ASSOCIATION OF SUBCLINICAL ATHEROSCLEROSIS WITH LIPOPROTEIN PARTICLES, ALCOHOL CONSUMPTION, AND LONG-CHAIN N-3 POLYUNSATURATED FATTY ACIDS AMONG HEALTHY MIDDLE-AGED MEN IN AN INTERNATIONAL POPULATION-BASED STUDY

by

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ABSTRACT

This dissertation includes three manuscripts examining the determinants of subclinical atherosclerosis among asymptomatic middle-aged men from four races/ethnicities. The present study sought to examine: 1) Do differences in the distribution of NMR-measured lipoproteins account for differences in the prevalence of coronary artery calcification (CAC) between Caucasians residing in the US (US White) and Japanese residing in Japan? 2) Is alcohol consumption associated with aortic calcification among middle-aged men? and 3) Are serum levels long chain n-3 polyunsaturated fatty acids (LCn-3PUFAs) inversely related to aortic calcification among middle-aged men? We examined the proposed research questions using data from the **E**lectron-Beam Tomography, **R**isk Factor **A**ssessment among **J**apanese and **U**.S. **M**en in **P**ost-World War II Birth Cohort (ERA-JUMP) study.

The major findings were: 1) in a population-based sample of 570 middle-aged men, US White compared to Japanese had significantly different NMR-measured lipoprotein particle distributions. The US White had significantly higher prevalence of CAC ≥10 compared to Japanese after adjustment for cardiovascular risk factors [Odds ratio = 3.25; 95% CI= 1.55, 6.84], and this difference was partially attenuated with further adjustment for lipoprotein levels [Odds ratio = 2.58; 95% CI= 1.16, 5.77]. In a multiethnic population-based study of 1033

asymptomatic men aged 40-49 years, after adjusting for cardiovascular risk factors and patential confounders: 2) the heavy drinkers had significantly higher expected aortic calcification score compared to nondrinkers [Tobit ratio (95% CI) = 2.15 (1.01, 4.57); Odds ratio (95% CI) =1.60 (1.07, 2.41)]; and 3) one standard deviation increase in total LCn-3PUFAs (3.3%), EPA (1.3%), and DHA (2.1%) (using Tobit regression) was associated with 29% (95% CI = 0.51, 1.00), 9% (95% CI = 0.68, 1.23), and 35% (95% CI = 0.46, 0.91) lower expected aortic calcification score respectively.

Adequately powered longitudinal studies are warranted: 1) to systematically examine the specific reasons for lower subclinical atherosclerosis among Japanese compared to western countries; 2) to further clarify the association between alcohol consumption and the incidence and the progression of atherosclerosis; and 3) to disentangle the differential effect of EPA and DHA on atherosclerosis, and the underlying biological mechanisms. From the public health importance, current study findings extend our understanding of NMR-measured lipoproteins, alcohol consumption, and LCn-3PUFAs related to subclinical atherosclerosis.

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1.0 INTRODUCTION

1.1 EPIDEMIOLOGY OF CORONARY HEART DISEASES/CARDIOVASCULAR DISEASES (CHD/CVD)

Cardiovascular diseases (CVD) are a group of disorders of the heart and blood vessels. They include coronary heart disease (CHD), cerebrovascular disease (CBVD), rheumatic heart disease, and other conditions. As per the American Heart Association (AHA) the term CHD includes "chronic progression of stable atherosclerotic plaque (occurring over years, resulting in angina), plaque instability (occurring over weeks to months), acute plaque rupture (occurring over seconds, resulting in acute coronary syndrome), thrombosis and coagulation (occurring over minutes to hours, resulting in acute MI), and ischemia-induced cardiac arrhythmia (occurring over seconds, resulting in CHD death)" [1]. The term sudden cardiac death (SCD) includes deaths resulting from "ischemia-induced ventricular fibrillation, arrhythmias caused by acute ischemia, and a range of cardiac arrhythmias arising from underlying structural heart disease rather than acute ischemia" [1].

CVD is the leading cause of death worldwide as well as in the United States (US) [2]. In 2010, CVD and CHD accounted for ~1 of every 3 deaths and ~1 of every 6 deaths in the US [2]. The estimated worldwide economic burden of CVD in 2010 was $863 billion USD (nearly equal to $125 USD per person) [2]. As per AHA, the annual cost of CVD in the US in 2010 was

$503.2 billion USD ($324.1 billion USD direct costs and $179.1 billion USD indirect costs) [2]. The estimated cost of CVD in 2010 in Europe was $153,194 million USD with $87,310 million USD direct costs and $65,884 million USD indirect costs [2]. It is expected that, in coming years, the developing countries mainly with the population of >100 million will face the enormous burden of CVD owing to its increasing rate [2].

In the US, from 2000 to 2010, the actual number of CVD deaths per year was reduced by 16.7%. However, the burden of disease is still high [2]. To further maintain the above mentioned 16.7% annual decline in CVD mortality in the US, the AHA launched a new idea of cardiovascular health. The AHA put forward the new set of goals for the decade 2010 to 2020: “By 2020, to improve the cardiovascular health of all Americans by 20%, while reducing deaths from CVDs and stroke by 20%” [3]. The AHA defined the ideal cardiovascular health as “the absence of clinically manifest CVD together with the simultaneous presence of optimal levels of all 7 metrics, including 4 health behaviors (not smoking; having sufficient physical activity; adopting a healthy diet pattern; and maintaining energy balance as represented by normal body weight) and 3 health factors (optimal total cholesterol, blood pressure, and fasting blood glucose, in the absence of drug treatment)” [3]. The seven metrics of cardiovascular health are depicted in table 1-1.

Table 1-1. Seven metrics of Cardiovascular Health defined by the American Heart Association

	Poor Health		Intermediate Health		Ideal Health	
Goal/Metric	Definition	Prevalence, %	Definition	Prevalence, %	Definition	Prevalence, %
Current smoking						
Adults >20 y of age	Yes	24	Former ≤12 mo	3	Never or quit >12 mo	73 (51 never; 22 former >12 mo)
Children 12–19 y of age	Tried prior 30 days	17			Never tried; never smoked whole cigarette	83
Body mass index						
Adults >20 y of age	≥30 kg/m^2	34	25–29.9 kg/m^2	33	<25 kg/m^2	33
Children 2–19 y of age	>95th Percentile	17	85th–95th Percentile	15	<85th Percentile	69
Physical activity						
Adults >20 y of age	None	32	1–149 min/wk moderate intensity or 1–74 min/wk vigorous intensity or 1–149 min/wk moderate+vigorous	24	≥150 min/wk moderate intensity or ≥75 min/wk vigorous intensity or ≥150 min/wk moderate+vigorous	45
Children 12–19 y of age	None	10	>0 and <60 min of moderate or vigorous activity every day	46	≥60 min of moderate or vigorous activity every day	44
Healthy diet score						
Adults >20 y of age	0–1 Components	76	2–3 Components	24	4–5 Components	<0.5
Children 5–19 y of age	0–1 Components	91	2–3 Components	9	4–5 Components	<0.5
Total cholesterol						
Adults >20 y of age	≥240 mg/dL	16	200–239 mg/dL or treated to goal	38 (27; 12 treated to goal)	<200 mg/dL	45
Children 6–19 y of age	≥200 mg/dL	9	170–199 mg/dL	25	<170 mg/dL	67
Blood pressure						
Adults >20 y of age	SBP ≥140 or DBP ≥90 mm Hg	17	SBP 120–139 or DBP 80–89 mm Hg or treated to goal	41 (28; 13 treated to goal)	<120/<80 mm Hg	42
Children 8–19 y of age	>95th Percentile	5	90th–95th Percentile or SBP ≥120 or DBP ≥80 mm Hg	13	<90th Percentile	82
Fasting plasma glucose						
Adults >20 y of age	≥ 126 mg/dL	8	100–125 mg/dL or treated to goal	34 (32; 3 treated to goal)	<100 mg/dL	58
Children 12–19 y of age	≥126 mg/dL	0.5*	100–125 mg/dL	18	<100 mg/dL	81

Some percentages do not appear to add up because of rounding.
SBP indicates systolic blood pressure; DBP, diastolic blood pressure.
*Estimate not reliable.

Derived from Lloyd-Jones et al. Circulation. 2010;121:586-613 [3]

Although public health has made tremendous progress in pharmacological and nonpharmacological interventions for the primary and secondary prevention of clinical CHD, nearly two-thirds of deaths among patients with acute MI occur before receiving any clinical care suggesting that even with advancement in clinical care services for CHD, these CHD deaths are unlikely to be significantly reduced. Thus, to reduce the burden of CHD morbidity and mortality, we must also look for strategies other than improvement in the clinical care for CHD. One of the

traditionally used approaches to reduce the burden of CHD is to lower the prevalence of the risk factors for CHD, or subclinical, or underlying atherosclerotic diseases in the population. A commonly used strategy to assess the cardiovascular risk of individuals is to screen them for traditional risk factors for CHD using scores/guidelines. Widely used scores/guidelines are the Framingham Risk Score (FRS - from the Framingham Heart Study in the US); the PROCAM score (from the Prospective Cardiovascular Münster (PROCAM) study in Germany), the European score (from the European risk prediction system); which assess the risk of coronary events in persons without previous CHD [4]. Each of these risk assessment algorithms, projects 10-year absolute risk of CHD, after considering the combination of traditional risk factors for CHD such as gender, smoking, and cholesterol [4]. However, because these scores/guidelines predict CHD risk only moderately well, researchers are exploring many subclinical CHD markers which can detect structural or functional changes associated with the underlying pathophysiological processes of atherosclerosis, and therefore, to identify patients who would benefit most from intensive prevention efforts [5-7].

1.2 ATHEROSCLEROSIS AND ITS ASSESSMENT

1.2.1 Introduction

Atherosclerosis is a systemic chronic inflammatory disease process in which fatty deposits, inflammatory cells, and scar tissue accumulate within the walls of arteries. It is the major underlying cause of most clinical cardiovascular events. Atherosclerosis remains asymptomatic for a longer period. Atherosclerotic lesions are classified into two major categories: stable

atherosclerotic plaque (rich in extracellular matrix and smooth muscle cells) and unstable atherosclerotic plaque (also known as vulnerable plaque - rich in macrophages, extracellular matrix, and foam cells). Most plaques are asymptomatic until enough closure of the lumen of an artery occurs. Symptoms and complications start occurring after severe narrowing of an artery, obstructing blood supply to vital organs. Commonly, plaque fissure or rupture of the unstable atherosclerotic plaques in the coronary arteries leads to fatal thrombosis and CHD mortality. As atherosclerosis is the major determinant of CHD, researchers are trying to comprehend and describe the underlying pathophysiological processes leading to atherosclerosis and to find newer interventions to prevent and delay atherosclerosis and its complications [8].

