *ba*PWV.

Results: As compared to quartile 1 of *ba*PWV, the multivariable-adjusted odds ratio (95% confidence-interval [CI]) for presence of CAC in the combined sample was 1.72 (1.00, 2.96) for 2nd quartile, 1.92 (1.10, 3.34) for 3rd quartile, and 2.25 (1.24, 4.09) for 4th quartile (p-trend = 0.01). The odds for CAC were higher by 20% per 100 cm/s increase ($p<0.01$), or by 38% per standard-deviation increase ($p<0.01$) in *ba*PWV. There was no difference in this association by race.

Conclusion: *ba*PWV is cross-sectionally associated with CAC among healthy middle-aged men. The association is consistent among different races. Longitudinal studies are needed in the West to determine its CVD predictive ability.

3.2 INTRODUCTION

Coronary heart disease (CHD) remains the leading cause of mortality in the United States (US) in spite of a significant decline in age-adjusted CHD over the past 5 decades.[1] The burden of CHD is likely to increase by 16% over the next 20 years due to an aging population and increased survival after suffering from a coronary event.[99] One of the current strategies for further reducing CHD is to identify and implement prevention strategies among individuals who are at an intermediate CHD risk according to the current risk prediction tools, and who would benefit most from CHD prevention therapy.[29]

Coronary artery calcification (CAC), assessed by computed-tomography scan (CT), is a strong independent predictor of future CHD events among asymptomatic individuals. Use of CAC imaging is currently recommended among individuals who are at low to intermediate risk i.e.10-year risk of cardiovascular disease (CVD) risk between 6%-20%.[14] However, CAC imaging exposes an individual to ionizing radiation, which may limit its wider applicability as a screening tool. Also, the cost-effectiveness of routine CT imaging among asymptomatic individuals is not yet established.[18]

Brachial-ankle pulse wave velocity (*ba*PWV) is a highly reproducible measure of arterial stiffness that has shown promise as a predictor of future CVD among east Asian populations.[67] Unlike carotid-femoral pulse wave velocity (*cf*PWV), which is a measure of central arterial

stiffness, *ba*PWV likely is a combined measure of central and peripheral arterial stiffness.[221] Although *cf*PWV is considered as gold standard marker for central arterial stiffness and a predictor of future CHD, despite much work, it has not gained acceptance into clinical practice in the US[222] – possibly due to operator training and expertise, and also patient's discomfort with groin exposure. In contrast, *ba*PWV is currently used routinely in Japan and South Korea to assess CVD risk. *ba*PWV is convenient to measure in a clinic, requires little technical expertise, and, unlike cfPWV, does not require exposure to the inguinal region. The author's research group has previously reported a significant association between *ba*PWV and the presence of CAC among obese post-menopausal women in the US.[223] However, the utility of *ba*PWV in the US remains to be thoroughly examined.[224,225]

Therefore, the association between *ba*PWV and presence of CAC was examined in the Electron beam computed tomography and Risk factor Assessment among Japanese and U.S. Men in the Post-World War II birth cohort (ERA JUMP Study), an international study of subclinical atherosclerosis among 40-49 year old men. The hypothesis was that *ba*PWV is significantly associated with presence of CAC in this healthy sample of middle-aged men.

3.3 METHODS

3.3.1 Participants

During 2002–2006, a population-based sample of 1,335 men aged 40–49 years, with no clinical CVD or other severe diseases, was obtained from 4 centers: 310 Whites and 107 Blacks from Pittsburgh, Pennsylvania, US; 303 Japanese Americans from Honolulu, Hawaii, US; 313

Japanese from Kusatsu city, Shiga, Japan; and 302 Koreans from Ansan, Gyeonggi-do, South Korea as previously described.[118] Written informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of the following institutions: the University of Pittsburgh, Pittsburgh, Pennsylvania, US; the Kuakini Medical Center, Honolulu, Hawaii, US; Shiga University of Medical Science, Otsu, Japan; and Korea University, Seoul, South Korea.

3.3.2 Pulse Wave Velocity Assessment

At the start of the study, staff from the University of Pittsburgh's Ultrasound Research Laboratory visited the Honolulu site to train the sonographers in Honolulu and from South Korea for PWV measurements. In addition, continuous quality control measures were implemented for all the sites, including Japan. PWV measurements were automatically generated using a noninvasive and automated waveform analyzer (VP2000, Omron, Japan). This device provides automated measures of *ba*PWV on both right and left sides – average of the two sides was used. Following 10 min of rest in a supine position, occlusion and monitoring cuffs were placed around both arms and both ankles of the participant. The arm cuffs were placed on the skin or over light clothing, and the ankle cuffs were directly placed over the skin. ECG electrodes were placed on both wrists and a phonocardiogram i.e. a microphone for detecting heart sounds was placed on the left edge of the sternum. The path length for *ba*PWV was calculated using height-based formulae.[71] PWV was calculated as the distance between arterial sites divided by the time between the feet of the respective waveforms. Intra-class correlations (ICC) for re-examination of *ba*PWV was 0.97 within technician, and 0.91 between technicians.[73]

3.3.3 Coronary Artery Calcification

CAC scanning was performed with a GE-Imatron C150 EBT scanner (GE Medical Systems, South San Francisco, California) at all centers as published elsewhere in detail.[121] Briefly, a standardized protocol was used to perform CAC scanning; 30–40 contiguous, 3-mm-thick transverse images from the level of the aortic root to the apex of the heart were obtained during maximal breath holding by using electrocardiogram triggering (60 percent of the R-R interval) so that each 100 millisecond exposure was obtained during the same phase of the cardiac cycle.[121] One trained reader at the University of Pittsburgh read the images using a DICOM (Digital Imaging and Communications in Medicine) workstation and software by AccuImage (AccuImage Diagnostic Corporation, San Francisco, California). The software program implements the widely accepted Agatston scoring method.[226] Coronary artery calcification was considered to be present when 3 contiguous pixels (area = 1 mm^2) greater than 130 Hounsfield Unit (HU) were detected overlying the vessels of interest. A coronary calcium score (CCS) was then calculated for each region of interest by multiplying the area of all significant pixels by a grade number (1, 2, 3, 4) indicative of the peak computed tomography number (HU). The individual region of interest scores was then summed for a total CCS. The reader was blinded to the participant's characteristics and the study centers. ICC for re-examination of electron-beam computed tomography scans was 0.98. Presence of CAC was defined as ≥ 10 Agatston Unit (AU) as a score between 0-10 is likely to be noise;[121] a cut-off value of 10 AU maximizes the positive predictive value of CAC for underlying plaque disease by minimizing any contribution from beam hardening or motion artifact.

3.3.4 Risk Factor Assessment

All participants underwent a physical examination, completed a lifestyle questionnaire, and a laboratory assessment as described previously.[118,201,202] Body weight and height were measured while the participant was wearing light clothing without shoes. Body-mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Blood pressure was measured – after the participant emptied his bladder and sat quietly for 5 min – twice on right arm with an automated sphygmomanometer (BP-8800, Colin Medical Technology, Komaki, Japan) using an appropriate sized cuff; average of the two measurements was used. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg or use of anti-hypertensive medications.[203] Venipuncture was performed early in the clinic visit after a 12-hour fast. Blood samples were stored at −80°C and shipped on dry ice from all centers to the University of Pittsburgh. Serum lipids were determined using the protocol standardized by the Centers for Disease Control and Prevention.[204] Serum glucose was determined by using hexokinase–glucose-6-phosphate-dehydrogenase enzymatic assay. Diabetes was defined as individuals with fasting glucose ≥126 mg/dl or use of medications for diabetes.[206] Alcohol drinking was defined drinking two or more times per week. Smoking was measured as history of ever smoking. Use of blood pressure-lowering, diabetes, and lipid-lowering medication were ascertained through questionnaire. Data collection procedures were standardized across all centers.

Following participants were excluded: those taking antihypertensive medications (n=140), those with extreme outliers i.e. CAC >1,000 AU (n=3), and those with extremely high *ba*PWV i.e. *ba*PWV >2,000 cm/s (n=4). The final sample for this study consisted of 1,171 men (281 Whites, 83 Blacks, 235 Japanese Americans, 292 Japanese in Japan, and 280 Koreans) with complete data.

3.3.5 Statistical Methods

Descriptive characteristics of the sample population were examined, including demographics, CVD risk factors, *ba*PWV, and CAC. Comparisons of population characteristics were made using t-tests for continuous variables, and chi-square tests for categorical variables. Non-parametric tests were used for skewed variables. Logistic regression was used to examine the association between *ba*PWV (per 100 cm/s increase, per 1 SD increase) and presence of CAC (≥ 10 AU) – in an unadjusted model (crude model), in an age-race adjusted model (Model I), as well as in multivariable models adjusted for CVD risk factors including race, age, SBP, LDL-c, ever smoking, and diabetes (Model II), and further adjusting for HDL-c, BMI and alcohol drinking (Model III). Unadjusted analysis was also performed after stratification by race-ethnicity. Further, *ba*PWV was categorized into quartiles and its association with the presence of CAC was examined keeping lowest quartile of *ba*PWV as the reference category. Similar to the previous analysis, the association was examined using unadjusted model, race-adjusted, and multivariable-adjusted models in logistic regression. Interaction analysis was performed to examine the differences in the association of *ba*PWV with CAC; further, unadjusted analyses was performed after stratification by race. All data analysis was performed using SAS/STAT software, version 9.3 of the SAS System, Cary, NC, USA.

3.4 RESULTS

Participants with CAC (n=207) were slightly older (46.0 vs. 44.9 years, $p<0.001$), had higher BMI (27.6 vs. 25.5 kg/m^2, $p<0.001$), SBP (126.4 vs. 122.7 mmHg, $p<0.001$), LDL-c (136.0 vs. 126.4 mg/dl, $p<0.001$), and triglycerides (148 vs. 129 mg/dl, $p<0.01$) than those with no CAC

(n=964). Prevalence of diabetes was also higher among those with CAC (9.7% vs. 5.6%, p=0.03). Further, *ba*PWV was significantly higher among those with CAC (1392.6 vs 1317.0 cm/s among those with no CAC, p<0.001). (Table 3-1) The mean (SD) of *ba*PWV (cm/s) among individual races was: Whites 1313.9(151.3), Blacks 1374.2(164.0), Japanese Americans 1414.4(160.5), Japanese in Japan 1296.1(175.6), and Koreans 1294.8(138.1). The prevalence of CAC increased successively with increasing *ba*PWV. (Figure 3-1) With every 100 cm/s increase in *ba*PWV, the odds for presence of CAC increased by 30% (p<0.001) in the crude model, 27% (p<0.001) after adjustment for age and race (Model I), and 19%-20% (p<0.01) in multivariable-adjusted models (model II and model III). Similarly, with every 1 SD increase in *ba*PWV, the odds for CAC increased by 61% (p<0.001), 52% (p<0.001), and 38% (p<0.01) in crude, age-race adjusted and multivariable adjusted models respectively. (Table 3-2) When examining *ba*PWV by quartiles, a significant trend was seen for increasing prevalence of CAC with increasing *ba*PWV quartiles in unadjusted and age-race adjusted models, as well as after multivariable-adjustment.

Among individual race-ethnicities, the prevalence of CAC was: Whites (24.9%), Blacks (15.7%), Japanese Americans (28.1%), Japanese in Japan (10.3%), and Koreans (10.0%). There was no significant interaction between race and *ba*PWV in the model for the presence of CAC (p=0.28). When analysis was performed after stratifying by race, in unadjusted model, the association between *ba*PWV and CAC prevalence was significant among Whites (OR: 1.30 per 100 cm/s increase; p<0.01), Blacks (OR: 1.63 per 100 cm/s increase, p<0.01), and Koreans (OR: 1.36 per 100 cm/s increase; p=0.03); the association was not significant among Japanese Americans and Japanese in Japan although the odds for presence of CAC were higher with higher *ba*PWV. (Table 3-2) When examining *ba*PWV by quartiles in individual races, the analysis could not be performed among Blacks due to sample size limitations. Among other races, the p-trend for

higher presence of CAC with higher quartiles of *ba*PWV was significant among Whites in unadjusted models. (Table 3-3)

3.5 DISCUSSION

This study reports that *ba*PWV iss associated with coronary atherosclerosis among middle-aged men without CVD. This association was independent of traditional CVD risk factor and did not differ by race. This is the first international study to examine the association between *ba*PWV and coronary atherosclerosis among healthy middle-aged men.

Previous studies have reported similar association of *ba*PWV with CAC among 504 obese postmenopausal women in the US,[223] and among 654 patients without CVD in Taiwan.[227] In the US, the author's group has previously shown that among 504 obese postmenopausal women, the multivariable-adjusted odds ratio for presence of any CAC (>0 AU) were 1.94 (95% CI 1.01, 3.70), 2.90 (95% CI 1.50, 5.58), and 2.21 (95% CI 1.11, 4.42) for quartiles 2, 3, and 4 respectively as compared to quartile 1 of *ba*PWV; the strength of association was stronger for *ba*PWV than for *cf*PWV.[223] Similarly, in the Taiwanese study, the mean (±SD) CAC score increased (35.7 ± 173.6, 100.2 ± 249.7, and 227.6 ± 412.5, p-trend<0.001) with increasing *ba*PWV (<1400 cm/s, 1400 cm/s-1800 cm/s, and >1800 cm/s). Further, addition of *ba*PWV to Framingham Risk Score (FRS) improved the area under curve (AUC) for prediction of CHD from 0.676 to 0.728.[227] Several clinical studies in eastern Asia have shown an association of *ba*PWV with other intermediate CVD outcomes. For example, *ba*PWV is reported to be positively associated with coronary stenosis in Korea,[228-230] Japan,[231] Taiwan[227], and China.[232] The present study's sample population was healthier than these previous studies i.e. the present sample was younger and population-based.

Additionally, the mean *ba*PWV in the present sample population was less than the *ba*PWV reported from these studies. Examining this association among healthy men allowed the assessment of the utility of *ba*PWV as an early marker of CVD in an asymptomatic population.

*ba*PWV likely measures arterial stiffness over the central and peripheral arterial tree. In contrast, *cf*PWV – a widely accepted marker of arterial stiffness – primarily measures stiffness in the central aortic trunk. Increased smooth muscle in the peripheral arteries, as compared to aorta, may lead to increased velocity of transmission of the pulse wave in the peripheral vasculature. This at least partly explains the increased PWV seen in the brachial-ankle region as compared to the *cf*PWV. However, given that aorta forms a large portion of the brachial-ankle region, 58% of the variation in *ba*PWV can be explained by aortic PWV.[233] Further research is needed to examine pathogenesis of arterial stiffness especially within the peripheral muscular arteries.