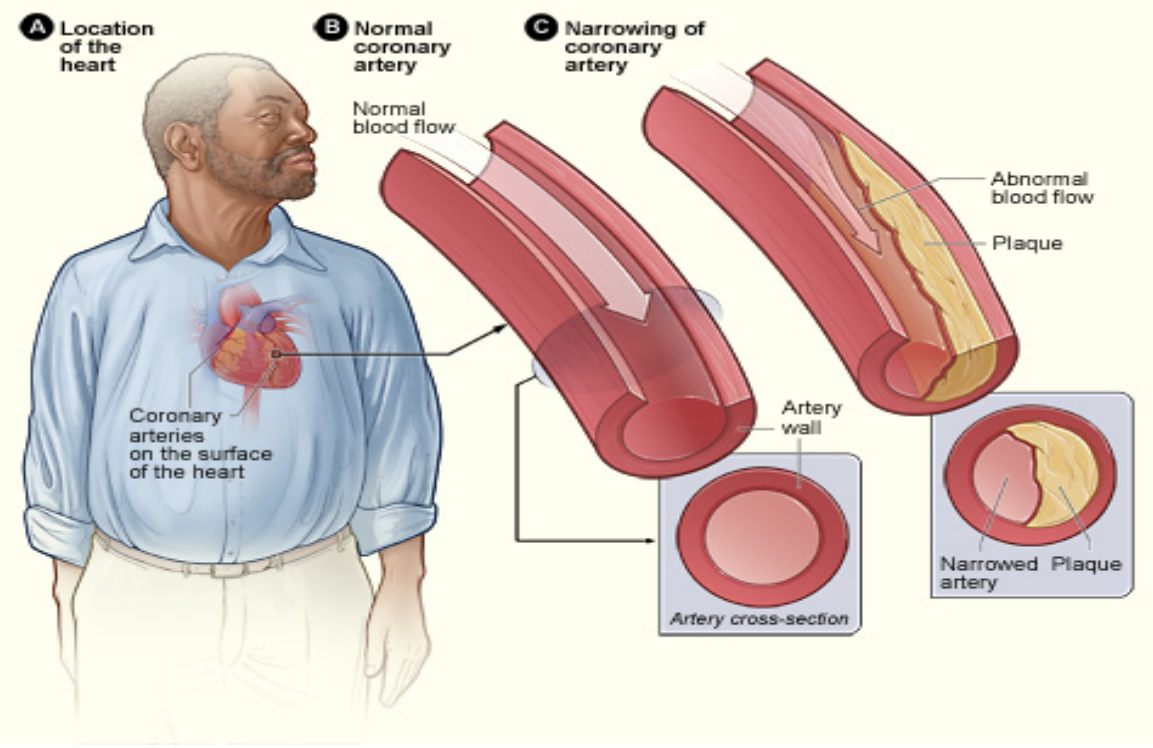

Figure 1-1. Location of the heart, normal coronary artery, and narrowing of coronary artery
"Figure A shows the location of the heart in the body. Figure B shows a normal coronary artery with normal blood flow. The inset image shows a cross-section of a normal coronary artery. Figure C shows a coronary artery narrowed by plaque. The buildup of plaque limits the flow of oxygen-rich blood through the artery. The inset image shows a cross-section of the plaque-narrowed artery" [8].

Derived from National heart, lung, and blood institute website: https://www.nhlbi.nih.gov/health/health-topics/topics/atherosclerosis/ Accessed on May 16, 2017

1.2.2 Pathophysiology of Atherosclerosis

The development of Atherosclerosis is a complex process. In humans, the intimal layer of arteries is much better developed than in most other animal species. As early as the first year of life of a human, the intima of the human arteries contains smooth muscle cells [9]. Atherosclerosis is initiated when endothelial cells of intimal layer of a vessel wall become activated by risk factors such as low-density lipoproteins particles (LDL-P), elevated blood pressure, toxins from cigarette smoking etc [10]. Activated endothelial cells express adhesion and chemoattractant molecules that recruit inflammatory leukocytes such as monocytes and T lymphocytes into the intima [9]. At the same time, extracellular lipid starts to deposit in the intimal layer of an artery. Monocytes migrated to the artery wall mature to macrophages and bind with modified lipoproteins. By ingesting these modified lipoproteins, macrophages become lipid-laden foam cells. Accumulated inflammatory leukocytes and resident vascular wall cells secrete inflammatory cytokines and growth factors which further augment leukocyte recruitment and cause smooth muscle cell migration and multiplication [9]. As the atherosclerotic lesion advances, inflammatory mediators induce expression of a potent procoagulant (tissue factor), and of matrix-degrading proteinases that thin out the fibrous cap of plaque. If the fibrous cap breaks at the point of thinning, coagulation factors in the blood may come in contact with the tissue-factor containing lipid core resulting in thrombosis on a non-occlusive atherosclerotic plaque. If there is an imbalance between prothrombotic and fibrinolytic mechanisms predominating at that specific region at that particular time, blocking thrombus resulting in acute coronary syndrome may occur. When the 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 a healing response, leading to increased collagen

deposition and smooth muscle cell growth. Thus, the fibro-fatty lesion containing lipid-laden foam cells can result into advanced fibrous and often calcified plaque, which may cause significant narrowing, and therefore symptoms of stable angina pectoris. In some cases, blocking thrombi arise not from rupture of the fibrous cap but the minor erosion of endothelial layer, and can result in acute coronary events if an imbalance occurs between prothrombotic and fibrinolytic mechanisms at that site (Figures 1-2 and 1-3) [9].

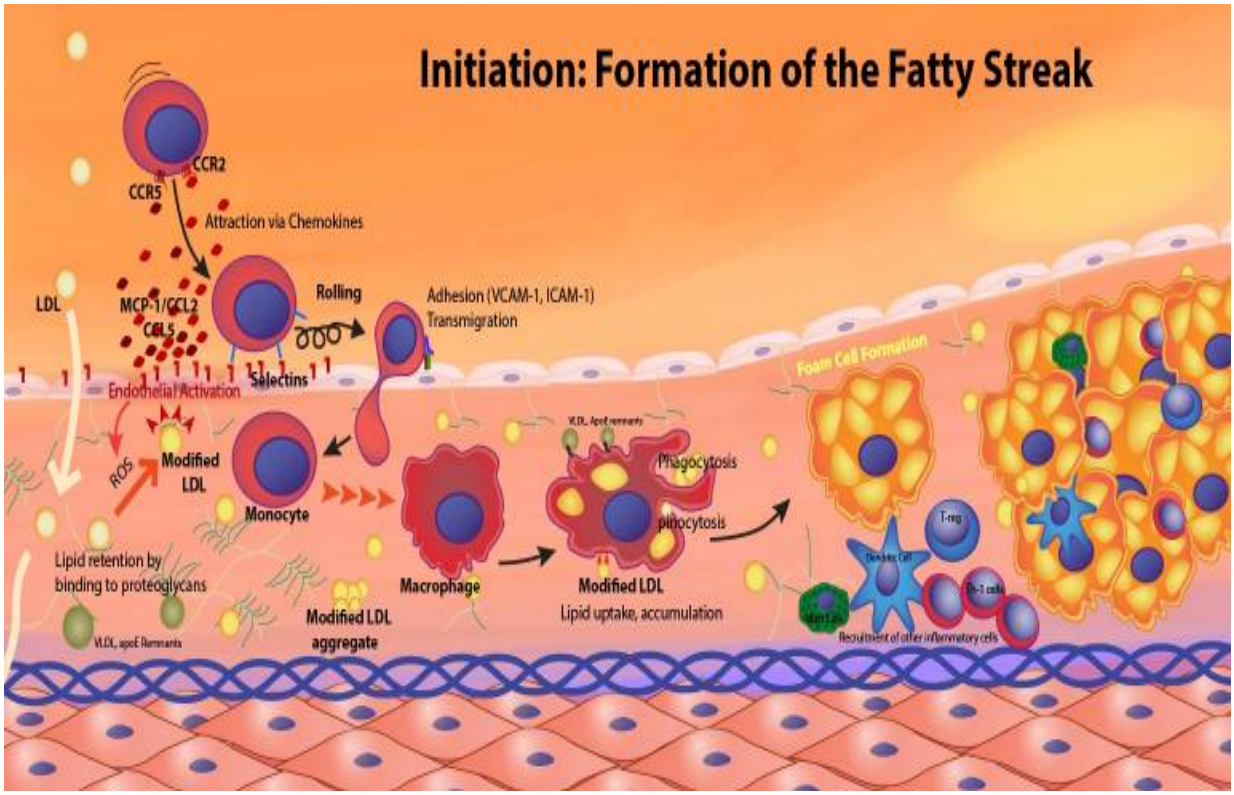

Figure 1-2. LDL-initiated development of atherosclerotic lesion in the vascular wall

Derived from Linton et al. The Role of Lipids and Lipoproteins in Atherosclerosis (2015):[11] Copyright © 2000-2017, MDText.com, Inc.

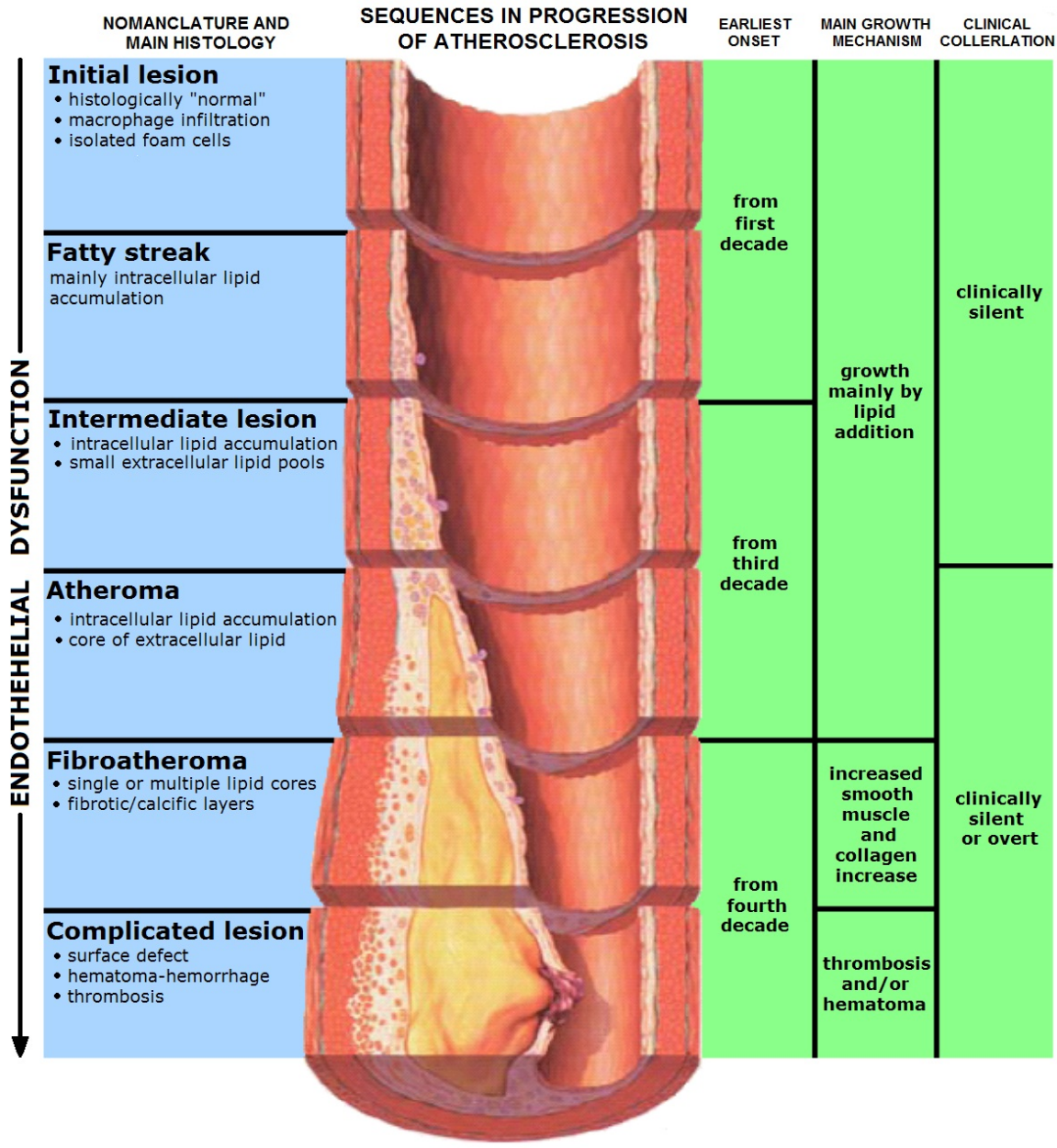

Figure 1-3. Sequences in the progression of Atherosclerosis

Derived from Stary et al. Circulation. 1995; 92:1355-1374. [12] Copyright © 1995, American Heart Association, Inc.