Several factors may account for the association between *ba*PWV and coronary atherosclerosis. Firstly, the pathogenesis of arterial stiffness possibly shares some common risk factors with atherosclerosis[234,235] – this is more likely in the peripheral muscular arteries than in the central aortic trunk. This author has previously shown that atherogenic lipoprotein particles are associated with *ba*PWV and femoral-ankle pulse wave velocity, but not *cf*PWV.[78] Secondly, arterial stiffness increases the mechanical stress on the arterial wall. This triggers molecular cascades that further triggers the growth of microvasculature within the vessel walls, and ultimately leads to microvasculature remodeling and damage.[236] These microscopic changes may be a fertile ground for the formation of atherosclerotic lesions. Finally, arterial stiffening reduces the cushioning effect of the aortic trunk that helps in maintaining coronary blood flow during the diastole. It increases cardiac afterload, thus increasing left-ventricular mass and requirement for coronary blood flow.[237]

*ba*PWV has a potential for wide clinical application. A recent meta-analysis reported that *ba*PWV is an independent predictor of total cardiovascular events, cardiovascular mortality, and all-cause mortality. However, 17 out of 18 studies used in this meta-analysis were conducted in eastern Asia. *ba*PWV measurement is inexpensive, can be performed in a physician's office, requires little operator's expertise, and does not require exposure to the inguinal area. Currently, it is widely used in clinics in Japan and South Korea to assess the risk of future CVD. However, several concerns need to be addressed before it can be used as a clinical tool in the US, most important of which are determination of normal reference values and applicability among Western populations.

These results should be interpreted in light of certain study limitations. As the present study is cross-sectional, temporality of the association between *ba*PWV and CAC cannot be determined; a scenario in which CAC leads to increase in *ba*PWV, however, seems unreasonable. Results of this study are not generalizable to older populations as the sample consisted of middle-aged men within a relatively narrow age range. However, use of this sample population allows examination of the study hypothesis in the age-group in which *ba*PWV is most likely to be useful as an early screening tool. Presence of CAC was defined as ≥ 10 AU as CAC score between 0 and 10 is likely to be noise. Few participants in the present study had clinically significant CAC (≥400 AU) (n=19),[238] and thus, association of *ba*PWV with clinically significant CAC could not be tested. The limited sample size within each race/ethnicity limits the ability to perform multivariable adjustment in race-specific analysis in this study.

3.6 CONCLUSION

In conclusion, in a population-based sample of healthy middle-aged men without CVD, *ba*PWV is independently associated with coronary atherosclerosis measured by CAC imaging. This association was consistent among Whites and Blacks in the US. Longitudinal studies to examine ability of *ba*PWV to predict coronary atherosclerosis and CVD events in the US population are needed.

3.7 TABLES AND FIGURES

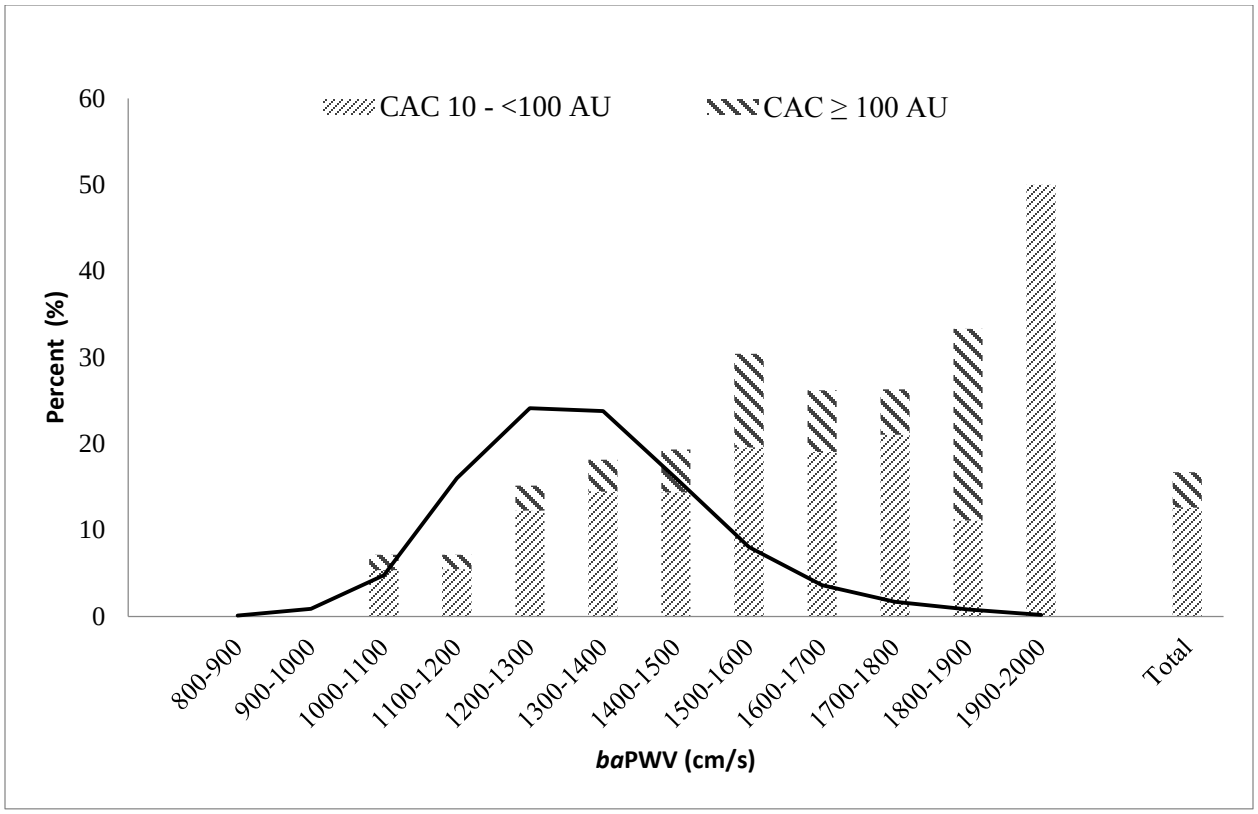
Table 3-1 Presence of cardiovascular risk factors among 40-49 year old men in the ERA JUMP Study, by presence of CAC (≥10 AU)

Population characteristic	CAC (n=207)	No CAC (n=964)	p-value
Age (years)	46.0 (2.6)	44.9 (2.9)	<0.001
BMI (kg/m^2)	27.6 (4.5)	25.5 (3.8)	<0.001
Race/ethnicity (%)			<0.001
Whites	24.9	75.1	
Blacks	15.7	84.3	
Japanese Americans	28.1	71.9	
Japanese in Japan	10.3	89.7	
Koreans	10.0	90.0	
Systolic Blood Pressure (mmHg)	126.4 (12.6)	122.7 (13.3)	<0.001
Hypertension (%)[$]	16.4	13.6	ns
Glucose (mmol/l)	5.9 (0.8)	5.8 (1.0)	ns
Diabetes (%)[‡]	9.7	5.6	0.03
Pack years of smoking[†]	0.8 (0.0, 21)	1.1 (0.0, 18.9)	ns
Ever Smoking (%)	53.6	54.0	ns
Ethanol intake (g/day)[†]	8.2 (0.4, 28.4)	7.5 (0.2, 24.7)	ns
Alcohol drinker (%)	49.3	47.3	ns
LDL-cholesterol (mmol/l)	3.5 (0.9)	3.3 (0.9)	<0.001
HDL-cholesterol (mmol/l)	1.3 (0.4)	1.3 (0.3)	ns
Triglycerides, mmol/l[†]	1.7 (1.1, 2.4)	1.5 (1.0, 2.1)	<0.01
***ba*PWV (cm/s)**	1392.6 (162.3)	1317.0 (160.7)	<0.001
CAC Score[†]	42.3 (20.1, 96.5)	NA	
CAC Score ≥ 100 AU (%)	23.7	NA	

Values are mean ± SD unless otherwise mentioned.
$ - Hypertension was defined as presence of one or more of following – i) Systolic blood pressure (BP) ≥140 mmHg, ii) Diastolic BP ≥90 mmHg, or iii) use of antihypertensive medication
‡ - Diabetes was defined as either glucose ≥7 mmol/l or use of diabetic medication, or both
† -Median (IQR)
ns = not significant

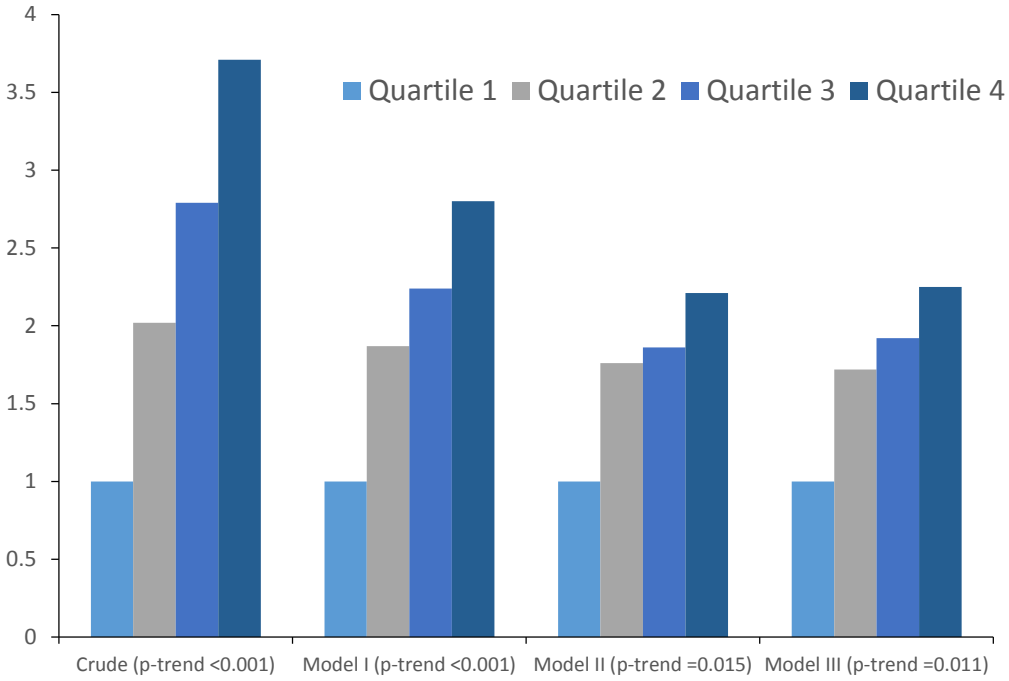
Table 3-1 contd.

CAC = Coronary Artery Calcification, AU = Agatston unit, LDL = low-density lipoprotein cholesterol, HDL = high-density lipoprotein cholesterol, *ba*PWV = brachial-ankle pulse wave velocity, BMI = body-mass index, IQR = inter-quartile range



CAC = coronary artery calcification, AU = Agatston unit, *ba*PWV = brachial-ankle pulse wave velocity

Figure 3-1 Prevalence of CAC (10- <100 AU and ≥100 AU) with *ba*PWV



Model I: Age, Race-adjusted.
Model II: Model I + systolic blood pressure, low-density lipoprotein cholesterol, ever smoking and diabetes
Model III: Model II + high-density lipoprotein cholesterol, alcohol drinking and body-mass index

Figure 3-2 Association between *ba*PWV and CAC: odds ratios for presence of CAC (≥10 AU) with increasing quartiles of *ba*PWV (n=1,171)

Table 3-2 Association between *ba*PWV and CAC: odds ratio for presence of CAC (≥ 10 AU) using continuous variables of *ba*PWV

	***ba*PWV (per 100 cm/s)**	***ba*PWV (per SD change)**	**P-value**
Crude	1.31 (1.20, 1.44);	1.61 (1.37, 1.90);	p<0.001
Model I	1.24 (1.12, 1.37);	1.46 (1.22, 1.74);	p<0.001
Model II	1.19 (1.06, 1.34);	1.36 (1.10, 1.67);	p=0.004
Model III	1.20 (1.06, 1.35);	1.38 (1.12, 1.70);	p=0.003
Race-Specific Analysis**			
	*ba*PWV (per 100 cm/s)	*ba*PWV (per SD change)	P-value
Caucasian (n=281)	1.30 (1.07, 1.58);	1.49 (1.11, 2.01);	p=0.009
African American (n=83)	1.83 (1.22, 2.73);	3.03 (1.45, 6.33);	p=0.003
Japanese American (n=235)	1.17 (0.98, 1.40);	1.32 (0.97, 1.81);	p=0.078
Japanese in Japan (n=292)	1.15 (0.94, 1.41);	1.31 (0.88, 1.93);	p=0.183
Koreans (n=280)	1.36 (1.03, 1.79);	1.55 (1.04, 2.31);	p=0.030

Model I: Age, Race-adjusted.
Model II: Model I + systolic blood pressure, low-density lipoprotein-cholesterol, ever smoking, alcohol drinking, and diabetes
Model III: Model II + high density lipoprotein cholesterol, body-mass index
CAC = coronary artery calcification, *ba*PWV = brachial-ankle pulse wave velocity, AU = Agatston unit, SD = standard deviation
** Race-specific analysis was unadjusted for confounders. No significant interaction was present by race

Table 3-3 Odds ratio for presence of CAC (≥ 10 AU) with increasing quartiles of *ba*PWV

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-trend
Caucasian (n=251)	1	3.13 (1.22, 8.04)	4.00 (1.54, 10.39)	4.22 (1.57, 11.33)	0.02
Japanese American (n=202)	1	0.69 (0.20, 2.44)	1.24 (0.40, 3.86)	1.58 (0.53, 4.69)	0.31
Japanese in Japan (n=281)	1	2.38 (0.84, 6.73)	1.84 (0.56, 6.00)	2.19 (0.70, 6.88)	0.41
Korean (n=277)	1	1.11 (0.34, 3.59)	2.69 (0.96, 7.56)	1.61 (0.43, 6.06)	0.20

* Unadjusted models
** Analysis could not be performed due to small sample size among Blacks
CAC = Coronary Artery Calcification, *ba*PWV = brachial-ankle pulse wave velocity, SD = Standard deviation
Analysis could not be performed among African-Americans due to limited sample size

4.0 EQUOL PRODUCER STATUS, BUT NOT SERUM ISOFLAVONES, IS ASSOCIATED WITH LOWER CORONARY ATHEROSCLEROSIS AMONG HEALTHY JAPANESE MEN

4.1 ABSTRACT

Objective: Epidemiological studies in Asia, where consumption of soy isoflavones is regular, implicate that isoflavones are protective against coronary heart disease (CHD), especially among post-menopausal women. Equol is a derivative of daidzein, a common soy isoflavone, and is said to have stronger anti-atherosclerotic properties than other isoflavones. Individuals who have intestinal bacteria to convert daidzein to equol are commonly termed as "equol producers". The aim of this study was to examine a cross-sectional association of serum levels of soy isoflavones and equol producer status with the presence of coronary artery calcification (CAC) among men in Japan.

Materials and Methods: A population-based sample of 303 men aged 40-49 years in Japan was recruited in 2002-06. Risk factors of coronary heart disease were measured. Coronary calcium score (CCS) was calculated with the Agatston's method; the presence of CAC was defined as a CCS $\geq$ 10. Stored samples at -80°C were analyzed to determine serum levels of isoflavones and equol. Serum level of equol ≥83 nM was used as the criterion to determine equol producer status. The association of tertiles of isoflavones and equol producer status with the prevalence of CAC was analyzed using multiple logistic regression model. Further analyses was performed after

excluding very heavy drinkers of alcohol (≥69 grams/day) as these men are previously reported to have much burden of coronary atherosclerosis.