1.2.3 Biomarkers of Atherosclerosis as Predictors of CHD/CVD

A biomarker is a biologic feature that can be used to measure the presence or progress of disease or the effects of treatment. The Food and Drug Administration (FDA) defines a biomarker as “a

characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention " [13].

Extensive research has been conducted to evaluate the non-invasive measurements of subclinical atherosclerosis, which include coronary artery calcification (CAC), aortic calcification, carotid intima thickness (CIMT), carotid artery plaque, ankle-arm index, aortic pulse wave velocity, and flow-mediated dilation. These measures allow the identification of potential candidates for the primary and secondary prevention of CVD events. Although these measurements, in general, predict CVD events independent of the cardiovascular risk factors, controversy exists in applying these measures as routine screening tools [14].

1.2.3.1 Coronary Artery Calcification (CAC)

The presence of coronary calcification is the hallmark of atherosclerosis, and it can be assessed non-invasively by cardiac computed tomography [15]. Among all noninvasive measures of subclinical atherosclerosis, CAC is the most examined measure. CAC is defined as “a hyperattenuating lesion above a threshold of 130 Hounsfield units and with an area of at least three adjacent pixels (1 mm^2)” [15]. CAC is commonly quantified using the Agatston’ score method [16]. CAC reflects the accumulated burden of atherosclerosis over a lifespan, and it is highly correlated with CHD and CVD events [15]. Several prospective cohort studies have shown that independent of other traditional cardiovascular risk factors, the presence and extent of CAC indicates a higher risk of cardiovascular and total mortality. (Table 1-2)

Kondos and colleagues followed 8855 initially asymptomatic adults 30 to 76 years old for 37±12 months. Independent of traditional cardiovascular risk factors, cardiac events were 10.5 times higher among men with CAC and 2.6 times higher among women with CAC

compared to no CAC [17]. Greenland and colleagues in South Bay Heart Watch Study followed 1461 asymptomatic adults for 8.5 years. During a median 7 years of follow-up, compared with a CAC= 0, a CAC of >300 was predictive of MI or CHD and prediction of CHD risk was higher in patients with an FRS greater than 10% compared to an FRS less than 10% [18]. LaMonte and colleagues in the Aerobics Center Longitudinal Study [19] followed 10,746 adults 22 to 96 years of age, free of known CHD for 3.5 years. In an asymptomatic population of men and women, CAC was a significant predictor of cardiac events after adjustment for conventional CHD risk factors. Similarly, other prospective observational studies like the Rotterdam study [20], the St. Francis Heart Study [21], the Prospective Army Coronary Calcium Project [22], and the Multi-Ethnic Study of Atherosclerosis (MESA) study [23] also reported that independent of traditional cardiovascular factors, participants with higher CAC score at baseline had increased hazards of CHD and CVD compared to absent CAC. Thus, CAC can provide incremental prognostic information on CHD/CVD beyond that defined by a single or a combination of traditional cardiovascular risk factors.

Thus, based on the results from community-based studies of CAC in asymptomatic adults, it is evident that the traditional cardiovascular risk factors could miss a significant proportion of patients by categorizing them as of intermediate or low-risk. Non-invasively measured CAC by Electron Beam Computed Tomography (EBCT) can improve CHD/CVD risk prediction based on the FRS alone, particularly among asymptomatic adults in the intermediate risk category (10-20% 10-years risk of coronary events based on FRS) in whom clinical decision making could be tricky. Therefore, many expert groups believe that it is reasonable to use CAC testing in asymptomatic intermediate-risk persons. To account for the limitations of the FRS/traditional cardiovascular risk factors in predicting CHD/CVD, the AHA/American College

of Cardiology (ACC), and the National Cholesterol Education Program (NCEP) support the use FRS followed by the calcium score in asymptomatic intermediate-risk persons. Thus, the presence of calcification in the coronary bed could help find out the potential candidates for primary and secondary prevention of CVD events.

Table 1-2. Selective prospective cohort studies reporting CAC as a predictor of CHD/CVD events

Author-Year-Location-Study name	Populations	Follow-Up	CAC Definition /Categories and Outcome	Point Estimates (95% CI)	Conclusion/ Interpretation
Kondos 2003 US [17]	Community-based, N=8855, age= 30-76 years, 74% men, 95% Caucasians	37±12 months	CAC: 0, ≥1 AU; quartile of positive CAC score; Outcome: Death, MI, revascularization (catheter based intervention or CABG)	All events= 224; CAC >0 vs. CAC= 0 - Men: 10.5 (3.85, 28.40); - Women: 2.57 (1.06, 6.23);	The presence of any detectable CAC provided incremental prognostic information in addition to age, the presence of hypercholesterolemia, hypertension, diabetes, and cigarette smoking.
Greenland 2004 US, South Bay Heart Watch Study [18]	Community-based, N=1461, majority men, mean age ~65 years, 85% Caucasians	8.5 years	CAC: 0, 1-100, 101-300, ≥301 AU; Outcome: Non-fatal MI and CHD death	Non-fatal MI and CHD death= 84; CAC >300 vs. CAC= 0: 3.90 (2.10, 7.30);	Across categories of FRS, CAC score improved risk prediction among patients at intermediate and high risk but not at low risk.
LaMonte 2005 US, The Aerobics Center Longitudinal Study [19]	Community-based, N=10746, age= 22-96 years, 64% men, 97% Caucasians	3.5 years	CAC: 0, 1-38, 39-249, ≥250; 0, >0, ≥100, ≥400, Log transformed CAC; Outcome: Hard CHD events (Nonfatal MI, death from coronary cause), revascularization (CABG and PCI)	Total cardiac events= 368; CAC >0 vs. CAC= 0: 31.7 (13.3, 75.4);	Independent association of CAC with CHD events above traditional risk factors.
Vliegenthart 2005 Netherland, The Rotterdam Study [20]	Community-based, 42.5% men, mean age= 71.1 ± 5.7 years, N=1795	3.3 years	CAC: 0-100, 101-400, 401-1000, >1000; Outcome: PTCA, CABG, MI, Stroke, CHD mortality	Coronary events= 50; Compared to CAC 0-100, - CAC 101-400: 3.1 (1.2, 7.9); - CAC 401-1000: 4.6 (1.8, 11.8); - CAC >1000: 8.3 (3.3, 21.1);	CAC further improved CHD prediction from the FRS model, even in the elderly.
Arad 2005 US, The St. Francis Heart Study [21]	Community-based, N=4613, age= 50-70 years, 65% men, 88% Caucasians	4.3 years	CAC: 0, 1-99, 100-399, ≥400; Outcome: Coronary death, Nonfatal MI, revascularization (PCI and CABG)	All coronary events= 129; Compared to CAC= 0, - CAC 100-399: 10.2 (4.8, 21.6); - CAC >400: 26.2 (12.6, 53.7);	Calcium score predicts CAD events independent of standard risk factors and CRP, and refines FRS.
Taylor 2005 US, The Prospective Army Coronary Calcium Project (PACC study) [22]	Healthy army personnel, N=1983, mean age ~43 years 82% men, 70% Caucasians	3 years	CAC: 0, 1-9, 10-44, ≥45; tertiles of CAC; Outcome: CHD events	CHD events= 9; CAC >0 vs. CAC= 0: 10.75 (2.23, 51.84);	Presence of CAC was associated with 11-fold higher risk of CHD. CAC screening could be an incremental tool in the identification

Table 1-2. Continued

					of individuals at increased risk for CHD above FRS.
Becker 2008 Germany [24]	Preventive cardiology clinic patients, N=1726, mean age=57.7 years, 59% men	40.3 months	CAC: Log transformed CAC; 0, >0 – 75th percentile, >75th percentile of CAC; Outcome: Non-fatal MI or CHD death	Non-fatal MI= 114; CHD death= 65; CAC >75th percentile vs. CAC <75th percentile: - MI=2.25; - CHD death= 2.44;	Higher CAC score predicted higher MI and CHD mortality. No cardiac events among those with CAC= 0.
Detrano 2008 US, The MESA Study [23]	Community-based, N=6722, mean age= 62.2 years, 47.2% men, 38.6% Caucasians, 27.6% Blacks, 21.9% Hispanics, and 11.9% Chinese	3.8 years	CAC: 0, 1-100, 101-300, >300; Outcome: CHD (MI, + angina)	Any coronary event= 162; Compared to CAC= 0, - CAC 1-100: 3.61 (1.96, 6.65); - CAC 101-300: 7.73 (4.13, 14.47); - CAC >300: 9.67 (5.20, 17.98); - Doubling of calcium score =1.26 (1.19, 1.33);	CAC added incremental value to the prediction of CHD over that of the standard coronary risk factors.
Erbel 2010, Germany [25]	Community-based, N=4129, age = 45-75 years, 47% men	5 years	Coronary deaths + Nonfatal MI	Coronary events= 93; Compared to CAC= 0, - CAC 100-399: 2.80 (1.31, 5.99); - CAC >400: 6.40 (3.12, 13.12);	Addition of CAC to traditional cardiovascular risk factors results in a high reclassification rate in the intermediate-risk patients.
Abbreviations – AU, Agatston' unit; CAC, coronary artery calcification; CABG, coronary artery bypass graft; CAD, coronary arterial disease; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; CRP, C-reactive protein; FRS, Framingham Risk Score; MI, myocardial Infarction; PCI, percutaneous coronary intervention; PTCA, percutaneous transluminal coronary angioplasty;					

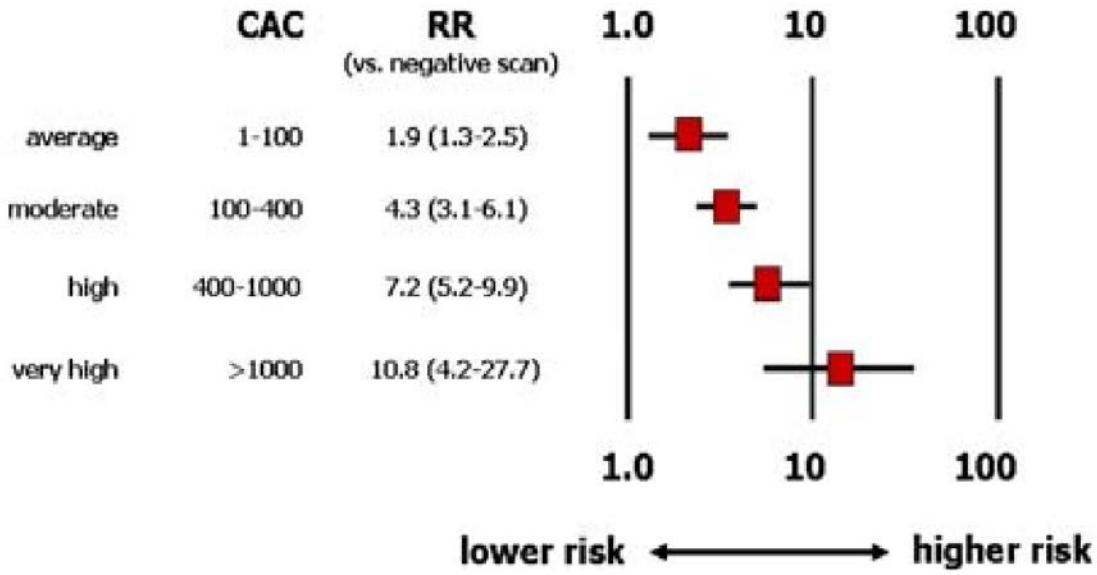

Figure 1-4. Increase in relative risk (95% CI) for CHD mortality and MI with increasing CAC scores in asymptomatic persons compared to asymptomatic persons without CAC

1.2.3.2 Aortic Calcification

Compared to coronary calcification, aortic calcification is a less studied measure of atherosclerosis. Aortic calcification is defined as "using computed tomography, any part of the aorta with a density of 130 Hounsfield units or more and with an area of at least three adjacent pixels (1 mm^2)" [26]. Data assessing the relationship between aortic calcification and CHD are limited. Moreover, heterogeneity exists in available data because of differences in study designs, populations studied, imaging techniques used to measure and quantify aortic calcification, and the outcome measures used to report findings, which hinders the synthesis of evidence. Atherosclerosis develops in the aorta before it develops in other vascular beds like the femoral, carotid, or coronary arteries and it appears to have a similar association with traditional

cardiovascular risk factors and the same clinical significance as CAC [27, 28]. The presence and extent of aortic atherosclerosis/calcification may identify subjects at increased risk for CHD [29].

Several prospective studies have been carried out to assess the presence of aortic calcification and its association with the subsequent development of cardiovascular events (Table 1-3). In the MESA study, Criqui and colleagues followed 1974 men and women aged 45-84 years for 5.5 years [30]. Participants having an abdominal aortic calcification score in the fourth quartile compared to first and second quartiles combined had 2.5 times higher hazards of hard CHD/CVD events and 5.5 times greater hazards of CVD mortality [30]. Budoff and colleagues in the MESA study followed 6807 participants aged 62±10 years for 4.5 years. Investigators found that presence of thoracic calcification was a significant predictor of coronary events independent of CAC [31]. Similarly, Irribarren et al. [32], Wilson et al. in the Framingham Heart Study [33], Meer et al. in the Rotterdam study [34], Rodondi et al. in the Study of Osteoporotic Fractures (SOF) [35], Shousboe et al. in the Clodronate British Study [36], reported that presence of calcification in the aorta was associated with CHD/CVD after adjustment for traditional cardiovascular risk factors.

A recent systematic review and meta-analysis of nine longitudinal studies suggested that aortic calcification is an independent predictor of future cardiac events (Figure 1-4). For coronary events, the RR (95% CI) for the middle tertile (moderate calcification) and higher tertile (severe calcification) compared to lowest tertile (mild calcification) were 1.43 (1.17, 1.77) and 1.92 (1.54, 2.38) respectively. There was a uniform increase in the risk of future cardiac events with increase in aortic calcification score (Figure 1-5) [27]. In conclusion, the addition of aortic calcification to traditional risk factor models for the general population could improve the

prediction and therefore risk reclassification of CHD/CVD [30]. Thus, aortic calcification can play a significant role in influencing the primary prevention strategy for CHD/CVD.

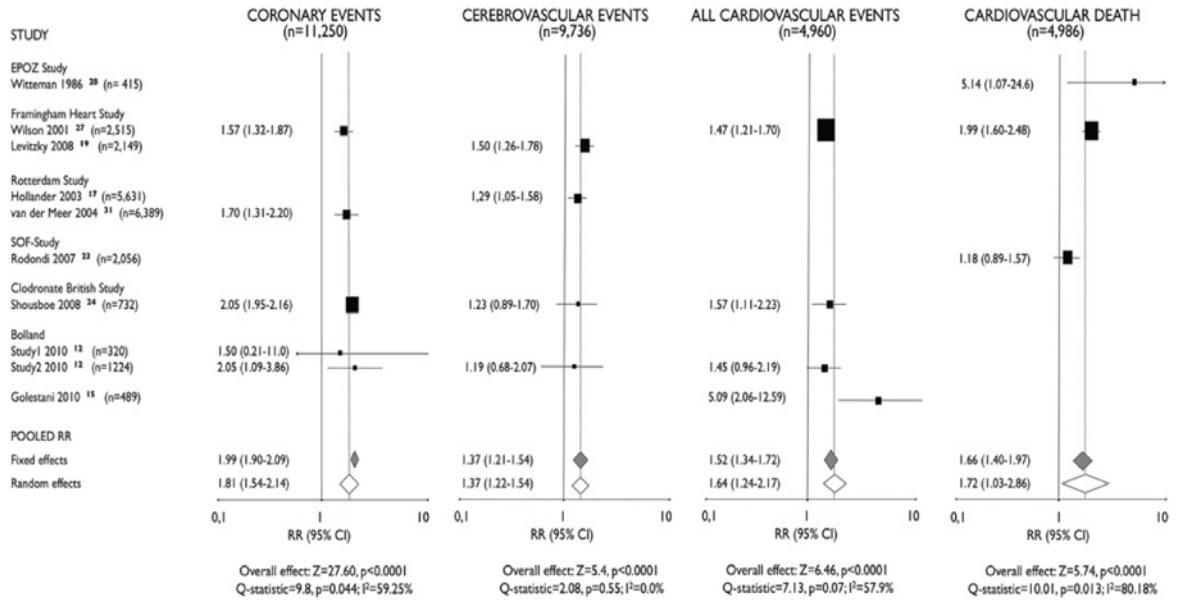

Figure 1-5. Association of aortic calcification with different cardiovascular end-points

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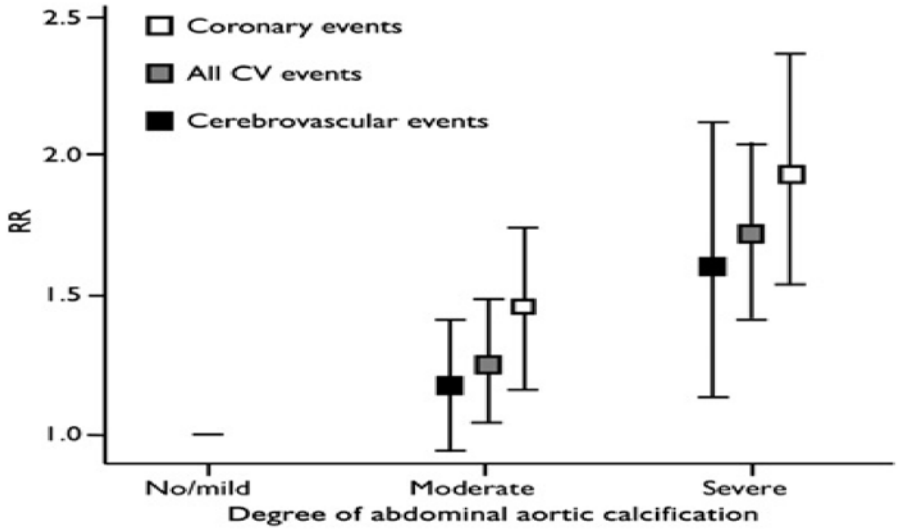

Figure 1-6. Pooled relative risk for cardiovascular end points per tertiles of abdominal aortic calcification

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Table 1-3. Studies examining the association between aortic calcification and CHD/CVD

Author-Year-Location-Study name	Populations	Type of Study/ Follow-Up	Aortic Calcification Categories	Point Estimate (95% CI)	Conclusion/ Interpretation
Witteman 1986, Netherland, EPOZ study [37]	Community based, N=2957, mean age=68.2, 46% men	Nested case control, FU=6-9 years	Abdominal aorta, presence of aortic calcification; Outcome: CV mortality	CV mortality= 83; Point estimate= NA;	Aortic calcification was associated with a 6-fold increased risk of CVD death in men independent of major CVD risk factors.
Iribarren 2000, US [32]	Community based, N=116309, age= 30-89 years, 48% men, 80% Caucasians, 13% blacks	Prospective cohort study, FU= 28 years	Aortic arch, presence of aortic calcification; Outcome: CHD	CHD: - Men=1063 events: 1.27 (1.11, 1.45); - Women= 1571 events: 1.22 (1.07, 1.38);	Aortic arch calcification was independently related to CHD risk above traditional risk factors.
Wilson 2001, US, The Framingham Heart study [33]	Community based, N=2515, mean age= 60.6 years, 42% men, mostly Caucasians	Prospective cohort study, FU = 22 years	Abdominal aorta, Tertiles of abdominal aortic calcification; Outcome: Incident CHD, incident CVD, CVD mortality	Incident CHD= 454; Incident CVD= 709; CVD mortality= 365; Third tertile vs. first tertile: - CHD: 1.91 (1.48, 2.47); - CVD: 1.70 (1.38, 2.09); - CVD mortality: 2.26 (1.66, 3.09);	Among middle-aged men and women abdominal aortic calcification was associated with CHD, CVD, and CVD mortality after adjustment for traditional cardiovascular risk factors.
Hollander 2003, Netherland, The Rotterdam study [38]	Community based, N=6931, age=69.5 years, 39.7% men	Prospective cohort study, FU= 6.1 years	Abdominal aorta, presence of aortic calcification; Outcome: Incident CVD	Incident CVD= 378 events; 1.6 (1.1, 2.5);	Aortic calcification was a stronger predictor of incident stroke than a carotid plaque or ankle-arm indexes. It has additional value to classic risk factors in predicting stroke.
Van der Meer 2004, The Rotterdam study [34]	Community-based, N=6389, mean age >55 years, 39.1% men	Prospective cohort study, FU= 7-10 years	Abdominal aorta, presence of aortic calcification; Outcome: Incident MI	Incident MI= 258; Compared no aortic calcification, - Moderate aortic calcification: 1.83 (1.21, 2.76); - Severe aortic calcification: 1.94 (1.30, 2.90);	Aortic calcification had higher hazard of incident CHD compared to no aortic calcification independent of traditional CV risk factors and other measures of atherosclerosis.
Rodondi 2007, The SOF Study [35]	Community based, N=2056, mean age = 72.0 years, all women, mostly Caucasians	Prospective cohort study, FU=13 years	Abdominal aorta, presence of aortic calcification; Outcome: CV mortality	All-cause mortality= 844; CV mortality= 321; - All-cause mortality: 1.37 (1.15, 1.64); - CV mortality:1.18 (0.80, 1.57);	Aortic calcification was associated with higher hazard of cardiovascular mortality after adjusting for traditional cardiovascular risk factors.
Shousboe 2008 UK, The Clodronate	Community-based, N=732, mean age =	Nested case control study, FU=	Abdominal aorta, tertile of aortic calcium score	Top tertile vs. bottom tertile:1.74 (1.19, 2.56);	AAC score was associated with incident CHD after