Results: The median (interquartile range) of isoflavones and equol were 482 (192, 1,082) nM and 10 (0, 33) nM, respectively. A median (75th, 90th and 95th percentile) of CCS was 0 (1.5, 15.4, and 40.9). About 16% of the participants were equol producers. These was no significant association between tertiles of soy isoflavones and CAC. After excluding very heavy alcohol drinkers (n=31), the prevalence of CAC was much lower among equol producers (2.3%) than among non-producers (10.9%). After multivariable adjustment, the odds for presence of CAC were significantly lower among equol producers (OR=0.10, 95% CI: 0.01, 0.96) than among equol non-producers.

Conclusions: Among men in Japan, there was no association between serum isoflavones and CAC. However, equol producers had a significantly lower CAC than equol non-producers, suggesting that the anti-atherosclerotic properties of isoflavones in Japanese in Japan may be mediated through equol.

4.2 INTRODUCTION

While several dietary nutrients have been examined for their anti-atherosclerotic properties,[239] the contribution of soy isoflavones in preventing CHD is not completely understood. Soy isoflavones such as daidzein and genistein are regular components of diet in east Asian countries such as Japan due to regular consumption of soy products such as tofu and miso. While daidzein and genistein are obtained directly from plant sources, equol is a structurally similar isoflavone derivative produced by the action of intestinal bacteria on daidzein.[158] CHD rates are

much lower in Japan as compared to the US, despite having very high smoking prevalence, higher blood pressure, and similar levels of serum total cholesterol and diabetes prevalence, which are all independent risk factors of CHD.[240] It is thus possible that soy isoflavones and equol contribute to the lower CHD rates in Japan.

Anti-atherogenic properties of soy isoflavones are reported in animal and *in vitro* studies.[140,141,148,241] The association of dietary intake of soy isoflavones with incident CHD is reported in several large prospective cohort studies.[131,132,242] These studies in Asian countries where dietary intake of soy and isoflavones is regular and high reported a significant inverse association, especially among post-menopausal women.[131,242] In contrast, a study in western countries, where the dietary intake of soy isoflavones is negligible compared to the dietary intake in Asia, reported no significant association.[132] It is, therefore, possible that soy isoflavones exert their anti-atherogenic properties only at levels currently consumed in east Asian countries.

Soy isoflavones are a class of compounds derived mostly from soybeans and classified as phytoestrogens because of their structural similarity to estrogen. Common isoflavones found in nature are genistin, daidzin, and glycitin, i.e. glucoside forms of genistein, daidzein and glycitein respectively. While dietary isoflavones i.e. genistein and daidzein are traditionally examined by most studies for their anti-atherosclerotic effects, [130,131] equol – a daidzein derivative – is said to have the most potent anti-atherosclerotic effect of all isoflavones. [161] Equol has higher anti-oxidant properties, higher bioavailability, and slower clearance rate compared to other isoflavones.[161,166-169] However, only some individuals have the intestinal bacteria to produce equol from daidzein. Individuals with regular intake of soy isoflavones and with the capability to produce equol are likely to benefit most from the anti-atherosclerotic properties of equol. These individuals, commonly termed as “equol producers” in the scientific literature, may have lower atherosclerosis

than equol "non-producers." It is reported that 10-50% of Japanese are equol producers,[170-174,178] while 25-30% of Americans can produce equol. [174,243] However, consumption of soy and soy products is low in the US i.e. the mean consumption of isoflavones in the US is <2mg/day.[145,146] In contrast, their average consumption in Japan is 25-50 mg/day.[145] Thus, it is unlikely that soy isoflavones have any clinically significant anti-atherosclerotic effects at the levels currently consumed in the US. In contrast, equol producers in east Asian countries including Japan may have clinically significant lower atherosclerosis than non equol-producers.

Although only a fraction of the population can convert daidzein into equol, equol is now available as a dietary supplement.[244] The first randomized controlled trial of equol supplement was conducted in Japan. In this crossover trial among 54 overweight or obese Japanese men (n=16) and women (n=38) conducted over 12 weeks, investigators found that equol supplementation resulted in a significant reduction in glycosylated hemoglobin (HbA1c), LDL-c and cardio-ankle vascular index, a measure of arterial stiffness.[178] Given the effects of equol on CVD risk factors, equol can potentially provide an exciting opportunity to further reduce CHD in the US.

Coronary artery calcification (CAC) on computed tomography scanning is a strong independent predictor of CHD.[19] No previous studies, however, have examined if the presence of CAC is associated with soy isoflavones or equol. The hypothesis for the current study is that among apparently healthy men in Japan, the presence of CAC has a significant inverse association with serum levels of dietary isoflavones, a reliable biomarker of their dietary intake in a population where soy isoflavone consumption is high and regular.[245] Further hypothesis was that the presence of CAC would be lower among equol producers than equol non-producers in Japan. The hypotheses were tested by examining serum levels of isoflavones and equol in a population-based sample of 303 men in Japan from the ERA JUMP Study.[120]

4.3 METHODS

4.3.1 Study Population

During 2002 to 2006, 303 men aged 40-49 years were randomly selected from Kusatsu, Shiga, Japan, as previously reported. [120] All participants were without clinical cardiovascular disease or other severe diseases. Informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of Shiga University of Medical Science, Otsu, Japan and University of Pittsburgh, Pittsburgh, US.

All participants underwent a physical examination, lifestyle questionnaire, and laboratory assessment as described earlier.[120] Body weight and height were measured while the participant was wearing light clothing without shoes. Body-mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Blood pressure was measured – after the participant emptied his bladder and sat quietly for 5 min – twice on right arm with an automated sphygmomanometer (BP-8800, Colin Medical Technology, Komaki, Japan) using an appropriate sized cuff; average of the two measurements was used. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg or use of anti-hypertensive medications.[203] Exercise habit was assessed as at least 1 hour of exercise/week. Education was ascertained as years of schooling. Venipuncture was performed early in the clinic visit after a 12-hour fast. Blood samples were stored at −80°C and shipped on dry ice from all centers to the University of Pittsburgh. Serum lipids were determined using the protocol standardized by the Centers for Disease Control and Prevention.[204] Serum glucose was determined by using hexokinase–glucose-6-phosphate-dehydrogenase enzymatic assay. Diabetes was defined as individuals with fasting glucose ≥126 mg/dl or use of medications for diabetes.[206] Alcohol

intake was categorized as nil, 1-23 grams/day, 24-46 grams/day, 46-69 grams/day and >69 grams/day. Heavy alcohol drinking was defined as > 69 grams/day of alcohol intake. Use of blood pressure-lowering, diabetes, and lipid-lowering medication were ascertained through questionnaire.

4.3.2 Measurements of serum isoflavones

The serum levels of two major forms of dietary isoflavones: daidzein and genistein, were measured using the serum samples stored at -80°C at University of Pittsburgh in 2009-12 with a modified method of Pumford.[246] In brief, daidzein-d_4 and genistein-d_4(Cambridge Isotope Laboratories, Andover, MA) and equol-d_4 (Medical Isotopes Inc., Pelham, NH) were added to samples and the samples incubated with β-glucuronidase Type H-1(Sigma – Aldrich; St. Louis, MO) at 37˚C, overnight. The samples were extracted twice with diethylether and the pooled extracts dried under nitrogen. MTBSTFA + 1% TBDMCS, (Thermo-Fisher Scientific, Inc; Waltham, MA) was added and the samples heated at 70˚C for 4 hours. The samples were cooled and heptane added. The samples were analyzed by GC-MS in the SIM mode: Trace Ultra GC/DSQ II (Thermo-Fisher). Column: TR-5MS, 30 m x 0.25 mm; 0.25 µm film (Thermo-Fisher). Injection port: 280˚C; column: 200˚C for 1 minute; 25˚C/minute; 320˚C for 8 minutes; transfer line: 320˚C. Carrier gas helium at 1.5 mL/min. Ions monitored (m/z): 425/482; 234/470 and 555 for daidzein, equol and genistein, respectively; 428/485, 236/474 and 559 for daidzein-d_4, equol-d_4 and genistein-d_4, respectively. Coefficients of variation: 6.2% and 7.5% for daidzein (m/z 428 and m/z 485 and 6.7% for genistein (m/z 555).

4.3.3 Measurement of CAC

As described in the previous chapter, the scanning for CAC was done using a GE-Imatron C150 Electron Beam Tomography scanner (GE Medical Systems, South San Francisco, US). Images were recorded over a disc in Japan and shipped to the University of Pittsburgh to be read by a trained reader using a DICOM (Digital Imaging and Communications in Medicine) workstation and software by AccuImage (AccuImage Diagnostic Corp., San Francisco, US). The software program implements the widely accepted Agatston scoring method. The reproducibility of non-zero CCS from this laboratory had an intraclass correlation of 0.98.

4.3.4 Statistical analyses

Total serum levels of isoflavones defined as the sum of daidzein and genistein were used because serum levels of these two isoflavones were highly correlated, *i.e.*, a Spearman correlation coefficient of 0.90 ($p<0.01$). Tertiles of total serum isoflavones were used as the main independent variables in the model because their distribution was highly skewed to the right. To examine the trend association of tertiles of total isoflavones with risk and other factors, general linear model for the continuous variables and Cochran-Armitage trend test for binary variables was used.

As described in the previous chapter, a cutoff point of CCS of 10 Agatston unit (AU) was used to define the presence of CAC. To analyze the association of CAC with tertiles of serum isoflavones, logistic regression to examine the association of prevalence of CAC with tertiles of total isoflavones was used. For the analysis, the following models were used - (i) an unadjusted analysis, (ii) after adjustment for age, exercise, education, smoking status and alcohol intake (Model I), (iii) Model I + further adjustment for BMI, LDL-c, HDL-c, diabetes and hypertension

(Model II), and (iv) Model II + further adjusted for serum levels of omega-3 fatty acids (Model III).

In a previous report from this sample population, it has been reported that heavy alcohol drinkers, i.e. those consuming ≥69 grams/day of alcohol, had more than ten times higher risk of CAC presence than moderate drinkers (23-45 grams/day of alcohol), and about four times higher risk of CAC presence than never drinkers. This alcohol intake is equivalent to about 4.5 shots of alcohol/day. This level of consumption is extremely high and not commonly seen in the US. For example, in the ERA JUMP study, the prevalence of heavy alcohol drinking was about 10% among Japanese in Japan as compared to less than 1% among Whites in Pittsburgh and less than 6% among Japanese Americans in Honolulu. Due to very high prevalence of CAC among heavy alcohol drinkers (≥69 grams/day or ~4.5 shots of alcohol),[247] this association was examined after excluding very heavy alcohol drinkers (n=31) in a subsequent analysis.

To examine the association of equol producer status with the presence of CAC, a criteria recommended by Setchell et al.,[161] i.e. a serum level of ≥83 nM, was used as a cut-off point for defining equol producer status. Examination of the association between equol producer status and presence of CAC was performed by using multiple logistic regression and adjusting for (i) age, BMI and alcohol intake (Model I) (ii) hypertension, diabetes, LDL-c, and HDL-c (Model II), (iii) Model II + log-transformed serum levels of soy isoflavones (Model III), and (iv) Model II + serum levels of omega-3 fatty acids (Model IV). Similar to the exclusion done in the previous analysis, this analysis was further performed after excluding very heavy alcohol drinkers. As a sensitivity analysis, different cut-off points of ≥20 nM and ≥40 nM for defining equol producer status were used.[130,180] A two-sided p-value <0.05 was considered significant. All the analyses were performed using SAS/STAT software version 9.3 of the SAS System, Cary, NC.

4.4 RESULTS

Descriptive characteristics of the study population by tertiles of soy isoflavones are presented in Table 4-1. With increasing tertiles of soy isoflavones, no significant trend existed for BMI, SBP, DBP, hypertension, diabetes, education, alcohol drinking or exercise. With increasing tertiles of soy isoflavones, there was a significant increase in glucose, and a marginally significant increase in age and serum triglycerides. CAC was present in 10.6% of the individuals in tertile 1, 15.4% in tertile 2 and 8.7% in tertile 3 of isoflavones (p-trend = ns).

Table 4-2 presents the descriptive characteristics of the study population by equol producer status. About 16% of the individuals (n=49) were equol producers (≥83 nM). There were no significant differences in traditional cardiovascular risk factors such as age, BMI, SBP, LDL-c, HDL-c, diabetes, hypertension, exercise or education by equol producer status. While 12.0% of equol producers were heavy alcohol drinkers (≥69 grams/day), the prevalence was 9.9% among non-producers (p=ns). The prevalence of CAC was lower among equol producers (8.0%) than non-producers (12.3%), but the difference was not statistically significant (p=0.39). When heavy drinkers of alcohol were excluded from the analysis (n=24), there was a marginally significant lower prevalence of CAC among equol producers (2.3%) than non-producers (10.6%) (p=0.08).

Table 4-3 presents results of multiple logistic regression analysis to examine association between serum levels of soy isoflavones and presence of CAC. Independent of other risk factors, the odds for CAC were higher, although not statistically significant, among those in tertile 2 of soy isoflavones, while the odds were non-significantly lower among those in tertile 3, as compared to tertile 1 of soy isoflavones. The results did not materially change when heavy alcohol drinkers were excluded from the analyses (Table 4-4).

In an unadjusted analysis, equol producers had 38% lower odds for presence of CAC than equol non-producers (OR=0.62, 95% CI: 0.21, 1.84). After multivariable adjustment (Model II), the association became stronger, although still was not statistically significant (p=0.21). This association remained similar after adjustment for log-transformed serum levels of soy isoflavones (log-transformed) (Model III) or after adjustment for serum omega-3 fatty acids (Model IV). (Table 4-6)

When heavy alcohol drinkers were excluded, the odds for presence of CAC were 80% lower among equol producers than among non-producers in an unadjusted analysis. (Table 4-7) After multivariable adjustment for cardiovascular risk factors, the association strengthened and became marginally significant (Model II) (OR=0.14, 95% CI: 0.02, 1.17). When further adjustment was done for log-transformed serum levels of soy isoflavones (Model III) or with serum levels of omega-3 fatty acids (Model IV), the association became significant with odds of CAC presence as much as 90% lower (OR=0.10, 95% CI: 0.01, 0.97) among equol producers than among non-producers.

In a supplementary analysis, alternate cutoff points of ≥20 nM and ≥40 nM were used to define equol producer status. The prevalence of those with equol ≥20nM was 40.3% and those with equol ≥40 nM was 22.4%. There was no statistically significant association between equol producer status and presence of CAC using these alternate definitions of equol producers, although the odds for CAC presence were about 50% lower among equol producers than among equol non-producers when using a cut-off value of 40 nM to define equol producer status. (Table 4-8 and Table 4-9)

4.5 DISCUSSION

In a population-based study among healthy middle-aged Japanese men in Japan, serum levels of soy isoflavones were not associated with presence of coronary atherosclerosis. In contrast, equol producers had much lower prevalence of CAC than equol non-producers – the association was marginally significant in the whole sample population, and became statistically significant after excluding very heavy alcohol drinkers. These results indicate that while soy isoflavones might not have anti-atherosclerosis properties, equol provides significant benefit against atherosclerosis. This is the first study to report significant inverse association of serum levels of equol with coronary atherosclerosis among men.

There was no significant association between serum levels of soy isoflavones and CHD risk factors. Several systematic reviews and meta-analyses have found similar observations that the effect of isoflavones on risk factors, if present, is modest.[248-251] Although there was a significant positive association between serum isoflavones and glucose seen in the present study, no such trend was seen with diabetes. Further, no association of other traditional risk factors was seen with equol producer status.

A nested case-control study having 377 cases and 753 controls in China had similar findings as this present study. In this study on Shanghai Women Health study and Shanghai Men Health Study cohorts, although urinary levels of isoflavones were not associated with CHD outcomes, urinary equol was inversely associated with CHD in women but not in men. As compared to quartile 1 of equol, participants in quartile 4 of equol had 54% lower odds of CHD, and there was a significant linear trend towards lower CHD with increasing quartiles of equol ($p=0.02$). Reasons for not replicating similar association among men might be shorter duration of

follow-up and lower prevalence of equol producers among men as compared to women.[194] Further, equol supplementation significantly reduced HbA1c, LDL-c and CAVI in a small 12-week randomized crossover trial in Japan.[178] Findings of this trial as well as the current study need to be validated through well-powered epidemiological studies and trials.

Although no previous study has examined the association between soy isoflavones and CAC, previous studies have also reported no significant association between soy isoflavones and another measure of atherosclerosis i.e. carotid IMT.[130,252-254] In the Women's Isoflavones Soy Health (WISH) trial among 350 postmenopausal women, isoflavones supplementation significantly reduced IMT progression only among women who were within 5 years of menopause (n=68). Also, there was no statistically significant difference in IMT progression by equol producer status, although the study was not statistically powered to examine this hypothesis.[130]

Setchell et al. have recommended using 83 nM as the cut-off point in the serum while defining equol producer status.[161] Alternate definitions of 20 nM and 40 nM were used in sensitivity analyses to define equol producer status. Although there were substantially lower odds for CAC among equol producers using the cut-off at 40 nM, the finding was not statistically significant. In contrast, no association was seen when the cut-off was kept at 20 nM. It is likely that several equol non-producers are misclassified as equol producers using these alternate definitions, and thus the lack of association between equol producer status and coronary calcification.

Participants with very high alcohol intake were excluded from the analysis in this study. A J-shaped association between alcohol intake and coronary calcification has previously been reported in this study sample.[247] As is also reported in the previous chapter of this manuscript,

Japanese in Japan have higher prevalence of alcohol drinking than other population groups. An intake of ≥69 grams/day is extremely high and is equivalent to about 4.5 shots of whiskey per day. Such heavy alcohol drinking increases the risk for CHD which is unlikely to be completely attenuated by anti-atherosclerotic properties of any single known dietary component including soy isoflavones or equol. When the analyses was performed after including heavy drinkers but adjusting for alcohol intake in the statistical models, the odds for presence of CAC were lower among equol producers than among equol non-producers, but not statistically significant.

Results of this study should be interpreted in light of the relevant study limitations. Serum levels of isoflavones and equol may not provide a true estimate of their past exposure. This is cross-sectional study among healthy middle-aged men in Japan, thus, temporality and generalizability cannot be inferred. The criteria to define equol producer is not clear especially when measuring equol in the serum. Also, previous studies have determined equol producer status after giving a daidzein challenge to the participants. As this study is among Japanese men living in Japan, an assumption is made that they were consuming isoflavones in the past, and that absence/low equol in the serum is due to absence of intestinal bacteria that convert daidzein to equol.

4.6 CONCLUSION

To conclude, this is the first population-based study to report lower coronary atherosclerosis among equol producers than among non-producers, with significant differences seen among those who were not very heavy alcohol drinkers. This inverse association was not seen with soy isoflavones, thus indicating independent anti-atherosclerotic properties of equol.

4.7 TABLES AND FIGURES

Table 4-1 Characteristics of the participants according to tertiles of serum isoflavones

	Tertile 1 (n=102)	Tertile 2 (n=99)	Tertile 3 (n=102)	P for trend
Range of isoflavones (nM)	**0–257**	**258-837**	**838–7,640**	
Age (years)	44.8 (2.9)	45.0 (2.6)	45.5 (2.8)	0.077
Body-mass index (kg/m^2)	23.5 (3.3)	23.9 (3.1)	23.7 (2.8)	0.668
Systolic BP (mmHg)	124.6 (15.2)	124.2 (17.6)	126.1 (15.5)	0.508
Diastolic BP (mmHg)	76.2 (11.2)	76.5 (13.2)	76.9 (11.3)	0.658
Hypertension (%)	23.1	31.1	25.2	0.721
Total cholesterol (mg/dl)	215.4 (40.7)	219.8 (33.8)	216.2 (33.2)	0.871
LDL-cholesterol (mg/dl)	134.4 (39.6)	133.5 (34.4)	128.7 (33.5)	0.253
HDL-cholesterol (mg/dl)	53.2 (13.2)	55.4 (13.7)	53.6 (13.9)	0.842
Triglycerides (mg/dl)	132.5 (92.5, 169)	136.5 (106, 180)	147 (110, 203)	0.085
Glucose (mg/dl)	104.6 (11.3)	105.7 (15.0)	110.3 (26.2)	0.028
Diabetes (%)	5.8	4.7	7.8	0.549
Current smokers (%)	55.8	44.3	47.8	0.129
Pack years of smoking (years)	18.0 (1.8, 30.0)	16.8 (4.9, 28.0)	21.8 (5.5, 29.0)	0.813
Education (years)	14.0 (2.2)	14.4 (2.0)	14.4 (1.9)	0.159
Current drinkers (%)	66.4	64.2	70.9	0.490
Heavy Alcohol drinkers (%)	11.5	12.3	6.8	0.261
Medication for				
Hypertension (%)	1.9	7.6	6.8	0.121

Table 4-1 contd.

Lipids (%)	2.9	3.8	3.9	0.696
Exercise (%)	23.1	30.2	26.2	0.607
Coronary calcium score (50th, 75th, 90th and 95th percentile)	(0, 1.8, 20.8, 37.8)	(0, 1.7, 18.0, 43.1)	(0, 1.5, 8.0, 31.3)	
Prevalence of coronary calcium score ≥10 (%)	10.6	15.4	8.7	0.682

Current drinkers were defined as individuals who drank alcohol ≥2 times a week. Heavy drinkers were defined as alcohol intake ≥ 69 grams/day. Exercise was defined as exercise ≥ 1 hour a week.
BP: blood pressure, LDL: low-density lipoprotein cholesterol, HDL: high-density lipoprotein

Table 4-2 Characteristics of the participants by Equol producer status (≥83 nmol/L) (n=303)

	Equol Producer (n=49)	Equol non-producer (n=254)	P-value
Soy Isoflavones	794.4 (286.9, 1326.3)	422.4 (180.5, 942.0)	0.005
Age (years)	45.7 (2.6)	45.0 (2.8)	0.107
Body-mass index (kg/m^2)	23.9 (2.8)	23.7 (3.2)	0.697
Systolic BP (mmHg)	123.0 (16.1)	125.2 (15.8)	0.368
Diastolic BP (mmHg)	76.0 (11.5)	76.5 (11.9)	0.798
Hypertension (%)	20.4	27.7	0.287
Total cholesterol (mg/dl)	218.7 (40.4)	216.8 (36.5)	0.741
LDL-cholesterol (mg/dl)	136.1 (33.2)	131.9 (35.9)	0.406
HDL-cholesterol (mg/dl)	53.4 (10.8)	54.1 (14.2)	0.682
Triglycerides (mg/dl)	132 (110, 167)	139 (102, 183)	0.452
Glucose (mg/dl)	107.3 (16.2)	106.9 (19.4)	0.911
Diabetes (%)	2.0	7.0	0.185
Current smokers (%)	42.9	50.8	0.120
Pack years of smoking	18 (4, 28)	18 (3, 29)	1.000
Current drinkers (%)	75.5	65.2	0.257
Heavy alcohol drinkers (%)	12.0	9.9	0.615
Medication for			
Hypertension (%)	6.1	5.5	0.855
Lipids (%)	4.1	3.5	0.846
Education (years)	14.5 (2.3)	14.2 (2.0)	0.535
Exercise (≥1 hr/wk) (%)	30.6	26.2	0.521
Coronary calcium score (50th, 75th, 90th and 95th percentile)	(0, 2.6, 6.7, 17.5)	(0, 1.5, 23.7, 43.1)	0.945
Prevalence of coronary calcium score ≥10 (%)	8.2	12.6	0.380

Current drinkers were defined as individuals who drank alcohol ≥2 times a week. Heavy drinkers were defined as alcohol intake ≥ 69 grams/day. Exercise was defined as exercise ≥ 1 hour a week.

BP: blood pressure, LDL: low-density lipoprotein cholesterol, HDL: high-density lipoprotein cholesterol

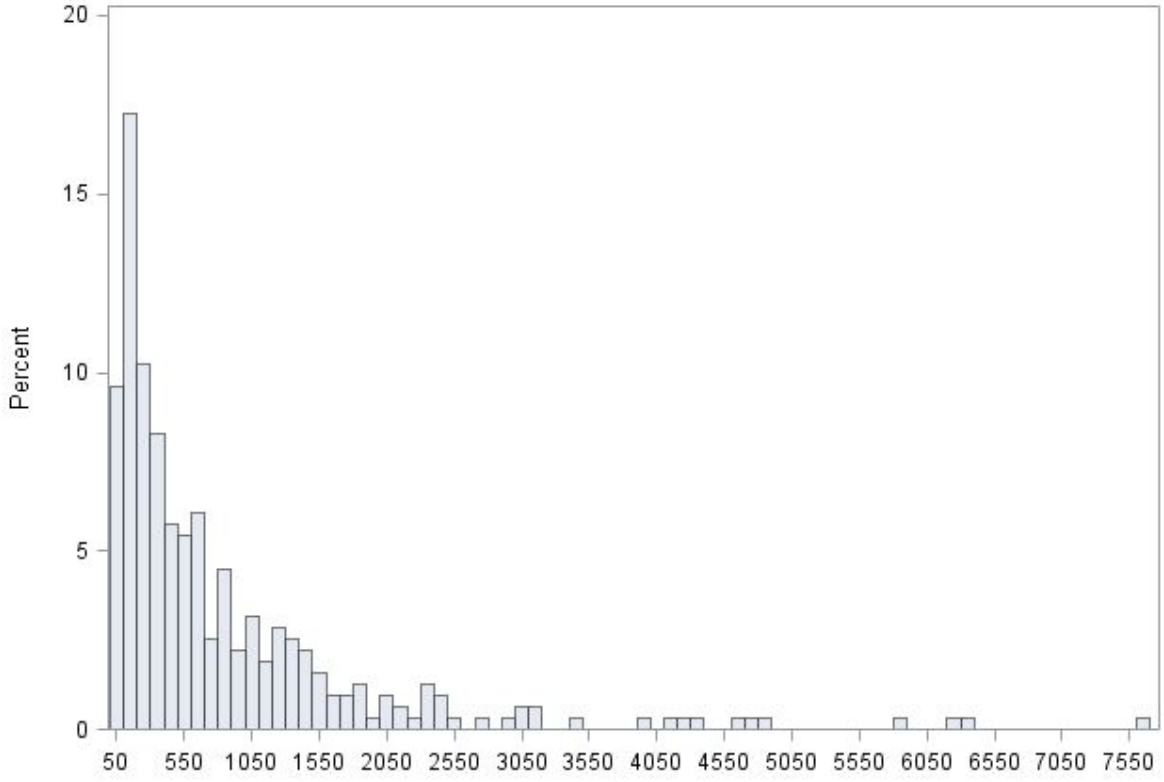


Figure 4-1 Distribution of serum isoflavones among Japanese in Japan (nM)

Table 4-3 Multivariable-adjusted odds ratio (95% CI) for the presence of coronary artery calcification according to tertiles of serum isoflavones among men in Japan (n=303)

	Tertile 1	Tertile 2	Tertile 3
Crude	1.0	1.54 (0.68, 3.49)	0.81 (0.32, 2.04)
Model I	1.0	1.65 (0.68, 4.03)	0.85 (0.32, 2.26)
Model II	1.0	1.56 (0.60, 4.04)	0.83 (0.28, 2.44)
Model III	1.0	1.55 (0.60, 4.03)	0.83 (0.28, 2.43)

Model I: Adjusted for age, exercise, education (years), smoking status, and alcohol intake
Model II: Model I + Further adjusted for body-mass index, LDL-c, HDL-c, hypertension and diabetes
Model III: Model II + Further adjusted for serum levels of omega-3 fatty acids

Table 4-4 Multivariable-adjusted odds ratio (95% CI) for the presence of coronary artery calcification (%) according to tertiles of serum isoflavones among men in Japan (n=272)

	Tertile 1	Tertile 2	Tertile 3
Crude	1.0	1.84 (0.69, 4.92)	0.96 (0.32, 2.84)
Model I	1.0	2.23 (0.79, 6.28)	0.98 (0.32, 3.02)
Model II	1.0	2.62 (0.83, 8.25)	1.32 (0.38, 4.56)
Model III	1.0	2.69 (0.84, 8.58)	1.36 (0.39, 4.71)

*Analysis was performed after removing heavy alcohol drinkers from the sample
Model I: Adjusted for age, exercise, education (years), smoking status, and alcohol intake
Model II: Model I + Further adjusted for body-mass index, LDL-c, HDL-c, hypertension and diabetes
Model III: Model II + Further adjusted for serum levels of omega-3 fatty acids
*After removing heavy alcohol drinkers (≥69 grams/day)