Table 1-3. Continued

British Study [36]	80.1 years, all women, all Caucasians	4.0 years	Outcome= Incident CHD/CVD		adjusting for traditional risk factors and prior stroke.
Levitzky 2008 US, Framingham Heart Study [39]	Community based, N=2149, 39.5% men, mean age=60.0±8.0 years, mostly Caucasians	Prospective cohort study, FU = 32 years	Abdominal aorta; tertiles of aortic calcium score; Outcome: Incident CHD/CVD	CHD events=702; CVD events = 1121; Top tertile vs. Bottom tertile: - CHD events: 1.59 (1.26, 2.00); - CVD events: 1.64 (1.37, 1.97);	Multivariable adjusted hazard ratio for the third versus first AAC tertile was, 1.59 for CHD, and 1.64 for CVD.
Bolland 2010 New Zealand [40]	Community-based, N=1471, mean age=74.2 years, all women	Prospective cohort study, FU = 4.4 years	Abdominal aorta, presence of aortic calcification; Outcome: Incident MI	Incident MI= 49 events; 2.30 (1.25, 4.22);	The presence of AAC independently associated with MI in women after adjustment for cardiovascular risk.
Bolland 2010 New Zealand [40]	Community based, N=323, mean age = 57.2, all men	Prospective cohort study, FU = 3.3 years	Abdominal aorta, presence of aortic calcification; Outcome: Incident MI	Incident MI= 6 events; 5.32 (1.07, 26.60);	The presence of AAC independently associated with MI in men after adjustment for cardiovascular risk.
Golestani 2010 Netherland [41]	Community based, N=489, mean age=69.0 years, 37.2% men	Nested case control study, FU = 2.8	Abdominal aorta, presence of aortic calcification; Outcome: Incident CVD	Point estimate= NA;	AAC-positive, low-AAC, and high-AAC compared to the control group had higher risk for CVD incidence after adjusting for traditional risk factors for CVD.
Budoff 2011 US, The MESA Study [31]	Community based, N=6807, 47% men, mean age 62 ± 10 years, 39.0% Caucasians, 27.0% Blacks, 22% Hispanics, and 12% Chinese	Prospective cohort study, FU = 4.5 years	Thoracic aorta, 0, ≥1, Log (thoracic calcium score + 1); Outcome- MI, resuscitated cardiac arrest, CHD death	For cardiac events= 232; 3.04 (1.60, 5.76);	Presence of thoracic aortic calcification was a significant predictor of future coronary events only in women independent of CAC.
Criqui 2014 US, The MESA study [30]	Community-based, N=1974, 45-84 years, ~50% men, 40% Caucasians, 21% Blacks, 26% Hispanics, and 13% Chinese	Prospective cohort study, 5.5 years	Abdominal aorta, 3 groups of percentiles of AU score, 0 to 50th, 51st to 75th, and 76th to 100th, quartiles of AAC; Outcome- CHD, CVD events, CVD mortality, total mortality	Hard CHD= 50; Hard CVD= 83; CVD mortality= 30; Total mortality=105; >75th percentile vs. AAC ≤50th percentile: - hard CHD: 4.06; - hard CVD: 4.00; - CVD mortality: 7.83; - total mortality: 3.51;	AAC showed higher AUCs for CVD mortality and total mortality compared to CAC. AAC was an independent predictor of hard CHD and CVD events.

Abbreviations: AAC, abdominal aortic calcification; AU, Agatston' unit; AUC, the area under receiver operating characteristic curve; CAC, coronary artery calcification; CABG, coronary artery bypass graft; CAD, coronary arterial disease; CHD, coronary heart disease; CI, confidence interval; CV, cardiovascular; CVD, cardiovascular disease; CRP, C-reactive protein; FU, follow-up; FRS, Framingham Risk Score; MI, myocardial infarction; PCI, percutaneous coronary intervention; PTCA, percutaneous transluminal coronary angioplasty;

1.2.3.3 Carotid Intima-Media Thickness (CIMT)

CIMT is a measurement of the thickness of tunica intima and tunica media of the wall of a carotid artery. CIMT can be measured non-invasively using a B-mode ultrasound scan of the carotid artery. To date, available evidence is mixed relevant to the role of CIMT in the prediction of CHD/CVD [42-44]. Based on the available evidence, the United States Preventive Services Task Force (USPSTF) recommended against the routine use of CIMT for risk stratification of individuals at intermediate cardiovascular risk [45]. In 2013, the AHA/ACC task force also recommended against the routine measurement of CIMT in clinical practice for the first atherosclerotic cardiovascular event risk assessment [46].

1.2.3.4 Carotid Plaque

Epidemiological studies suggest a link between the presence of an atherosclerotic plaque and CHD/CVD [44, 47]. Investigators from the MESA study reported that the presence of carotid plaque was independently associated with CHD events after adjustment for FRS and added incremental prognostic value to traditional cardiovascular risk factors for the prediction of CHD events [47]. Similar to the MESA study findings, investigators of the Atherosclerosis Risk in Communities (ARIC) study reported that CHD prediction from traditional cardiovascular risk factors could be improved by the addition of carotid plaque [44]. Similarly, a meta-analysis of eleven community-based studies showed that compared with CIMT carotid plaque could more accurately predict CHD [48].

1.2.3.5 Ankle Brachial Index (ABI)

ABI is the ratio of the blood pressure at the ankle to the blood pressure in the upper arm (brachium). Normal ABI value in a healthy adult range from 0.9-1.3. An ABI value <0.9

indicates arterial disease, and it is an indicator of generalized atherosclerosis [49]. Measurement of the ABI may improve the accuracy of the FRS in the prediction of a cardiovascular risk [49]. A meta-analysis of sixteen prospective cohort studies reported that a low ankle brachial index (≤0.9) was associated with a higher hazard of total mortality, cardiovascular mortality and major coronary event [49].

1.3 EPIDEMIOLOGY OF CHD – COMPARISON BETWEEN THE US AND JAPAN

In 1960, the internationally conducted Seven Countries Study with participants aged >50 years from Japan, the US, and Europe revealed for the first time that Japan had the lowest CHD mortality. This finding was mainly attributed to low serum levels of total cholesterol in Japan i.e. 165 mg/dL in Japan vs. 240 mg/dL in the US in the 1960s [50]. Further studies in Japanese migrants to the US, [the Ni-Hon-San Study [51, 52] and the Honolulu Heart program [53]] revealed increase rates of CHD mortality among Japanese Americans. In 1965, the Ni-Hon-San Study was initiated to assess the relationship between environmental factors and CHD/stroke among participants from Japan, Honolulu, and San Francisco. This study revealed higher rates of CHD among participants from Honolulu compared to Japanese in Japan [51, 52]. In 1965, the Honolulu Heart program [53] was begun to compare CVD mortality between Japanese in Japan and Japanese people living in Hawaii, US. Investigators reported that in addition to higher rates of CVD and type 2 diabetes mellitus (DM) among Japanese American compared to Japanese in Japan, Japanese American also had a worse CHD risk factor profile [53].

Findings from the Seven Countries Study, Ni-Hon-San, and the Honolulu Heart Program led the formulation of the hypothesis that with Western culture adoption, CHD rates among

Japanese in Japan would increase to the levels in the US. Over the period of the next three decades (1960 to 1990), with Western acculturation, intake of saturated fat increased among Japanese in Japan which steadily increased serum total cholesterol levels. In the year 2008, population levels of total cholesterol among Japanese in Japan aged 50-69 years were higher than the US population [54]. However, even if the intake of saturated fat has increased among Japanese in Japan from the 1960s to 1990s, CHD mortality has declined since the 1970s ('Japanese paradox'). Currently, Japan has the lowest CHD mortality among developed countries. This 'Japanese paradox' provides an opportunity to explore various risk factors responsible for differences in the CHD mortality burden between Japan and the US.

1.4 THE ERA-JUMP STUDY

The **E**lectron-beam computed tomography, **R**isk Factor **A**ssessment among **J**apanese and **U**S **M**en in the **P**ost-World War II Birth cohort (ERA-JUMP) study was the first international study initiated to assess the prevalence and risk factors associated with subclinical atherosclerosis among 300 Japanese men in Kusatsu, Japan, 300 US White and 100 Black men in Pittsburgh, US, and 300 Japanese American men in Honolulu, US. The ERA-JUMP study enrolled men aged 40-49 years old, free of clinical CVD or other severe diseases between 2002 and 2006. In Japan, participants were randomly selected using basic residents' register. In Pittsburgh, White and Black study participants were randomly selected from the voter registration list. In Honolulu, study participants were randomly selected from the offspring of the members of the Honolulu Heart Program cohort [55]. Basic characteristics of the ERA-JUMP study participants are shown in table 1-4 [55-58].

Results of the first four years of the ERA-JUMP Study [55-57] have documented that (i) the Japanese in Japan had the lowest levels of subclinical atherosclerosis in the coronary and carotid arteries among all race-ethnicities, (ii) associations of CAC and CIMT with traditional risk factors were similar across populations, (iii) as compared to US White, Japanese American had similar or higher levels of subclinical atherosclerosis, and (iv) variations in traditional risk factors did not account for the difference in subclinical atherosclerosis between the Japanese in Japan and US White.