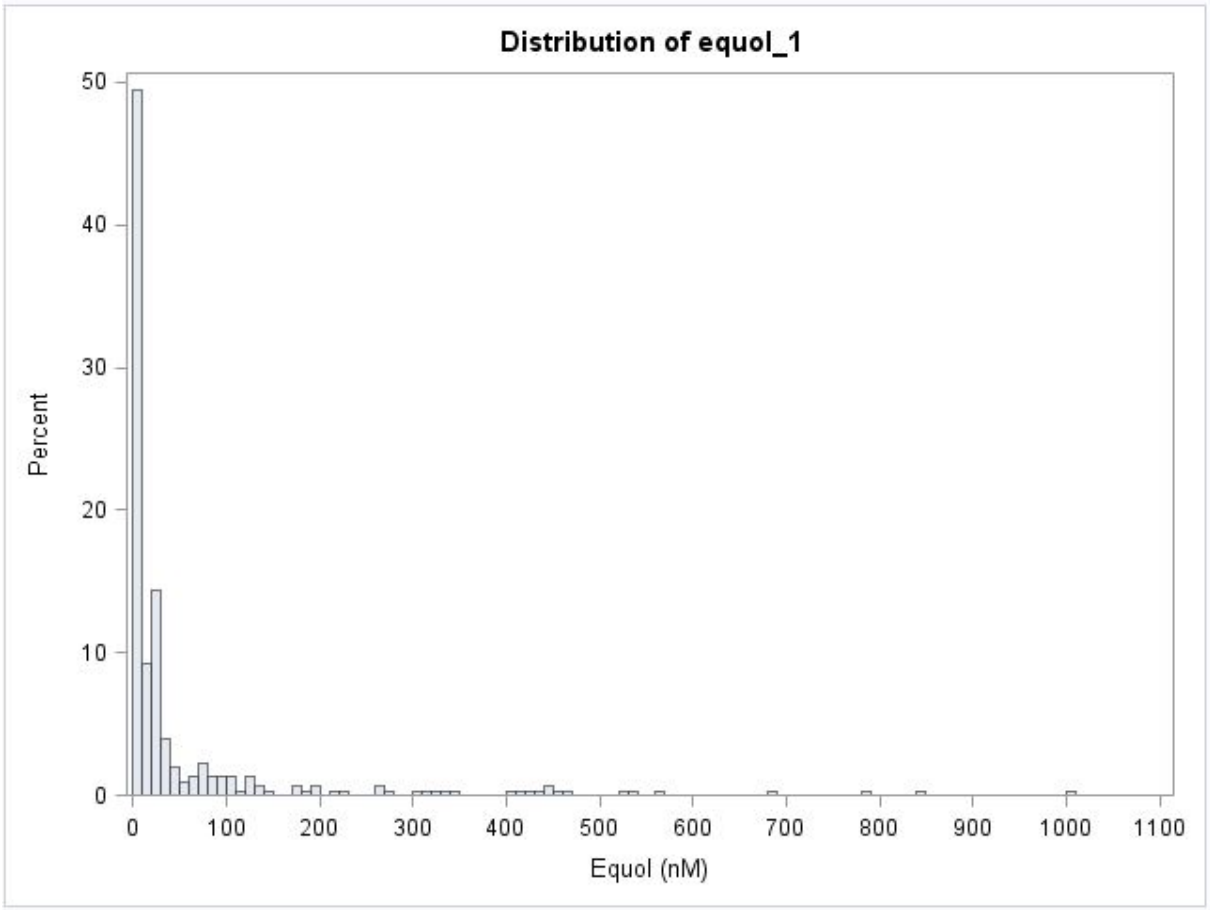


Figure 4-2 Distribution of serum equol among Japanese men in Japan (nM)

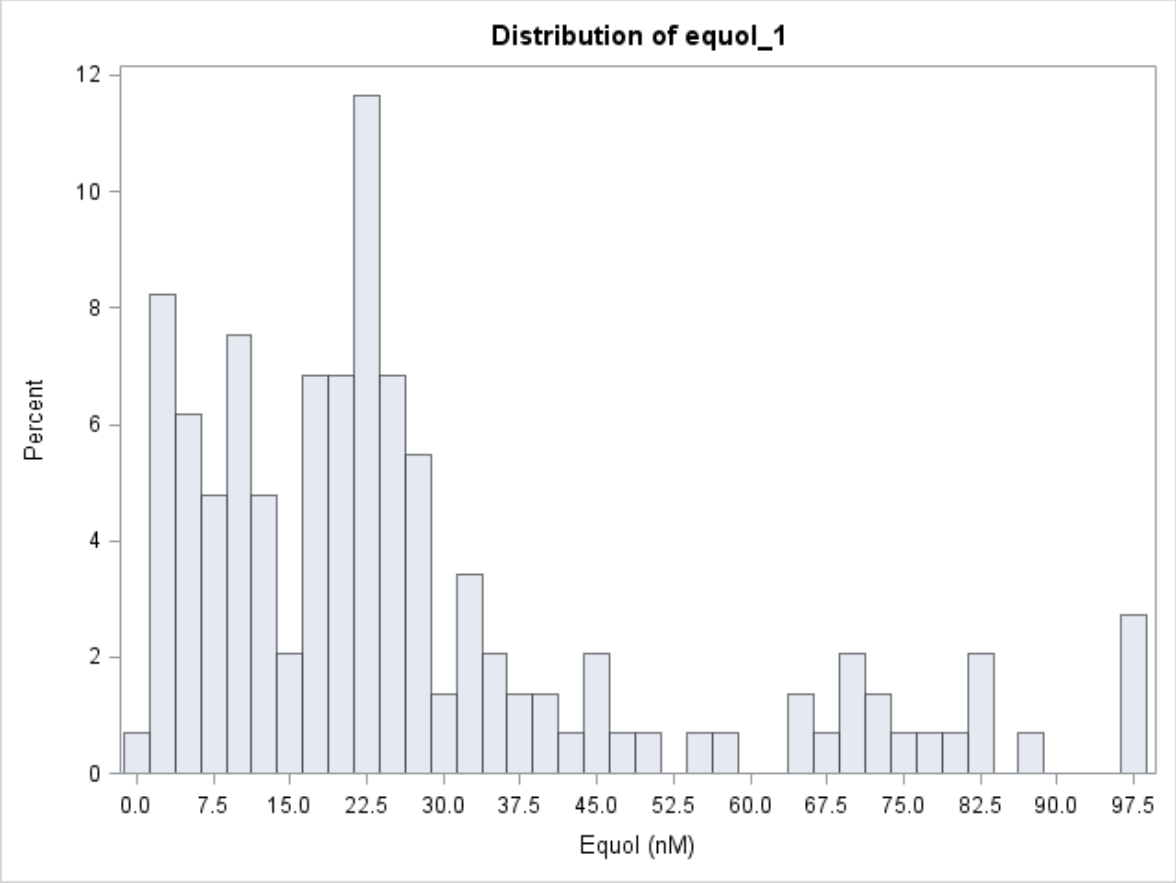


Figure 4-3 Distribution of serum equol among those with equol more than 1 nM and less than 100 nM (n=146)

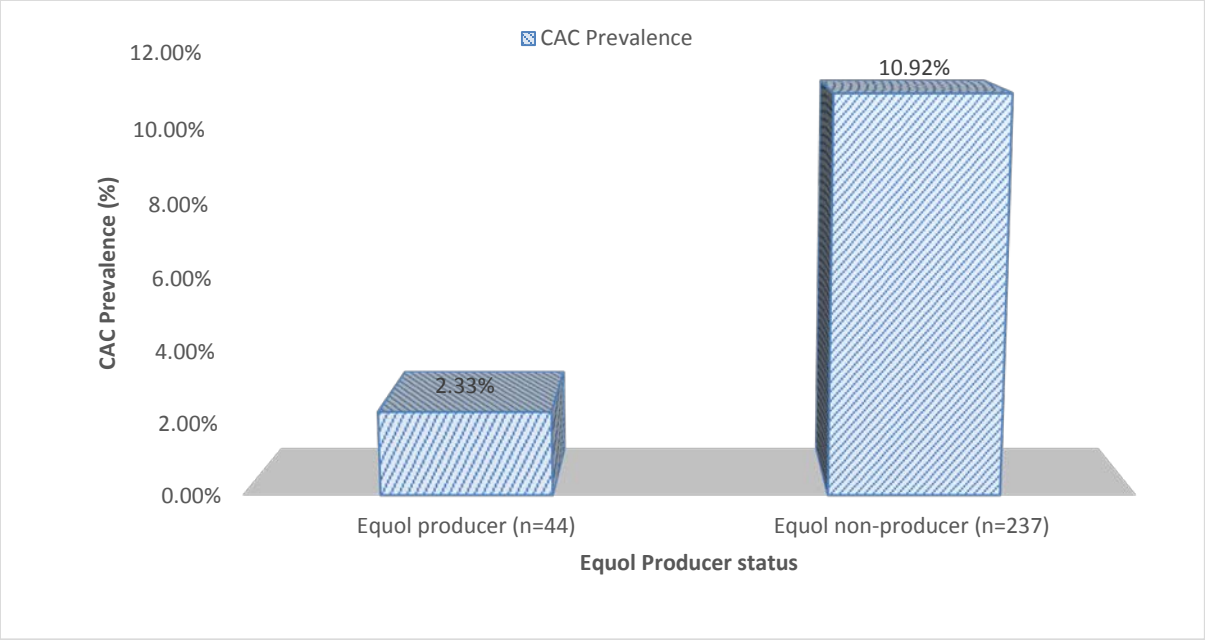
Table 4-5 Characteristics of the participants by Equol producer status after excluding heavy alcohol drinkers (≥83 nmol/L) (n=272)

	Equol Producer (n=43)	Equol non-producer (n=229)	P-value
Soy Isoflavones	803.7 (279.0, 1346.6)	445.5 (186.7, 1030.7)	0.014
Age (years)	45.6 (2.6)	44.9 (2.8)	0.121
Body-mass index (kg/m^2)	23.8 (2.7)	23.6 (3.2)	0.820
Systolic BP (mmHg)	123.1 (16.6)	124.6 (15.6)	0.635
Diastolic BP (mmHg)	75.0 (11.7)	76.0 (11.8)	0.753
Hypertension (%)	18.6	25.8	0.317
Total cholesterol (mg/dl)	218.6 (33.5)	217.3 (36.1)	0.833
HDL-cholesterol (mg/dl)	52.0 (10.3)	53.5 (13.8)	0.433
LDL-cholesterol (mg/dl)	137.1 (33.3)	133.5 (35.3)	0.539
Triglycerides (mg/dl)	128 (108, 161)	139 (103, 181)	0.359
Glucose (mg/dl)	105.8 (7.4)	106.5 (19.1)	0.681
Diabetes (%)	0.0	6.1	0.136
Current smokers (%)	41.9	48.5	0.246
Pack years of smoking	18.0 (3.0, 29.0)	18.0 (2.2, 28.0)	0.771
Education	14.4 (2.3)	14.3 (2.0)	0.660
Current drinkers (%)	72.1	62.0	0.207
Medication for			
Hypertension (%)	2.3	4.8	0.698
Lipids (%)	4.6	2.6	0.616
Coronary calcium score (50th, 75th, 90th and 95th percentile)	(0, 1.0, 4.6, 6.7)	(0, 1.0, 13.1, 36.6)	0.707
Prevalence of coronary calcium score ≥10 (%)	2.3	10.9	0.092

Current drinkers were defined as individuals who drank alcohol ≥2 times a week. Heavy drinkers were defined as alcohol intake ≥ 69 grams/day. Exercise was defined as exercise ≥ 1 hour a week.

BP: blood pressure, LDL: low-density lipoprotein, HDL: high-density lipoprotein

*After removing heavy alcohol drinkers (≥69 grams/day)



* After removing heavy alcohol drinkers (≥69 gms/day)

Figure 4-4 Prevalence of coronary calcification by Equol producer status (n=272)*

Table 4-6 Multivariable-adjusted odds ratio of the presence of CAC by equol producing status in men in Japan (n=303)

	Odds Ratio	P value
Crude	0.62 (0.21, 1.83)	0.384
Model I	0.50 (0.16, 1.62)	0.248
Model II	0.46 (0.14, 1.55)	0.210
Model III	0.44 (0.13, 1.53)	0.197
Model IV	0.46 (0.13, 1.59)	0.220

Model I: Adjusted for age, body-mass index and alcohol intake
Model II: Model I + Further adjusted for hypertension, diabetes, LDL-c and HDL-c
Model III: Model II + further adjusted for serum levels of soy isoflavones (log)
Model IV: Model II + further adjusted for serum levels of omega-3 fatty acids

Table 4-7 Multivariable-adjusted odds ratio for the presence of CAC by equol producing status (≥83 nM) among men in Japan (n=272)

	Odds Ratio	P value
Crude	0.19 (0.03, 1.47)	0.113
Model I	0.16 (0.02, 1.23)	0.078
Model II	0.14 (0.02, 1.17)	0.069
Model III	0.10 (0.01, 0.94)	0.044*
Model IV	0.10 (0.01, 0.96)	0.046*

Model I: Adjusted for factors significantly associated in the univariate model – age, BMI
Model II: Further adjusted for other traditional risk factors – hypertension, diabetes, LDL-c and lipid medication
Model III: Further adjusted for serum levels of soy isoflavones
Model IV: Further adjusted for serum levels of omega-3 fatty acids
*After removing heavy alcohol drinkers (≥69 grams/day)

Table 4-8 Multivariable-adjusted odds ratio for the presence of CAC by equol producing status (≥20 nM) among men in Japan (n=272)

	Odds Ratio	P value
Crude	1.09 (0.48, 2.47)	0.838
Model I	1.02 (0.44, 2.36)	0.966
Model II	1.06 (0.43, 2.59)	0.901
Model III	0.99 (0.40, 2.46)	0.982
Model IV	1.01 (0.40, 2.53)	0.978

Model I: Adjusted for factors significantly associated in the univariate model – age, BMI
Model II: Further adjusted for other traditional risk factors – hypertension, diabetes, LDL-cholesterol and lipid medication
Model III: Further adjusted for serum levels of soy isoflavones
Model IV: Further adjusted for serum levels of omega-3 fatty acids
*After removing heavy alcohol drinkers (≥69 grams/day)

Table 4-9 Table. Multivariable-adjusted odds ratio for the presence of CAC by equol producing status (≥40 nM) among men in Japan (n=272)

	Odds Ratio	P value
Crude	0.77 (0.28, 2.14)	0.618
Model I	0.62 (0.22, 1.80)	0.382
Model II	0.54 (0.18, 1.65)	0.280
Model III	0.48 (0.15, 1.50)	0.209
Model IV	0.49 (0.16, 1.54)	0.224

Model I: Adjusted for factors significantly associated in the univariate model – age, BMI
Model II: Further adjusted for other traditional risk factors – hypertension, diabetes, LDL-cholesterol and lipid medication
Model III: Further adjusted for serum levels of soy isoflavones
Model IV: Further adjusted for serum levels of omega-3 fatty acids
*After removing heavy alcohol drinkers (≥69 grams/day)

5.0 DISCUSSION

This manuscript examined several subclinical disease biomarkers and their risk factors in a population sample of apparently healthy middle-aged men drawn from three different countries i.e. the US, Japan and South Korea. It also compares the prevalence of subclinical cardiovascular disease by comparing presence of one of the biomarkers, i.e. carotid plaque, among these population samples. An effort is made to examine the risk factors associated with subclinical cardiovascular disease. Finally, among Japanese population, where the intake of soy and soy products is regular, this manuscript examines the association between serum levels of soy isoflavones, a measure of the dietary intake of soy isoflavones, and coronary atherosclerosis examined as coronary artery calcification. The major findings of this dissertation is that Japanese, and to some extent, South Koreans have lower subclinical atherosclerosis than US men, and that among Japanese, equol producer status is associated with lower subclinical atherosclerosis. Additionally, this dissertation work indicates that among apparently health men, brachial-ankle pulse wave velocity, a biomarker of arterial stiffness, is associated with coronary artery calcification (CAC). Given its non-invasive measurement and clinical applicability, brachial-ankle pulse wave velocity deserves thorough examination as a biomarker of arterial stiffness and a predictor of future cardiovascular disease in the US.

This manuscript highlights several lessons that may be learned from Japan for further reducing the burden of CHD in the US. US have one of the highest rates of CHD among western

countries. In contrast, the CHD rates in Japan are one of the lowest is the world, in spite of high rates of smoking and higher blood pressure prevalent in Japan although the prevalence of obesity in much lower in Japan. Several studies have tried to examine the lower CHD in Japan, by comparing the CHD rates between Japanese in Japan, Japanese Americans living in the US and Whites living in the US. However, these previous studies were conducted when there was a significant difference in lifestyle between the US and Japan, i.e. the level of total cholesterol in the US was much higher than that in Japan. The present study is significant as it examines these populations in the 21st century and among men born after the World War II. With westernization of diet and lifestyle in Japan, these men have consumed increasingly western diet over their lifetime.

Previous studies have examined the differences in CHD between the US and Japan by examining the rates of CHD events.[100,103,108] This study examines these differences using newer technologies to evaluate the subclinical burden of atherosclerosis. This is especially important as increasing life expectancy is likely to increase the burden of atherosclerosis in the population. Also, with an ever-increasing availability of therapeutic options to prevent and delay the onset of CHD, identifying subclinical atherosclerosis informs is beneficial as it aids in the decision-making while instituting the therapy for CHD prevention. This study confirms the finding of lower atherosclerosis in Japan that was first reported by epidemiological studies in the 20th century.

East Asian men, especially Japanese in Japan, have lower atherosclerosis than men in the US. In the present study carotid plaque was used to compare the burden of atherosclerosis between these populations, as it has emerged as a predictor of CHD risk. This study confirms the findings from previous reports of lower subclinical atherosclerosis among Japanese in Japan as compared to the US men. Also, similar to the US, the CHD rates in Japan are declining for the past several

decades. The “Japanese paradox” i.e. declining CHD rates in spite of increasing total cholesterol levels has been attributed to the decline in smoking rates and blood pressure achieved in Japan.[255] Nonetheless, the extremely low CHD rates among Japanese in Japan remain a subject of examination.

This study also reports a relatively new biomarker of CVD i.e. brachial-ankle pulse wave velocity (*ba*PWV). *ba*PWV is a popular too for assessing CVD risk in east Asian countries including Japan. If the success of *ba*PWV is replicated in the US, it may turn out to be a clinically convenient predictor of CVD and may aid the clinicians in decision-making. *ba*PWV has a potential for wide clinical application. *ba*PWV measurement is inexpensive, can be performed in a physician’s office, requires little operator’s expertise, and does not require exposure to the inguinal area. However, several concerns need to be addressed before it can be used as a clinical tool in the US, most important of which are determination of normal reference values and applicability among Western populations

This study also reports that equol producers in Japan have lower coronary atherosclerosis than equol non-producers. Equol is producer by the action of intestinal bacteria on dietary isoflavone i.e. daidzein. Dietary intake of isoflavones is high in Japan, thus, it is likely that equol producers in Japan have had protective benefits from the anti-atherosclerotic properties of equol over the past several years. This is especially likely as equol producer status is stable in an individual. However, it is unlikely that at the current level of intake of isoflavones in the US, isoflavones or equol would have any protective effect. Thus, this hypothesis cannot be currently examined in the general population in the US. Nevertheless, as equol is now available as a dietary supplement in the US, anti-atherosclerotic properties of equol can be examined with a clinical trial in the US and elsewhere.

Strengths

This work makes several contributions to the scientific literature. This study uses standardized approach to compare the prevalence of carotid plaque across several countries. This is also the first study to examine the usefulness of brachial-ankle pulse wave velocity among apparently healthy men in the US. Finally, it indicates that a dietary factor i.e. soy isoflavones may be protective against atherosclerosis.

Several study strengths increase the impact and validity of these findings. All the data acquisition was done in a standardized manner under strict data quality control. Several established research laboratories in Pittsburgh, PA handled data acquisition and processing – (1) the Ultrasound Research lab at the University of Pittsburgh examined *ba*PWV, and kept quality control check over carotid plaque assessments that were made at individual sites. The Ultrasound Research Lab has a track record of examination of subclinical disease markers esp. vascular markers such as intima-media thickness, carotid-femoral pulse wave velocity and flow-mediated dilatation for several large epidemiological studies in the US, including the Study of Women's Health Across the Nation and the ERA JUMP study. It employs several trained full-time sonographers who are experts in assessing the subclinical markers under strict quality control. This lab has been one of the pioneers for establishing validity and reproducibility for some of these biomarkers. (2) The Cardiovascular Institute at the University of Pittsburgh Medical Center examined CAC after obtaining cardiac images from all the study sites. CAC assessment was performed by one trained technician with a low inter-scan variability for measurement. (3) The Heinz laboratory at the University of Pittsburgh examined the blood for all the serum markers used in this study including lipids, glucose, and dietary markers such as soy isoflavones and equol. The

Heinz lab, similar to the previous two labs has a long history of providing services to the research studies conducted at the University of Pittsburgh.

This study examines the research questions is a population of apparently healthy middle-aged men. These men were recruited directly from the population. Thus, the results of this study are applicable especially for middle-aged men, a section of the general population who is most likely to get potentially preventable coronary heart disease in the next 20 years. Studying this population provides the greatest opportunity to prevent any future reversal of declining trend of coronary heart disease in the population. In contrast, a study recruiting patients from the hospital, however clinically important, would not provide an insight into preclinical preventable phase of the disease.

Although the study sample size is relatively modest, it is sufficiently large esp. given the breadth of self-reported and laboratory assessments included in the study and the strict quality control under which these assessments were made. In a part of the first aim (chapter 2) and the second aim (chapter 3), data from all the groups could be combined for the analysis, thus providing large sample size to enable statistically powerful analysis. However, for parts of these chapter where the population was stratified by the race-ethnicity, certain limitations were present with regards to statistical power.

Limitations

Results from this dissertation should be interpreted in light of the study limitations. Certain general limitations were common to all the aims – low generalizability, cross-sectional design, and lack of more sophisticated biomarkers to assess subclinical atherosclerosis.

As the sample population in this study included only men that were 40-49 years of age, the study findings are not generalizable to other sections of the population. An important section of

the population that has gained importance in the recent scientific literature comprises of women transitioning through the menopause. Although slightly overlapping in age with the population of this study, the findings of this study should not be extrapolated to menopausal women. Further, number of chronic diseases such as hypertension and diabetes increase with age. These conditions when present in the same individual has a greater effect on the progression of atherosclerosis than that of the sum of their individual effects. Thus, our findings may not necessarily be extrapolated to older populations, both men and women. However, use of a wider age-range or both genders would have necessitated a much larger sample to be recruited into the study.

The cross-sectional nature of these studies slightly limits the impact of these results. Hill's criteria for assessing causal association puts emphasis on the temporality of association between a factor and a disease. Unfortunately, given its cross-sectional design, this dissertation research could not assess temporality which might be important for Aims 2 and 3. In the second aim of this manuscript, association between *ba*PWV and CAC, both subclinical measures of cardiovascular disease, was examined. An assumption was made that *ba*PWV would increase before appearance of CAC, and thus *ba*PWV was used as a predictor variable in the models while CAC was kept as the outcome variable. However, this limitation should not significantly impact the validity of the findings as the aim was to only examine the association between the two biomarkers with no intention to prove a causal relationship between the two pathophysiological processes that these biomarkers assess. However, this study design may be more important while interpreting the results of the third aim. In this chapter, as assumption is made that the dietary practices of the population have been consistent for the past several years at least with regards to intake of soy and soy products. Given the generally unreliable nature of other dietary assessment techniques such as food frequency questionnaires, serum levels of dietary factors such as isoflavones provide a

relatively accurate measure of their current intake, but provide little information about the past dietary habits.

Several studies have used more sophisticated cardiovascular disease biomarkers such as coronary angiography to examine coronary atherosclerosis and intravascular ultrasound to examine carotid atherosclerosis. Given the population-based design of this study, only non-invasive measures such as electron-beam computed tomography and carotid B-mode ultrasound were used. Although these biomarkers have been examined thoroughly in several studies, including population-based studies, other studies have started to show that these biomarkers, although qualitatively valid, may not be accurate in assessing the quantity of atherosclerosis in the vascular beds. Thus, a need exists for incorporating more sophisticated but non-invasive techniques for quantifying atherosclerosis.

6.0 PUBLIC HEALTH SIGNIFICANCE AND FUTURE RESEARCH

WHO describes public health as "all organized measures (whether public or private) to prevent disease, promote health, and prolong life among the population as a whole." Various functions of public health are described as (i) assessment and monitoring of the health of communities and populations at risk to identify health problems and priorities, (ii) formulation of public policies designed to solve identified local and national health problems and priorities, and (iii) to assure that all populations have access to appropriate and cost-effective care, including health promotion and disease prevention services.[256] To the extent of the abovementioned role of public health, this dissertation highlights several research areas that are of public health importance globally as well as in the US.

The first aim of this dissertation reports on higher atherosclerosis – an underlying factor for coronary heart disease (CHD) – among US men than among east Asian men. As these differences were independent of other traditional cardiovascular risk factors, this finding suggests that some other factor(s) might be responsible for higher atherosclerosis in US or lower atherosclerosis among east Asian men. Identification of such factor(s) might lead the way to lower the rising global epidemic of cardiovascular disease. Thus, larger longitudinal studies are needed to systematically examine reasons for lower carotid atherosclerosis in Japan as compared to the US.

Early identification of cardiovascular disease (CVD) using biomarkers is a well-established strategy to prevent the occurrence of clinical CVD. In this regards, several biomarkers have been examined extensively in the past few decades. Although some of these biomarkers have consistently been shown to be associated with future CVD, they have certain limitations such as side-effects, increased costs and generalizability. Thus, a search of an "ideal" CVD biomarker

continues. In this regard, brachial-ankle pulse wave velocity is a promising biomarker of CVD and needs to be thoroughly examined in the western populations.

The third and final aim of this dissertation examines the effects of a dietary component i.e. soy isoflavones on coronary atherosclerosis. Although dietary isoflavones are not associated with lower coronary atherosclerosis, equol – a product of the action of intestinal bacteria on isoflavones – has shown some benefit over coronary atherosclerosis, examined in this aim with coronary artery calcification. Equol is available as a dietary supplement. Thus, if this finding is replicated in larger longitudinal studies and clinical trials in the US and other countries with a high burden of atherosclerosis and CVD, it may lead to reduction of the public health burden of the CHD.

APPENDIX A: STUDIES EXAMINING ASSOCIATION BETWEEN TRADITIONAL CVD RISK FACTORS AND CAROTID PLAQUE

Table A-1 Studies examining association between Traditional CVD Risk Factors and Carotid Plaque

Author-Year	Study Population	Follow-Up	Carotid plaque measurement	Prevalence of carotid plaque	Risk factors associated with carotid plaque
Tell 1989 USA[257]	CV Diagnostic patients, 49.0% male, 90% White, 10% Blacks, in USA, Mean age: 63 years, Age range: 24-98 years	Cross-sectional study	Biosound compact scanner; plaque defined as thickening > 1.0 mm; calculation of plaque score based on severity of plaques	Not reported	Age and cigarette smoking consistently associated with plaque thickness
Handa 1990 Japan[258]	Japanese patients, 71% male, 59 ± 13 years, N=232	-do-	B-mode US, SSD-125 Tokyo Japan, and EUB-450, Tokyo, Japan	59% among CVD group and 41% among non-CVD group	Age, sex, hyperlipidemia, DM
Prati 1992 Italy[259]	Population-based, 46.7% male, Age range: 18 to 99 years, N=1348 (San Daniele Project)	-do-	Duplex US; plaque defined as a distinct area with mineralization or protrusion into the lumen	Men: 25.4%, Women: 26/4%	Above 40 years only: Age, SBP, HDL-c, smoking (pack years), alcohol intake

Table A-1 contd.

Author-Year	Study Population	Follow-Up	Carotid plaque measurement	Prevalence of carotid plaque	Risk factors associated with carotid plaque
Salonen 1994 Finland[260]	Population-based, all men, Age range: 40-59 years, N=182 from Seven Countries Study	FU: 30 years	B-mode US scan; plaque categories: (1) smooth arterial wall surface, (2) uneven arterial wall surface, (3) protrusion into lumen, (4) Large (≥25%) protrusion	45.1% had any plaque present	TC, smoking
Bonithon-Kopp 1996 France[261]	Population-based, 40.6% male, Age range: 59-71 years. The EVA Study	Cross-sectional study	B-mode US Aloka SSD-650; plaque defined as a localized structure that protruded into the lumen with IMT >1.0 mm	Men: 25.59%, Women: 16.5%	Age, hypertension, IMT
Bonora 1997 Italy[262]	Population-based, 59±11 years. The Bruneck study	FU: 5 years	B-mode US, plaque not expressly defined	42.5% had any plaque present	*Univariate*: 2-hr glucose, 2-hr insulin, SBP, DBP, hypertension, LDL-c, apoB, AT-III
Ebrahim 1999 UK[263]	Population-based, 53.2% male, 66 years. The British Regional Heart Study	Cross-sectional study	Advanced Tech. Lab. HDI 3000 triplex system. Plaque defined as localized IMT >1.2 mm	Me:57%, Women:58%	Men: current smoking, HDL-c Fibrinogen, Factor VII(age adjusted) Women: current smoking, manual labor, SBP, TC (age adjusted)
Spence 1999 Canada[264]	CVD patients, Atherosclerosis group: 51.4% male, 48.4±12.7 years, N=294; Control group: 55.2% male, 50.4±10.8 years, N=351	-do-	B-mode US. Plaque defined with plaque area on US	Mena plaque area: 0.55±0.83 cm^2	Age, sex, smoking, medications for lipid, medications for BP
Zureik 2000 France[220]	Population-based	FU: 4 years	B-mode US Aloka SSD-650; plaque defined as a localized structure that protruded into the lumen with IMT >1.0 mm	Occurrence of new plaque: 18.3%	*Univariate*: Hypertension, TC

Table A-1 contd.