Table 1-4. Levels of subclinical atherosclerosis and basic characteristics of study participants in the ERA-JUMP study, 2002-2006

	Japanese in Japan (n=313)	US White (n=310)	Japanese American (n=303)	African American (n=101)	P*
Subclinical atherosclerosis					
Coronary calcification (%)	11.6	26.3	32.0	20.4	¶§
Aortic calcification (%)	35.8	62.3	69.9	69.9	¶§
Carotid IMT (mm)	0.614 ± 0.080	0.670 ± 0.093	0.720 ± 0.113	0.746 ± 0.124	¶†§
Basic characteristics					
Age (years)	45.0 ± 2.8	45.0 ± 2.8	46.1 ± 2.8	45.0 ± 2.8	†§
Body mass index (kg/m^2)	23.6 ± 2.9	28.0 ± 4.4	28.0 ± 4.6	30.4 ± 6.6	†§
Visceral adipose tissue (cm^2)	80.1 ± 30.6	103.4 ± 44.2	104.2± 46.1	80.2 ± 36.1	¶§
SAT (cm^2)	81.6 ± 35.5	152.1 ± 67.7	139.5 ± 72.1	170.9 ± 93.6	¶§
Systolic BP (mmHg)	125.0 ± 16.1	122.6 ± 11.2	127.7 ± 12.5	126.7 ± 16.1	†
Hypertension (%)	26.5	15.2	33.0	33.0	¶§
LDL-C (mg/dL)	132.2 ± 35.9	134.7 ± 33.5	121.7 ± 32.7	128.9 ± 41.7	†§
Triglycerides (mg/dL)	154.9 ± 80.9	151.7 ± 99.9	184.5 ± 142.4	128.9 ± 41.7	†§
HDL-C (mg/dL)	54.1 ± 13.6	47.7 ± 12.7	50.7 ± 12.2	51.0 ± 16.0	¶†§
Glucose (mg/dL)	106.8 ± 18.7	101.7 ± 15.4	112.3 ± 21.0	102.7 ± 15.8	¶†§
Insulin (uU/mL)	10.3 ± 4.4	15.3 ± 8.3	15.1 ± 9.2	15.7 ± 8.6	¶§
Diabetes mellitus (%)	6.1	3.6	13.9	11.0	†§
Current smoking (%)	49.5	7.7	12.9	27.0	¶§
Pack years	19.7 ± 16.9	4.3 ± 10.5	4.4 ± 8.9	4.9 ± 7.7	¶§
Alcohol drinker (%)	67.1	44.2	37.3	35.0	¶†§
Ethanol (g/day)	26.8 ± 28.7	10.0 ± 14.0	17.8 ± 32.9	12.8 ± 21.6	¶†§
Years of education (years)	14.3 ± 2.0	17.0 ± 2.7	15.6 ± 1.8	14.4 ± 2.4	¶†§
Exercise (≥1 hour/week)(%)	26.5	73.2	n.c	69.2	¶
Hypertension meds (%)	5.4	8.7	20.5	19.0	†§
Lipid meds (%)	3.5	12.3	23.1	8.0	¶§
Diabetes meds (%)	1.9	1.0	6.6	3.0	†§
CRP (mg/dL)	0.7 ± 1.8	1.6 ± 2.3	1.3 ± 2.3	3.4 ± 6.2	¶§
Fibrinogen (mg/dL)	256.0 ± 65.6	291.3 ± 70.2	316.2 ± 72.8	315.3 ± 80.6	¶†§
Homocysteine (mg/L)	13.3 ± 6.6	8.4 ± 5.1	8.2 ± 3.2	8.4 ± 2.5	¶§
Adiponectin (μg/mL)	6.9 ± 4.0	11.1 ± 5.0	7.7 ± 4.3	7.9 ± 3.8	¶†
NMR lipoprotein particles					
Total VLDL (nmol/L)	92.7 ± 48.6	92.9 ± 44.0	108.7 ± 54.7	78.0 ± 50.6	†§
Large VLDL (nmol/L)	2.6 ± 5.4	4.5 ± 6.5	5.8 ± 8.1	3.2 ± 3.9	¶§
Total LDL (nmol/L)	1390 ± 442	1459 ± 394	1354 ± 482	1474 ± 467	†
Small LDL (nmol/L)	852 ± 512	898 ± 494	971 ± 509	913 ± 510	§
Total HDL (μmol/L)	35.5 ± 6.6	31.1 ± 5.7	36.3 ± 6.0	31.3 ± 6.2	¶†
Large HDL (μmol/L)	8.6 ± 4.0	5.0 ± 3.1	5.9 ± 3.3	5.6 ± 3.1	¶†§
VLDL size (nm)	44.1 ± 7.5	49.9 ± 7.7	49.3 ± 7.3	50.4 ± 7.1	¶†
LDL size (nm)	21.1 ± 0.8	20.9 ± 0.9	20.6 ± 0.9	20.9 ± 0.8	†§
HDL size (nm)	9.1 ± 0.5	8.6 ± 0.5	8.7 ± 0.4	8.7 ± 0.5	¶†§

Values are expressed as mean ± SD or %. Coronary calcification was defined as coronary calcium score ≥10. Aortic calcification was defined as aortic calcium score >0. IMT: intima-media thickness. SAT: subcutaneous adipose tissue, BP: blood pressure, Med: medication, CRP: C reactive protein. NMR: nuclear magnetic resonance. VLDL: very-low-density. n.c.: not collected. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or hypertensive medication. Diabetes was defined as fasting glucose ≥ 126 mg/dL or diabetes medication. Alcohol drinker was defined as those who drank alcohol ≥2 days/week.

¶: P<0.017 between the Japanese in Japan versus US Whites, †: P<0.017 between US Whites and Japanese Americans, §: P<0.017 between the Japanese in Japan versus Japanese Americans

1.5 THE ROLE OF LIPIDS AND LIPOPROTEINS IN ATHEROSCLEROSIS/CHD

1.5.1 Low-Density Lipoprotein Cholesterol (LDL-C)/LDL-P and Atherosclerosis/CHD

Seminal work by Anitschkow in 1913 showing the causal role of cholesterol in the development of atheroma in rabbits [59] and findings from the Seven Countries Studies [60] in the mid-20thcentury led to the formulation of the cholesterol hypothesis, i.e. lowering of LDL-C would reduce the burden of cardiovascular events. The next four decades of research work was mainly based on this hypothesis, and large RCTs of cholesterol-lowering drugs have further confirmed the cholesterol hypothesis [61]. Major outcome RCT, the Lipid Clinic Research trial reported for the first time a significant reduction in cardiac events with lowering of LDL-C [62]. A meta-analysis of 26 statin trials reported that with every one mmol/L decrease in LDL-C, there was 20% reduction in cardiovascular outcomes [63]. Depending on the available evidence on the relationship between LDL-C and risk of CHD/CVD, risk assessment, patients' stratification into different risk categories and treatment guidelines for hypercholesterolemia were mainly based on the level of LDL-C. However, in CVD/CHD patients, the risk of CVD/CHD persists (known as 'residual risk') even after achieving the recommended level of LDL-C [64]. This limitation, the inability to completely eliminate CVD/CHD risk by normalizing LCL-C, led researchers to shift their focus to another measure of LDL, specifically to the LDL particle (LDL-P) concentration, which has been reported to be a better measure of CHD/CVD risk than LDL-C [65-67].

LDL-P concentrations, as well as the distribution of their subclasses, can be measured by nuclear magnetic resonance (NMR) spectroscopy [68]. NMR-measured lipoprotein particles vary in size and cholesterol content. Therefore, enzymatically measured lipid concentration (LDL-C) is not virtually equal to lipoprotein particle concentrations measured by NMR spectroscopy [69].

At any given level of LDL-C, two individuals could have different LDL-P concentrations depending on their LDL-P subclasses distribution. Independent of the cholesterol content of lipoprotein particles; differences in lipoprotein particle numbers, average lipoprotein size, and lipoprotein subclass distribution are linked to atherosclerosis and CHD. Several studies have reported that NMR-measured small LDL-P, large LDL-P, and total LDL-P are significantly associated with subclinical atherosclerosis and CHD/CVD [65-67]. In fact, some studies have shown that NMR-measured lipoprotein particle number compared to enzymatically measured lipids are a stronger predictor of atherosclerosis and CVD events [65, 66, 70, 71] (Table 1-5). Thus, if the patient has higher concentrations of small LDL-P and total LDL-P despite normal LDL-C, residual risk of CHD/CVD persists.

It is very well known that LDL-P enters the arterial wall via a gradient-driven process, binds to arterial wall proteoglycans and undergoes oxidative modification, which is subsequently taken up by macrophages resulting in foam cell formation. Further activation of these foam cells and expansion of the inflammatory response via impaired acetylcholine-induced vasodilatation, reduced endothelial nitric oxide activity [72], and increased oxidative stress further enhances the propensity for initiation and promotion of atherosclerosis. Traditionally, among different LDL-P subclassess, small LDL-P is thought to be more atherogenic owing to their greater susceptibility to oxidation, arterial retention and greater uptake by macrophages. Whereas, few researchers have reported that the atherogenic potential of small LDL-P is due to increased total LDL-P. The amount of cholesterol deposition in the sub-endothelial space will largely be dependent on the concentration of total LDL-P. The more LDL-P particles that reaching the sub-endothelial space, modified by reactive oxygen species to be engulfed by macrophages, the greater the foam cell formation, and therefore, the greater the progression of the atherosclerotic plaque.

Table 1-5. Studies showing a robust association of LDL-P with atherosclerosis/CHD/CVD above LDL-C

Author/Year/ Location/ Study name	Populations	Type of study/Follow-up	LDL-P categories/Primary outcome	Point estimate (95% CI)	Conclusion/ interpretation
Kuller 2002 US, CHS study [73]	Community-based, N=683, mean age= 73± 5.5 years, all women, 95% Caucasian	Matched case-control study	Quartiles of LDL-P; Outcome: Incident MI	Incident MI= 434; 4th vs. 1st quartile of LDL-P: 3.34 (1.50, 6.50);	Small LDL-P, the size of LDL particles, and the greater number of LDL-P were related to incident CHD (MI and angina) among older women.
Cromwell 2007 US, The Framingham Offspring Study [65]	Community-based, N=3066, age=30-74 years, 47% men, mostly Caucasians	Prospective cohort study, FU=14.8 years	Continuous and Quartiles of LDL-P; Outcome: CVD (MI, angina pectoris, coronary insufficiency, CHD death, stroke, transient ischemic attack, intermittent claudication, or CHF)	CVD events= 531; A 1-SD increase in - LDL-P: 1.33 (1.17, 1.50); - LDL-C: 1.18 (1.02, 1.37);	The LDL-P number was more strongly related to incident CVD events than LDL-C.
Harchaoui 2007 UK, EPIC-Norfolk [74]	Community based, N=2888, mean age = 65 years, 63% men	Nested case-control study, FU= 6 years	Quartiles of LDL-P Outcome = CAD	CAD events= 1003; 4th quartile vs. 1st quartile - LDL-P: 1.78 (1.34, 2.37); - LDL-C: 1.22 (0.92, 1.61);	LDL-P was related to CAD on top of FRS as well as after adjusting for LDL-C.
Otvos 2011 US, The MESA study [66]	Community-based, N= 6814, mean age = 62 years, and 49% men, 39% Caucasians, 27.0% Blacks, 22.0% Hispanics, and 12.0% Chinese	Prospective cohort study, FU= 5.5 years	Continuous and tertiles of LDL-P; Outcome: CVD (MI, CHD deaths, angina, stroke, stroke death, CVD death), CIMT	Overall CVD events = 319; A 1-SD increase in - LDL-P: 1.35 (1.21, 1.50); - LDL-C: 1.28 (1.15, 1.43); CVD events in discordant group= 159; A 1-SD increase in - LDL-P: 1.41 (1.15, 1.75); - LDL-C: 1.17 (0.96, 1.42);	When LDL-P and LDL-C were discordant, LDL-P was more strongly associated with risk of CVD events and with carotid IMT than was LDL-C.
Prado 2011, US [70]	Hospital based, N=284, age=40-69 years, 68% men, 98% Caucasians	Cross-sectional	Continuous and tertiles of LDL-P Outcome= CAC	A 1-SD increase in - Small LDL-P: 1.35 (1.3, 1.5); - Large LDL-P: 1.08 (1.00, 1.17);	Independent of LDL-C, a 1-SD increase in total LDL-P, small, and large LDL-P were associated with higher odds of CAC.
Parish 2012, UK, Heart Protection Study [75]	Community-based, N=20000, age= 40-80 years	Prospective cohort study FU= 5.3 years	LDL-P as a continuous variable; Outcome= major occlusive coronary events (non-fatal MI and coronary death	Major occlusive coronary events in placebo arm= 1039; A 1-SD increase in - LDL-P: 1.11 (1.05, 1.17);	Equal association of LDL-P and LDL-C with major occlusive coronary events.