Author-Year	Study Population	Follow-Up	Carotid plaque measurement	Prevalence of carotid plaque	Risk factors associated with carotid plaque
Klein 2008 Canada[35]	CVD patients, 52.9% male, 53.4±12.0 years, N=876	Cross-sectional study	ATL Mark 9, ATL HDI 2000. Plaque defined as % stenosis	Carotid plaque area: 0.38 (0.11, 0.96) cm^2	Age, sex, SBP, Smoking(pack years) medication for lipid, DM
Wagenknecht 2009 USA[265]	Population-based 43% male, 19% blacks, Mean age >70 years. The ARIC study	FU: 18 years	Contrast-enhanced MRI + 3D Angiogram; Plaque composition analysis done only among those with wall thickness ≥1.5 mm	Lipid core observed in 42% (weighted)	18 Year predictors of lipid core: age, LDL-c, TC Concurrent predictors: LDL-c, glucose, BMI
Kelley 2011 USA[266]	Population-based, 42% male, 31% Blacks, Age range: 29-51 years, N=914. The Bogalusa heart Study	Cross-sectional study	Power vision Toshiba SSH-380, Carrollton, USA. Plaque defined as discrete, focal area 50% greater than the surrounding wall, ≥1.5 mm IMT	White men: 14%, White women: 8%, Black men: 9%, Black women: 9%	Age, race, smoking, hypertension, DM
Kuo 2012 USA[36]	Population-based, 40% male, 61% Hispanics, 19% Blacks, 17% white, Age: 69±9 years, N=1790. The NOMAS Study	-do-	Plaque defined as the total plaque area.	Average plaque area: 1.45±1.38 cm^2	Age, SBP, smoking, and DM
Herder 2012 Norway[38]	Population-based, 47.6% male, Age ~ 56 years, N=2743. The Tromso study		B-mode US Acuson Xp10 128 ART-upgraded. Plaque defined as a localized protrusion ≥50% thicker than the adjacent IMT. Total plaque area.	TPA: Men-7.42 mm^2, Women-4.73 mm^2	TC, SBP, smoking

Table A-1 contd.

Author-Year	Study Population	Follow-Up	Carotid plaque measurement	Prevalence of carotid plaque	Risk factors associated with carotid plaque
van den Bouwhuijsen 2012 Netherlands[267]	Participants with carotid wall thickening (≥2.5mm) in a previous survey, 52% men, 70.3±10.2 years, N=1006. The Rotterdam Study	-do-	1.5 T MR scanner, GE Healthcare, Milwaukee, US: 2D images, and 3D angiogram. Composition of plaque assessed in all plaque with IMT > 2.0mm	13.5% had more than 30% stenosis, intraplaque hemorrhage in 34.5%, and lipid core in 25.5% of participants	Hemorrhage: age, sex, hypertension, current smoking, IMT, carotid stenosis Lipid Core: Age, sex, dyslipidemia, IMT, carotid stenosis Calcification: age, hypertension, IMT, carotid stenosis
Herder 2012 Norway[38]	Population-based, 47.6% male, Age ~56 years, N=2743	FU: 13 years	B-mode US Acuson Xp10 128, ART-upgraded. Plaque defined as a localized protrusion ≥50% compared with adjacent IMT. The area of the plaques was outlined manually. Progression in plaque area calculated	Progression: Men: 0.82 mm^2/year Women: 0.56 mm^2/year	Age, sex, TC, SBP, smoking, medications for lipids, CVD
Ijas 2013 Finland[268]	Population survey, 45.4% males, 57.1±8.1 years, N=91 from Helsinki Carotid Endarterectomy study	Cross-sectional study	B-mode US	14.1% prevalence of plaque	Age, haptoglobin-2 allele
Khalil 2013 India[269]	Population-based, 60.3% male, Age ~36 years, N=600	FU: 6.9±1.0 years	B-mode US GE 5X3, Barrington, USA. Plaque defined as IMT >1mm	Men: 33.4%, Women: 25.6%	Sex, metabolic syndrome, education, SES, exercise
Blondon 2013 USA[37]	Population-based, 46.5% men, 60.4±9.4 years, N=3246 The MESA study	FU: 9.4 years	B-mode US. Plaque defined as localized thickening of IMT > 50% than the adjacent IMT.	47% prevalence of plaque at baseline, and 40.6% at FU among those with no plaque at baseline	PTH and Vit. D are not associated with/ predict plaque

Table A-1 contd.

Author-Year	Study Population	Follow-Up	Carotid plaque measurement	Prevalence of carotid plaque	Risk factors associated with carotid plaque
Sala Vila 2014 Spain[270]	High CVD risk participants, 46.3% male, Age ~66 years, N=164Sub-cohort of a clinical Trial (PREDIMED trial)	FU: 2.4 years	B-mode US Sequoia Acuson, Enlargen, Germany. Plaque defined as focal protrusions ≥1.2 mm thick.	76.8% prevalence of plaque at baseline. Mediterranean diet+nuts lead to delayed progression of mean plaque thickness.	Mediterranean diet, nuts
Hogberg 2014 Sweden[271]	Population-based, all men, 65 years, N=4657	Cross-sectional study	Carotid US Sequoia, Mountain view, USA. Plaque defined as focal IMT ≥2 mm	25.1% had plaque	*Univariate*: Smoking, hypertension, CAD, hypercholesterolemia, claudication, COPD, DM, TIA/Stroke
Sinning 2014 Germany[271]	Population-based, 50.8% male, 55.5±10.9 years, N=5000. The Gutenberg health study	-do-	iE33 US system. Plaque defined as protrusions ≥1.5mm into the lumen.	Plaque was present in 37.8%	?

CVD = cardiovascular disease, DM= diabetes mellitus, US= ultrasound, TC= total cholesterol, IMT= intima-media thickness, FU = follow-up, SBP=systolic blood pressure, DBP = diastolic blood pressure, LDL-c = low-density lipoprotein cholesterol, HDL-c = high-density lipoprotein cholesterol, UK = United Kingdom, MESA= multi-ethnic study of atherosclerosis, TIA= transient ischemic attack, CAD= coronary artery disease, COPD = chronic obstructive pulmonary disease

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APPENDIX B: ASSOCIATION OF CAROTID PLAQUE WITH OTHER SUBCLINICAL AND CLINICAL DISEASE MARKERS

Table B-1 Association of Carotid plaque with other subclinical and clinical disease markers

Author-Year	Study Population	Follow-Up	Carotid plaque measurement	Prevalence of carotid plaque	Risk factors associated with carotid plaque
Coronary Artery Calcification					
Oei 2002 The Netherlands[272]	Population-based, 46.3% men, ~71 years, N=2013. The Rotterdam Coronary Calcification Study	-do-	Carotid US Scan. Plaque defined as focal protrusion at least 50% thicker than the adjacent IMT	Men: 78%, Women: 59%	Carotid plaque significantly associated with CAC among men as well as women
Polak 2013 USA[273]	Population-based, 42.3% men, 63.6±10.4 years, N=2837. The MESA Study	FU: 2.4 years	B-mode US scan.	Plaque was present in 45.6%	Carotid plaque significantly predicted incidence of coronary calcification
Lee 2012 USA[274]	Population-based, 47.8% men, 62.15±10.2 years, N=6715. The MESA Study baseline data	Cross-sectional study	B-mode US scan, GE Logiq 700 US machine,. Peak-systolic velocities of <150 cms, 150-250 cm/s, and >250 cm/s were determined to correspond to <50%, 50-75% and >75 stenosis	Prevalence of stenosis (≥25%) was 15%	Carotid stenosis was associated with presence of CAC as well as CAC score

Table B-1 contd.

Author-Year	Study Population	Follow-Up	Carotid plaque measurement	Prevalence of carotid plaque	Risk factors associated with carotid plaque
Schroeder 2013 Canada[275]	Asymptomatic patients, 54% male, 53±10 years, N=50.	-do-	Carotid US scan, Siemens Antares. Carotid plaque defined as wall thickness that was increased as compared to the surrounding IMT	Prevalence of plaque: 28%	No association between US-assessed carotid plaque and presence of CAC
Coronary Angiogram					
Morito 2008 Japan[276]	Suspected CAD patients, 58.6% male, 68.5 ± 10.8 years, N=116	-do-	B-mode Carotid US scan, Toshiba Power vision 8000. Carotid plaque was defined as focal IMT ≥ 1.1 mm.	Mean number of plaque: 3 among those with coronary artery stenosis, and 1 among those with no coronary artery stenosis	Plaque score was associated with severity of coronary stenosis
Chang 2013 Taiwan[277]	Suspected CAD patients, 79.2% male, 61±11 years, N=120	-do-	B-mode US scan iE33 xMatrix, Philips, Andover, USA. Plaque defined as focal thickening that was at least 50% greater than the surrounding vessel wall, or a focal region with IMT ≥1.5 mm	Carotid plaque were present in 77.5%	Carotid plaque was associated with CAD (defined as coronary luminal narrowing >50%)
Intima-Media Thickness					
von Sarnowski 2010 Germany[278]	Population-based, 45-81 years, N=1922	FU: 5 years	Carotid US scan, Gateway VST, GE Diagnostics	Carotid plaque prevalence at baseline: 67%.	Among those with no carotid plaque at baseline, baseline IMT predicted the development of carotid plaque
Pulse Wave Velocity					
Selwaness 2014 The Netherlands[279]	Population-based, 43.4% men, 67.0±8.6 years, N=6527. The Rotterdam Study	FU: 10.9 years and 0.8 years	Carotid US scan, ATL Ultra-Mark IV. Carotid MRI scan. Carotid wall thickening reported as IMT ≥2.5 mm.	Prevalence of plaque: 39.6%	Higher PWV was associated with carotid plaque, intraplaque hemorrhage, and intraplaque calcification.

Table B-1 contd.

Author-Year	Study Population	Follow-Up	Carotid plaque measurement	Prevalence of carotid plaque	Risk factors associated with carotid plaque
CAC= coronary artery calcification, MESA= multi-ethnic study of atherosclerosis, US= ultrasound, CVD = cardiovascular disease, CAD= coronary artery disease, IMT= intima-media thickness, FU = follow-up, PWV = pulse wave velocity					

APPENDIX C: PREVALENCE OF CAROTID PLAQUE AND ITS ASSOCIATION WITH CARDIOVASCULAR RISK FACTORS

Table C-1 Prevalence of Carotid plaque and its association with cardiovascular risk factors

Author-Year	Study Population	Follow-Up	Carotid plaque measurement	Prevalence of carotid plaque	Risk factors associated with carotid plaque
Polak 1993 USA[280]	Population-based study, 43.2% men, ~73 years, N=5201	Cross-sectional study	Internal carotid artery US scan, SSA-270A Toshiba America Medical System, Tustin, USA. Carotid stenosis categorized as 0%, 1-24%, 25-49%, 50-74%, and >74% based on peak flow velocities on US	Prevalence of plaque: 68.2%	ICA stenosis was associated with Stroke as well as TIA
Hollander, 2002, The Netherlands[281]	Population-based, 39.9% male, 68.8 years, N=4217. The Rotterdam study	FU: 5.2 years	B-mode US scan. Plaque defined as a focal widening of vessel wall >50% relative to the adjacent segments	Prevalence of plaque: 60%	Carotid plaque increased the risk of lacunar stroke as well as stroke in the anterior circulation, but not in the posterior circulation.
Spence 2002 Canada[282]	Patients in the premature atherosclerosis clinic, ~55% male, N=1686	FU: 5 years	Carotid US scan. Plaque defined as local thickening of intima > 1mm in thickness.	Prevalence of plaque: 87.6%	Carotid plaque progression was associated with higher risk of Stroke.

Table C-1 contd.

Author-Year	Study Population	Follow-Up	Carotid plaque measurement	Prevalence of carotid plaque	Risk factors associated with carotid plaque
Touboul 2005 France[283]	Brain infarct patients (n=510) and controls (n=510). The GENIC study	Cross-sectional study	Carotid US scan. Plaque defined as a localized protrusion into the lumen > 1mm in thickness than surrounding IMT.	Prevalence among Cases: 61.4%, controls: 35.2%	Carotid plaque associated with Stroke risk
Roman 2012 USA[284]	Population-based, 35% male, 63±8 years, N=2441. The Strong Heart Study	FU: 7.7 years	Carotid US scan, Acuson 128 system. Plaque defined as focal thickness ≥ 50% thicker than the adjacent IMT	Plaque prevalence: 63.6%	Carotid plaque was associated with incident CVD.
Polak, 2013, USA[31]	Population-based, 47.4% male, 61.1±10.2 years, N=6562. The MESA Study	FU:7.8 years	Carotid US scan. Presence of carotid lesions graded as 0%, 1-24%, 25-49%, and >49%.	Plaque prevalence: 41.9%	Carotid plaque at baseline was associated with CHD
Ziegelbauer 2013 Germany[285]	Population-based, 48.7% male, 49.9±13.0 years. N=4995. The Carotid Atherosclerosis Progression Study (CAPS)	FU: 10 years	Internal carotid artery US scan, P700SE, Philips Medical System. Plaque defined as a focal protrusion of at least 1.8 mm	Prevalence of ICA plaque: 71%	ICA predicted any stroke or death as the outcome over 10 years
Wannarong 2013 Canada[286]	Stroke prevention clinic patients, baseline plaque area 40 to 600 mm^2, ~70 years of age. N=349	FU: 1 year	Philips duplex scanners ATL 5000 HDI, ATL, Bothel, USA. Plaque measure as total plaque area and total plaque volume	NA	Progression of total plaque volume but not total plaque area predicted stroke among patients
Park 2013 S Korea[287]	Consecutive patients with carotid US scan and coronary angiogram. 54.5% male, 59.5±10.6 years, N=1390.	Cross-sectional study	Carotid US scan, HP Sonos-5500. Plaque defined as a focal protrusion >50 thicker than the surrounding intima-media or IMT>1.2 mm.	Prevalence of plaque: 31%.	Carotid plaque was associated with cardiac death and major adverse cardiovascular event

Table C-1 contd.

Author-Year	Study Population	Follow-Up	Carotid plaque measurement	Prevalence of carotid plaque	Risk factors associated with carotid plaque
Hald 2014 Norway[288]	Population-based study, 47.8% male, 59.9 ±10.3 years, N=6257. The Tromso Study	FU:15.4 years	B-mode US scan, Acuson Xp10 128 ART scanner. Plaque defined as localized thickening more than 50% thicker than the adjacent IMT	Plaque prevalence at baseline: 47.9%	Carotid plaque area predicted MI but net VTE

US= ultrasound, IMT= intima-media thickness, CHD= coronary heart disease, CVD = cardiovascular disease, FU = follow-up, ICA = internal carotid artery, TIA= transient ischemic attack, VTE = venous thromboembolism.

APPENDIX D: STUDIES EXAMINING ASSOCIATION BETWEEN *ba*PWV AND SUBCLINICAL AND CLINICAL CARDIOVASCULAR DISEASE

Table D-1 Studies examining association between *ba*PWV and subclinical and clinical cardiovascular disease

Author-Year	Population	Follow-Up	*ba*PWV Measurement /Distance	Mean *ba*PWV	*ba*PWV definition for model	Outcome	Confounders	Results
Coronary Artery Calcification								
Venkitachalam, 2007 USA[223]	Obese postmenopausal women, 57.0±2.9 years N=441 (WOMAN Study)	Cross-sectional analysis	Form ABI/PWV; Colin Co Ltd	1434 ± 232 cm/s	Quartiles of *ba*PWV	Presence (>0 AU) and severity (>100 AU) of CAC	Age, SBP, WC, BMI, Smoking, HRT Use, race, glucose, insulin, TC, HDL-c, LDL-c, TG, and antihypertensive medications	*ba*PWV independently associated with presence and severity of CAC
Lemos 2007 Brazil[289]	Pre-dialysis patients (59% male), 54.4±11.5 years N=104	-do-	Complior SP apparatus Artech Medical, Panyin, France	1220 ± 340 cm/s	Continuous	Presence of CAC	None	Higher *ba*PWV among those with presence of CAC

Table D-1 contd.