Table 1-5. Continued

			other than from heart failure)	- LDL-C: 1.09 (1.03, 1.15);	
Zaid 2016, Japan, The Shiga Epidemiological Study of Subclinical atherosclerosis [71]	Community based, N=889, aged 40-79 years, all men	Cross-sectional	LDL-P as a continuous variable Outcome= CAC and CIMT	CIMT: β est (95% CI) for a 1-SD increase in - LDL-P: 24.60 (5.90, 43.30), - LDL-C: 8.70 (-10.10, 27.50); For CAC>0: OR (95% CI) a 1-SD increase in - LDL-P: 1.55 (1.15, 2.08); - LDL-C: 0.87 (0.65, 1.16);	Independent of LDL-C, LDL-P was significantly associated CIMT and CAC.
Abbreviations: AU, Agatston' unit; CAC, coronary artery calcification; CABG, coronary artery bypass graft; CAD, coronary arterial disease; CHD, coronary heart disease; CI, confidence interval; CIMT, carotid intima-media thickness; CV, cardiovascular; CVD, cardiovascular disease; CRP, C-reactive protein; FU, follow-up; FRS, Framingham Risk Score; LDL-C, low density lipoprotein cholesterol; LDL-P, low density lipoprotein particle; MI, myocardial Infarction; PCI, percutaneous coronary intervention; PTCA, Percutaneous transluminal coronary angioplasty; SD, standard deviation; US, United States					

1.5.2 Triglycerides/Very Low-Density Lipoproteins (VLDL-P) and Atherosclerosis/CHD/CVD

The prevalence of hypertriglyceridemia is increasing all over the world, and this is accompanied by an increase in the prevalence of obesity and type 2 diabetes [76, 77]. Usually, elevated serum levels of triglyceride-rich lipoproteins: chylomicrons, VLDL-P lead to hypertriglyceridemia [11]. Individuals with hypertriglyceridemia are more susceptible to cardiovascular complications [78], although how much CVD risk increases with increasing serum triglycerides is not clear. The Framingham study was the first study to document the significant association of triglycerides with CVD [79]. However, a debate is going around the independent role of triglycerides in CVDs and the utility of triglycerides measurement for cardiovascular management. The evidence available describing the association of triglycerides and CVD is mixed [78, 80-82]. In a meta-analysis of 68 prospective cohort studies, Angelantonio and colleagues reported that measurement of either total and HDL cholesterol levels or apolipoproteins is sufficient as a part of lipid assessment in vascular disease [83]. There is no need for further triglycerides assessment

above HDL-C or apolipoproteins as triglycerides assessment do not add any additional predictive value [83]. The conclusion was mainly based on the non-significant association of triglycerides with CVD after adjustment for traditional cardiovascular risk factors [83]. However, the PROCAM study [84] and the PROVE IT-TIMI 22 [85] study found a robust association of triglycerides with CVD after adjustment for traditional cardiovascular risk factors including LDL-C. Similarly, a meta-analysis of 29 long-term prospective cohort studies reported that participants in the highest tertile of triglycerides compared with the lowest tertile had a higher likelihood of CVD [OR (95% CI) =1.72 (1.56, 1.90)] after adjustment for traditional cardiovascular risk factors [80]. In another meta-analysis of randomized controlled trials, the authors reported an independent role of serum levels of triglycerides in CVDs. In the latter study, it was further shown that residual CVD risk declined with a reduction in triglyceride-rich lipoproteins [86].

1.5.2.1 Plausible mechanisms of action of triglycerides in atherosclerosis

i. Increase foam cell formation (hallmark of atherosclerosis)

In hypertriglyceridemia, there is increased secretion of triglyceride-rich lipoproteins: VLDL-P and chylomicrons [87]. Through the actions of lipoprotein lipase, VLDL-P and chylomicron can undergo partial hydrolysis to produce their remnants. VLDL-P and chylomicron remnants can acquire more cholesterol from HDL through the action of cholesterol ester transfer protein [88]. VLDL-P, chylomicrons, and their remnants particles contain a significant amount of cholesterol [89]. These cholesterol-rich lipid particles can easily cross the endothelial layer, invade the intimal layer of an artery and promote the formation of foam cell by depositing cholesterol in atherosclerotic lesions [90, 91].

ii. Increased production of toxic free fatty acids in cells of the arterial wall

Along with the increase in the concentration of cholesterol in triglyceride-rich lipoproteins VLDL-P and chylomicrons, within atherosclerotic lesions lipolysis of the triglycerides by lipoprotein lipase can also increase the production of toxic free fatty acids in cells of the arterial wall [92]. These toxic free fatty acids can further increase cell death and therefore increase in inflammation within the atherosclerotic lesions [92].

iii. Increased atherogenesis of triglycerides through the action of Apolipoprotein CIII content of VLDL and remnants

Serum level of Apolipoprotein CIII increases with increasing serum levels of triglycerides [93, 94]. Apolipoprotein CIII content of VLDL-P and remnants prevent their uptake by the liver, promote their arterial retention within the atherosclerotic lesion, and therefore, further increase their atherogenic potential [93].

1.5.3 High- Density Lipoprotein Cholesterol (HDL-C)/ High-Density Lipoprotein Particles (HDL-P) and Atherosclerosis/CHD/CVD

Decades of research have shown a significant inverse association of HDL-C with the risk of CHD/CVD [95]. However, recent Genome Wide Association Studies (GWAS) [96] and clinical studies [97, 98] raising the levels of HDL-C suggest different pictures. In a pooled analysis from six community-based cohorts, investigators did not observe a monotonic inverse linear association between HDL-C and CHD risk at very high levels of HDL-C [99]. Further, findings of two prospective cohort studies, IDEAL and EPIC-Norfolk cohorts, suggest an increased risk CVD at the higher end of the HDL-C and HDL particle size distributions after adjustment for

apo B and apo A-I [100]. Recent failures of drugs in clinical studies [101] that raised HDL-C without reducing the CVD risk diverted researchers' focus towards other measures of HDL specifically HDL particles (HDL-P) or HDL subclasses measured by NMR Spectroscopy.

Recent reports assessing the relationship between HDL-C with CHD risk suggest that the inverse association of HDL-C with CHD might be partially due to its metabolic correlations with atherogenic lipoprotein concentrations (LDL-P, VLDL-P, and triglycerides). However, total HDL-P remained inversely associated with CHD after adjusting for LDL-P, triglycerides, and HDL-P size [102]. Similarly, Mackey and colleagues in the MESA study among 5589 study participants aged 45-84 years reported that the significant, robust association of total HDL-P with CIMT and CVD after adjusting for traditional risk factors, LDL-P and HDL-C. Whereas, a significant univariate association of HDL-C with CIMT as well as CVD attenuated after adjusting for atherogenic lipoproteins [103]. In a Japanese study of 889 study participants aged 40-79 years, Zaid and colleagues in Japan among reported a robust association of HDL-P with measures of carotid atherosclerosis after adjusting for traditional cardiovascular risk factors and HDL-C. In contrast, the significant association of HDL-C was attenuated after adjustment for HDL-P [104]. Other investigators, Kuller et al. in a MRFIT study [105], Harchaoui et al. in an EPIC-NORFOLK study [102], Duprez et al. in the SMART study [106], Parish et al. in the Heart Protection Study [75], and Mora et al. in the JUPITER trial [107] also reported the robust significant association of HDL-P with CHD/CVD (Table 1-6).

In summary, HDL-C level may reflect neither HDL-P concentrations nor HDL function (e.g. cholesterol efflux capacity). Moreover, HDL-P concentration rather than HDL-C may be an index of the capacity of HDL for reverse cholesterol transport pathway, which mediates the efflux of cholesterol from peripheral cells to the liver [108]. Reverse cholesterol transport is an

essential step in preventing plaque formation and progression of atherosclerosis, and thus prevention of CHD/CVD [108, 109]. Thus, HDL-P and the cholesterol efflux capacity of HDL appeared to be better measures of CHD/CVD risk than HDL-C [101]. Other potentially cardiovascular protective effects of HDL include anti-inflammatory, antioxidative, anti-thrombotic, anti-infectious, antiapoptotic, intercellular communication, and pro-vasodilatory capacities [11].

Table 1-6. Studies showing the association of HDL-P with atherosclerosis/CVD/CHD

Author/Year/ Location/ Study name	Populations	Type of Study/Follow-up	HDL-P categories/ primary outcome	Point estimate	Conclusion/ interpretation
Kuller 2007, USA MRFIT [105]	Community-based, N=428 age= 35–57 year, all men, ~93% Caucasians	Matched case-control study, 214 CHD deaths were matched with 214 controls with metabolic syndrome who did not die of CHD;	HDL-P as a continuous variable; Outcome: CHD death	CHD deaths= 214; A 1-SD increase in medium HDL-P: 0.70 (0.55, 0.90);	Medium HDL-P and not HDL-C was a significant predictor of CHD.
Harchaoui 2009 UK, EPIC-NORFOLK Cohort [102]	Community-based, N=2223, age= 45-79 years, ~64% men	Nested case-control study, 822 men, and women diagnosed with CAD matched with 1401 healthy participants; matching variable= sex age, enrollment time; FU= 6-10 years	Quartiles of HDL-P; Outcome: first CAD event	CAD events= 822; for HDL-P, 4^{th} vs. 1^{st} quartile: 0.50 (0.37, 0.66);	HDL-P concentration was independently associated with CAD risk.
Duprez 2009 USA, The SMART study [106]	Community-based, N=728, Median age = 49, ~80% men, ~62% Caucasians and 38% Blacks	Nested case-control study, 248 CVD patients, matched with 480 controls, matching variables= age, gender, date of randomization; FU= 5.1 years	Quartiles of HDL-P; Outcome: Non-fatal CHD events (MI, coronary revascularization), fatal CVD	Non-fatal CHD events= 248; for 4^{th} vs. 1^{st} quartile: Total HDL-P: 0.30 (0.13, 0.65); Large HDL-P: 0.37 (0.17, 0.83);	Lower levels of total and large HDL-P significantly associated with increased risk of CVD independent of other CVD risk factors.
Mackey 2012 USA, The MESA Study [103]	Community based, N= 5598, Age-45 to 84 years, ~47% men, 37.6% Caucasians, 27.7% Blacks, 22.7% Hispanics, and 12.1% Chinese	Prospective cohort study, FU= 6 years	HDL-P as a continuous variable; Outcome: CIMT, incident CHD (MI, CHD death, resuscitated cardiac arrest, definite or probable angina)	CIMT: β-est (95% CI) for a 1-SD increase in - HDL-C: 5.7 (-8.2, 19.7); - HDL-P: -25.2 (-37.6, -12.8); Incident CHD: Events = 227; HR (95% CI) for a 1-SD increase in - HDL-C: 1.12 (0.86, 1.46); - HDL-P= 0.68 (0.54, 0.85);	HDL-P was significantly inversely associated with CIMT and incident CHD, independent LDL-P, triglycerides, LDL-C and HDL-C.
Parish 2012, UK, MRC/BHF Heart Protection Study [75]	Community based, N= 20000	Prospective cohort study, FU= 5.3 years	HDL-P as a continuous variable; Outcome= Non-fatal MI or coronary deaths	Major occlusive coronary events = 2187; A 1-SD increase in HDL-P: 0.88 (0.84, 0.92); HDL-C: 0.92 (0.87, 0.96);	Equal association of HDL-P and HDL-C with major coronary events.
Mora 2013 USA, The JUPITER trial [107]	Community based, N= 5367, median age =60 years, ~64% men, ~71% Caucasian, 12%, Hispanic 13%	Prospective study, FU = 2 years	HDL-P as a continuous variable; Outcome= incident CVD (MI, stroke, hospitalization for unstable angina, arterial revascularization, cardiovascular death)	Incident CVD= 82; HR (95% CI) for a 1-SD increase in - HDL-C: 1.03 (0.75, 1.41); - HDL-P: 0.72 (0.53, 0.97);	Among subjects treated with Rosuvastatin, HDL-P had a stronger association with CVD than HDL-C