Author-Year	Population	Follow-Up	*ba*PWV Measurement /Distance	Mean *ba*PWV	*ba*PWV definition for model	Outcome	Confounders	Results
Seo 2010 S Korea[229]	Suspected CAD (68% male), 63 ± 9 years, N=174	-do-	Colin VP-2000; Komaki Japan	1651± 354 cm/s	Continuous	CAC score	Sex, age, BMI, BP, HR, TC, glucose	*ba*PWV correlated in unadjusted analysis but not after adjustment
Liu 2011 Taiwan[227]	Health screening patients (45.3% male), 54.5±9.4 years N=654	-do-	Form ABI/PWV; Colin Co Ltd	Normal: 1439 ± 98 cm/s; CAD: 1594±307 cm/s	Categorical: <1400, 1400-1800, >1800 cm/s	CAC score	None	Mean CAC score significantly increased with increasing categories of baPWV
Nam 2012 S Korea[228]	Health screening patients, (63.9% male), 53.7±9.2 years, N=615	-do-	VP device; Omron, Japan	1427±234 cm/s	Continuous	CAC score	None	*ba*PWV significantly correlated with CAC score in an unadjusted analysis
Sung 2013 S Korea[290]	Health screening patients, 81.2% male, Age: 41.0±6.2 in no CAC group & 47.2±7.3 in the CAC group, N=6009	-do-	VP-1000 device; Omron, Japan	No CAC group: 1302.9±146.3 cm/s; CAC group: 1379.9±174.8 cm/s	Quartiles	Presence of CAC (>0 AU)	Sex, age, TG, HDL-c, LDL-c, glucose, SBP, alcohol (glass/day), Smoking (ever), physical activity (≥ 3 times/week), waist circumference, fatty liver	Odds for presence of CAC significantly higher in 4th vs. 1st quartile of *ba*PWV

Table D-1 contd.

Author-Year	Population	Follow-Up	*ba*PWV Measurement /Distance	Mean *ba*PWV	*ba*PWV definition for model	Outcome	Confounders	Results
Coronary Angiography								
Imanishi 2004 Japan[231]	CAD patients (?) (62.6% male) 63.5±11.8 years, N=123	Cross-sectional study	Form ABI/PWV; Colin Co Ltd Japan	Males: No CAD=1529±50 cm/s, CAD=1890±53 cm/s; Females, No CAD=1680±69 cm/s, CAD=1846±84 cm/s	Categorical: 1044-1470 cm/s, 1471-1693 cm/s, 1694-1986 cm/s, 1987-2643 cm/s	CAD defined as greater than 50% stenosis in each of three coronary arteries	Age, hypertension, DM, hyperlipidemia, chest pain	Odds for CAD significantly higher in highest vs. lowest category of *ba*PWV
Sakuragi 2005 Japan[291]	CAD patients, (73.3% male), Non-CAD: 63±8 years, CAD: 67±9 years; N=251	-do-	Form PWV/ABI, Nihon Colin, Japan	Non-CAD: 1623±395 cm/s, CAD: 1748± 389 cm/s	Continuous	Presence of CAD; one-, two-, or three-vessel disease	None	*ba*PWV is higher in the CAD group, but not different in patients with one-, two-, or three- vessel disease
Koji 2007 Japan[292]	CAD patients (77.0% male) 63±11 years N=396	-do-	Form ABI/PWV; Colin Co Ltd Japan	Not reported	Not reported	No. of diseased arteries with >50% stenosis	Age, gender, smoking and hypercholester-olemia	*ba*PWV significantly associated with number of diseased arteries.
Kwon 2011 Korea[230]	CAD patients (67.7% male). Age: *ba*PWV<1600 cm/s: 56.2±10.3 years, *ba*PWV≥1600 cm/s: 66.6±8.33 years	-do-	Colin VP-1000; Komaki, Japan	Not reported	<1600 cm/s, and ≥1600 cm/s	Diseased vessel: >50% stenosis	None	Higher *ba*PWV (>1600 cm/s) was associated with longer lesions and smaller lumen area

Table D-1 contd.

Author-Year	Population	Follow-Up	*ba*PWV Measurement /Distance	Mean *ba*PWV	*ba*PWV definition for model	Outcome	Confounders	Results
Xiong 2012 China[232]	Retrospective enrollment od CAD patients(61.1% male) . Age: *ba*PWV=61.8±9.4 years, *ba*PWV$_{mid}$= 64.5±10.6 years, *ba*PWV $_{hig}$= 67.6±10.6 years, N=321	-do-	VP device, Omron, Japan	SYNTAX$_{low}$:1474.9 ±357.7, SYNTAX$_{mid}$:1947.7 ±323.9, SYNTAX$_{hig}$:2428.8 ±440.7	*ba*PWV tertiles	SYNTAX score tertiles	Age, sex, BMI, current smoking, family history of CAD, DM< hypertension, hyperlipidemia, antihypertensive medication use, statins, SBP, DBP, MBP, BUN, sr. creatinine, TG, LDL-c, cysteine proteinase, LV ejection fraction	Significantly higher odds for higher SYNTAX score with increasing tertile of *ba*PWV
Seo 2010 S Korea[229]	Suspected CAD (68% male), 63 ± 9 years, N=174	-do-	Colin VP-2000; Komaki Japan	1651± 354 cm/s	Continuous	Modified Gensini score, luminal narrowing	Sex, age, BMI, BP, HR, TC, glucose	*ba*PWV is not correlated with CAD severity
Liu 2011 Taiwan[227]	Health screening patients (45.3% male), 54.5±9.4 years N=654	-do-	Form ABI/PWV; Colin Co Ltd	Normal: 1439 ± 98 cm/s; CAD: 1594±307 cm/s	Categorical: <1400, 1400-1800, >1800 cm/s	Coronary stenosis	None	Prevalence of coronary stenosis significantly higher with increasing categories of *ba*PWV
Chae 2013 S Korea[293]	Chest pain patients, 58.1% male, 61.5±9.9 years, N=651	-do-	VP device, Omron, Japan	1646.5±467.2 cm/s	Continuous	Significant CAD= >50% stenosis; 0,1,2,3 group depending on the number of vessels involved	Sex, age, BMI, hypertension, DM, smoking, dyslipidemia, anti-hypertensive medication use, HDL-c, LDL-c, glucose, HbA1c, SBP	*ba*PWV associated with significant CAD but not number of vessels involved

Table D-1 contd.

Author-Year	Population	Follow-Up	*ba*PWV Measurement /Distance	Mean *ba*PWV	*ba*PWV definition for model	Outcome	Confounders	Results
Nam 2012 S Korea[228]	Health screening patients, (63.9% male), 53.7±9.2 years, N=615	-do-	VP device; Omron, Japan	1427±234 cm/s	Continuous	Coronary stenosis >50%	Sex, age, hypertension, DM, current smoking, dyslipidemia	*ba*PWV significantly associated with significant coronary stenosis after multivariable adjustment
				Other disease Outcomes				
Munakata 2005 Japan[294]	ESRD patients, 67.6% male, 59.6±1.6 years, N=68	-do-	AT-form PWV/ABI, Colin, Komaki, Japan	1836±48 (?SE) cm/s	Continuous	IMT (max IMT, plaque score), Echo-cardiography (LVMI)	None	*ba*PWV significantly correlated with max IMT, plaque score but not LVMI
Takaki 2008 Japan[295]	Patients with chest pain, 61.5% male, 69±12 years, N=130	-do-	AT-form PWV/ABI, Colin, Komaki, Japan	1676±389 cm/s	Continuous	IMT, Echo-cardiography	None	*ba*PWV significantly correlated with max-IMT but not with EDCT
Sakuragi 2005 Japan[291]	CAD patients, (73.3% male), Non-CAD: 63±8 years, CAD: 67±9 years; N=251	-do-	Form PWV/ABI, Nihon Colin, Japan	Non-CAD: 1623±395 cm/s, CAD: 1748± 389 cm/s	Continuous	Echo-cardiography (LVEF)	Age, BMI, TC, TG, HbA1c, LAD, E wave velocity, Dct, Number of diseased vessels, PP, ABI	*ba*PWV is significantly inversely associated with LVEF
Otsuka 2013 Japan[296]	Health screening patients, all males, 38±9 years, N=387	-do-	Form PWV/ABI, Colin, Komaki, Japan	1277±181 cm/s	Continuous	Oscillometric measurement of brachial artery cross-sectional area (eA)	Age, BMI, SBP, HR, LDL-c, TG, glucose, uric acid, volume elastic modulus	*ba*PWV is not associated with eA independent of confounders

Table D-1 contd.

Author-Year	Population	Follow-Up	*ba*PWV Measurement /Distance	Mean *ba*PWV	*ba*PWV definition for model	Outcome	Confounders	Results
Nakamura 2003 Japan[297]	ESRD or PAD patients, 55.7% male, 62.3±1.3 years, N=97	-do-	Form PWV/ABI, Colin, Komaki, Japan	Males: 2047±86 cm/s, Females: 1881±78 cm/s	Continuous	Aortic calcification	Age, SBP	Aortic calcification length significantly associated with *ba*PWV
Cardiovascular disease event (since publication of meta-analysis in 2012 [67]								
Kawai 2013 Japan[298]	Hospital-based cohort (NOAH study), 56.1% male, 61.0±0.6 years, N=440	FU: 12.7 years	FCP-4731, Fukuda Denshi, Japan	1690±17 cm/s	<1750 cm/s, and ≥1750 cm/s	CVD, Stroke, and all-cause mortality	Age, Sex, DM, dyslipidemia, current smoking, and anti-hypertensive medications	*ba*PWV is an independent predictor of CVD+Stroke mortality
Yoon 2013 S Korea[299]	Health screening (?) patients, 54.4% male, 52.9±9.2 years, N=241	Retro-spective FU	VP-1000, Colin, Japan	1461.0±263.4 cm/s	Continuous	CHD, cerebrovascular disease, PVD, arrhythmia, and HF	Age, sex, baseline eGFR, eGFR change, smoking, DM, hypertension, statins, CVD, SBP, DBP, BMI, brachial artery PWV, hematocrit, glucose, albumin, calcium, phosphorus, TC, TG, LDL-c, CRP, albuminuria	*ba*PWV was independently associated with CVD events in group with low eGFR (<90) but not in eGFR ≥ 90 group
Huang 2013 Taiwan[299]	Hemodialysis patients	4.4±1.5 years	Form PWV/ABI, Colin, Komaki, Japan	FRS low risk: 1758.2±490.2 cm/s, intermediate risk: 2065.5±560.5 cm/s, high risk: 2082.3±430.0 cm/s	Continuous	CV events, CV mortality, all-cause mortality	None	Addition of *ba*PWV and ABI to FRS model improves prediction of all-cause but not CV mortality

Table D-1 contd.

Author-Year	Population	Follow-Up	*ba*PWV Measurement /Distance	Mean *ba*PWV	*ba*PWV definition for model	Outcome	Confounders	Results
Ishisone 2013 Japan[300]	General population, 88.2% male, 59±11 years, N=973	FU: 7.8 years	HEM-907, Omron, Japan	Not reported	Quartiles; >90th percentile; 1SD	CV events (stroke+ CHF+ MI+ CV death)	Age (>65), sex, obesity, hypertension, DM, hypercholesterolemia, current smoking	*ba*PWV (>90th percentile and 1 SD change significantly and independently predicts CV event)
Ninomiya 2013 Japan[301]	Population-based, 42.7% male, >40 years, N=2916	FU: 7.1 years	BP-203RPE II form PWV/ABI, Omron, Japan	Not reported	Quintiles	CHD (AMI, silent MI, SCD, CABG, angioplasty)	Age, sex, hypertension, DM, TC, HDL-c, obesity, smoking, alcohol, exercise	*ba*PWV associated independently with CV risk
Nagai 2013 Japan[302]	Geriatric patients, 41.6% male, Age: with vascular event-74±12 years, w/o vascular event-71±12 years, N=274	FU: 41±28 months	Form PWV/ABI, Omron, Japan	With vascular event-2250 ± 660 cm/s, w/o vascular event-1970± 650 cm/s	Tertile	Angina/MI, cerebral hemorrhage, subarachnoid hemorrhage, cerebral infarction, TIA, HF, renal failure, arteriosclerosis obliterans, aortic dissection	Age, sex	*ba*PWV predicted event independent of age, sex
Han 2013 S Korea[303]	Consecutive patients with *ba*PWV & echocardiography, 56.2% male, 61,7 ± 11.6 years, N=185	FU: 19.8 months	Form BP-203RPE II, Colin, Komaki, Japan	1724 ± 454 cm/s	<1704 cm/s, ≥1704 cm/s	CV event (ischaemic stroke, CAD, PAD, or AD)	Sex, age, ABI (≤0.9), hypertension, DM	*ba*PWV predicted independently CV event

Table D-1 contd.

Author-Year	Population	Follow-Up	*ba*PWV Measurement /Distance	Mean *ba*PWV	*ba*PWV definition for model	Outcome	Confounders	Results
Kuwahara 2014 Japan[304]	Hemodialysis patients, 61.0% male, 61.0±9.6 years, N=300	FU: 7 years	Form BP-203RPE II, Colin, Komaki, Japan	1870 ± 488 cm/s	/ 100 cm/s	Cardiac, cerebrovascular, and PAD events	Age, duration of hemodialysis, DM, ABI, rate of decrease in ABI, rate of increase in *ba*PWV, LDL-c, BMP, LVEF, LVMI	*ba*PWV not associated with any outcome in multivariable analysis
Takashima 2014 Japan[305]	Population-based cohort, 37.2% male, 58.9±13.0 years, N=4164	FU:6.5 years	Form BP-203RPE II, Omron	1548 ± 374 cm/s	/200 cm/s; <1400 cm/s, 1400-1790 cm/s, ≥ 1800 cm/s	CVD, Stroke, AMI	Age, sex, smoking, drinking, BMI, HDL-c, LDL-c, TG, HbA1c, HR, diabetes medication, anti-hypertensive medication, MBP	Higher *ba*PWV (≥ 1800 cm/s) significantly predicted CVD event
Sugamata 2014 Japan[306]	Patients with stable CAD, 72% male, 65 ± 12 years, N=923	FU: 64±30 months	VP-1000, Colin, Komaki, Japan	1651± 381 cm/s	Continuous; / 1SD change	Coronary event (cardiac death, non-fatal MI, refractory unstable angina pectoris)	Age, DM, LDL-c, number of diseased vessel, maxIMT, FMD	1 SD change in *ba*PWV significantly predicted coronary event

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