Table 1-6. Continued

Zaid 2015 Japan, The SESSA Study [104]	Community based, N=870, age=40-79 years, all men	Cross-sectional	Continuous and quartiles of HDL-P; Outcome= CIMT, Carotid plaque	For CIMT: β-est (95% CI) for a 1-SD increase in HDL-P: -22.8 (-37.9, -7.7), HDL-C: 1.0 (-12.6, 14.6); For carotid plaque: PR (95% CI) for a 1-SD increase in - HDL-P: -10.4 (-19.7, -1.1); - HDL-C: 3.7 (-4.5, 11.9);	HDL-P in comparison to HDL-C is more strongly associated with CIMT and carotid plaque.
Ditah 2015 Israel [110]	Community based, N=504, median age = ~62 years, ~65% men, 55% Arabs, 45% Jews	Cross-sectional	Tertiles of HDL-P; Outcome= CAC <100, ≥100	3rd vs. 1st tertile of - Total HDL-P: 0.42 (0.22, 0.79); - Med HDL-P: 0.36 (0.19, 0.69), - HDL-C: 0.59 (0.27, 1.29);	HDL-P and medium size HDL-P were more strongly associated with CAC than HDL-C.

Abbreviations: AU, Agatston' unit; CAC, Coronary artery calcification; CABG, coronary artery bypass graft; CAD, coronary arterial disease; CHD, coronary heart disease; CI, confidence interval; CIMT, carotid intima-media thickness; CV, cardiovascular; CVD, cardiovascular disease; CRP, C-reactive protein; FU, follow-up; FRS, Framingham Risk Score; HDL-C, high density lipoprotein cholesterol; HDL-P, high density lipoprotein particle; MI, myocardial Infarction; PCI, percutaneous coronary intervention; PTCA, percutaneous transluminal coronary angioplasty; SD, standard deviation;

1.5.4 Summary (Lipoproteins and Atherosclerosis/CHD/CVD) and Research Gap

The NMR-measured lipoprotein particles LDL-P, HDL-P, and VLDL-P are heterogeneous with respect to size and their cholesterol content. Lipoprotein particles have a differential association with CHD/CVD irrespective of their enzymatically cholesterol concentrations and suggested as alternative measures of CHD/CVD risk assessment [68]. We have previously reported differences in the distribution of lipoprotein particles between US White and Japanese in Japan [56]. We have also reported a higher prevalence of subclinical atherosclerosis among US White compared to Japanese in Japan [55]. We therefore hypothesize that variations in the distribution of lipoprotein particles between two populations may partially explain differences in the prevalence of subclinical atherosclerosis.

1.6 ALCOHOL

1.6.1 Introduction

All over the world, alcohol is one of the most commonly used recreational substances. The alcoholic drink contains ethanol which is a central nervous system depressant. Alcoholic beverages are usually categorized into three groups: beers (4%-6% alcohol by volume (ABV)), wines (9%–16% ABV), and spirits (20%-40% ABV). Sake is an example of rice wine, commonly consumed in Japan [111]. Alcohol in small doses can cause euphoria, reduced anxiety, and higher sociability; and in large doses can cause intoxication, stupor, and unconsciousness [111]. In the US, one "standard" drink contains ~12-14 grams of pure alcohol, which is found in 350 ml of regular beer containing ~5% ABV; 150 ml (one glass) of wine containing ~12% ABV; 45 ml of distilled spirits (rum, whiskey, vodka, gin etc.) containing ~40% ABV [111].

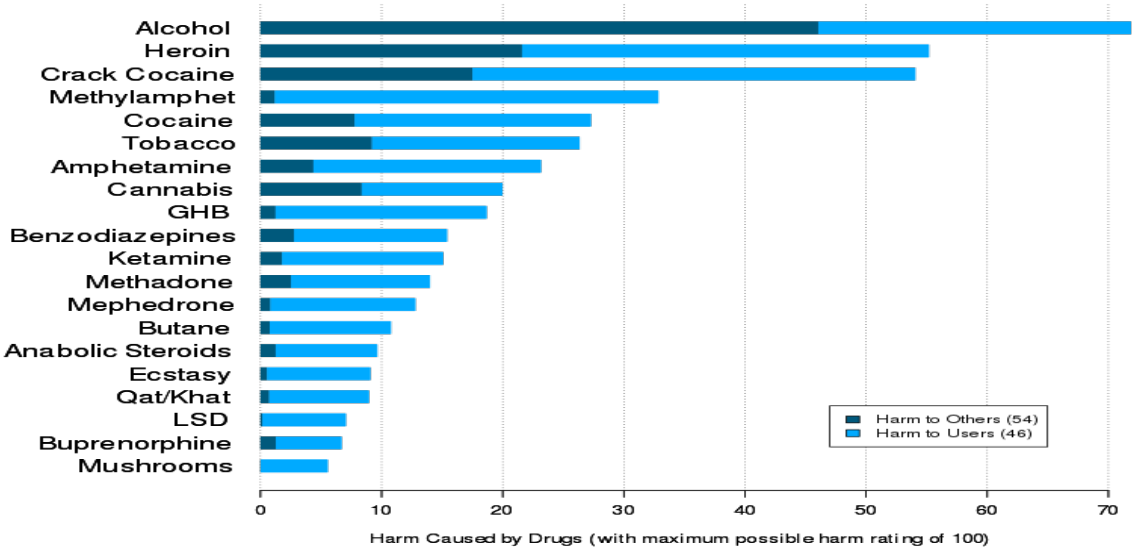

Figure 1-7. The levels of damage caused by recreational drugs

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Out of nine non-communicable disease targets to be attained by 2025, one of the important targets set by the WHO expert committee on non-communicable diseases was “At least 10% relative reduction in the harmful use of alcohol as appropriate, within the national context” [113]. Heavy alcohol consumption is associated with a risk of developing communicable diseases, non-communicable diseases, mental and behavioral disorders [113]. In 2012, approximately 6.0% of all deaths worldwide (~3,300,000 deaths) was attributed to alcohol consumption with >50% of these deaths being mainly from CVDs, diabetes, cancers, and liver cirrhosis [113].

Extensive research has established a direct causal link between high levels of alcohol consumption and the risk of the gastrointestinal tract, nasopharynx, and female breast cancers [114]. The International Agency for Research on Cancer has classified alcoholic drinks as a Group 1 carcinogen [115]. Nevertheless, the association between alcohol consumption and CHD/CVD is complicated due to differences in the patterns and types of alcohol consumed (which varies by country and population subgroups).

1.6.2 Alcohol and CHD

Epidemiological studies consistently reported a J-shaped or U-shaped association of alcohol with CHD. Light to moderate alcohol drinking (20-30 grams of alcohol per day) has a beneficial effect compared to no drinking or heavy drinking [116]. Several systematic reviews and meta-analysis have addressed the issue of the relationship between alcohol consumption and CHD and consistently reported a protective association of moderate alcohol consumption with CHD. Cleophas et al. in their meta-analysis of 12 major cohort studies and two case-control studies reported that alcohol consumption in the dose of 1-4 drinks/day was associated with a reduced

risk of mortality and CHD and this association did not vary based on the type of alcohol beverages [117]. Based on 28 major cohort studies, Corrao et al. reported that risk of CHD decreased with increasing alcohol intake from 0 to 20 g/day [RR (95% CI) = 0.80 (0.78, 0.83)]. This protective effect persisted up to an alcoholic consumption of 72 g/day [RR (95% CI) = 0.96 (0.92, 1.00)]. However, alcohol consumption ≥89 g/day was positively associated with CHD risk [RR (95% CI) = 1.05 (1.00, 1.11)] [118]. In another meta-analysis of 156 high-quality studies, Corrao et al. assessed alcohol consumption and the risk of 15 diseases. They observed a J-shaped association between alcohol consumption and CHD. Alcohol consumption of 20 g/day was associated with minimal risk of CHD. RR (95% CI) for 25 g/day, 50 g/day and 100 g/day for CHD were 0.81 (0.79, 0.83), 0.87 (0.84, 0.90), and 1.13 (1.06, 1.21) respectively [119]. Di Castelnuovo et al. in meta-analysis of 26 studies (case-control + cohort) reported that moderate beer consumption (up to 750 ml daily) was negatively associated with vascular diseases [RR (95% CI) = 0.78 (0.70, 0.86)]. They also reported a J-shaped association between various amounts of wine intake and vascular risk. The RR for wine drinkers compared to nondrinkers was 0.68 (95% CI, 0.59, 0.77). However, a statistically significant negative relationship was evident only up to intake of 150 mL of wine/day [120]. Roerecke et al. in their systematic review and meta-analysis of 44 observational studies (case-control + cohort studies) found that among men and women the lowest CHD mortality was seen at alcohol consumption of 31 g/day and 11 g/day respectively. The benefit persisted up to alcohol consumptions of 69 g/day [121]. When alcohol consumption was treated as categorical variable, among men a statistically significant cardioprotective relationship was seen for three standard drinks [RR (95% CI) = 0.78 (0.63, 0.97)], but not for one [(RR (95% CI) = 0.89 (0.79, 1.00)] or two drinks [RR (95% CI) = 0.86 (0.73,1.02)] per day. In women, for CHD mortality and CHD morbidity, a significant inverse

relationship was found for one and two standard drinks/day respectively [121]. Similarly, Ronksley et al. in a meta-analysis of 84 prospective observational studies reported that light to moderate alcohol consumption (1-2 drinks/day) was related to a lower risk of cardiovascular outcomes. In random effects models, among alcohol drinkers compared to nondrinkers, the RR (95% CI) for CVD mortality was 0.75 (0.70, 0.80); for incident CHD it was 0.71 (0.66, 0.77) (Figure 1-8); and for CHD mortality it was 0.75 (0.68, 0.81) (Figure 1-9) [116].

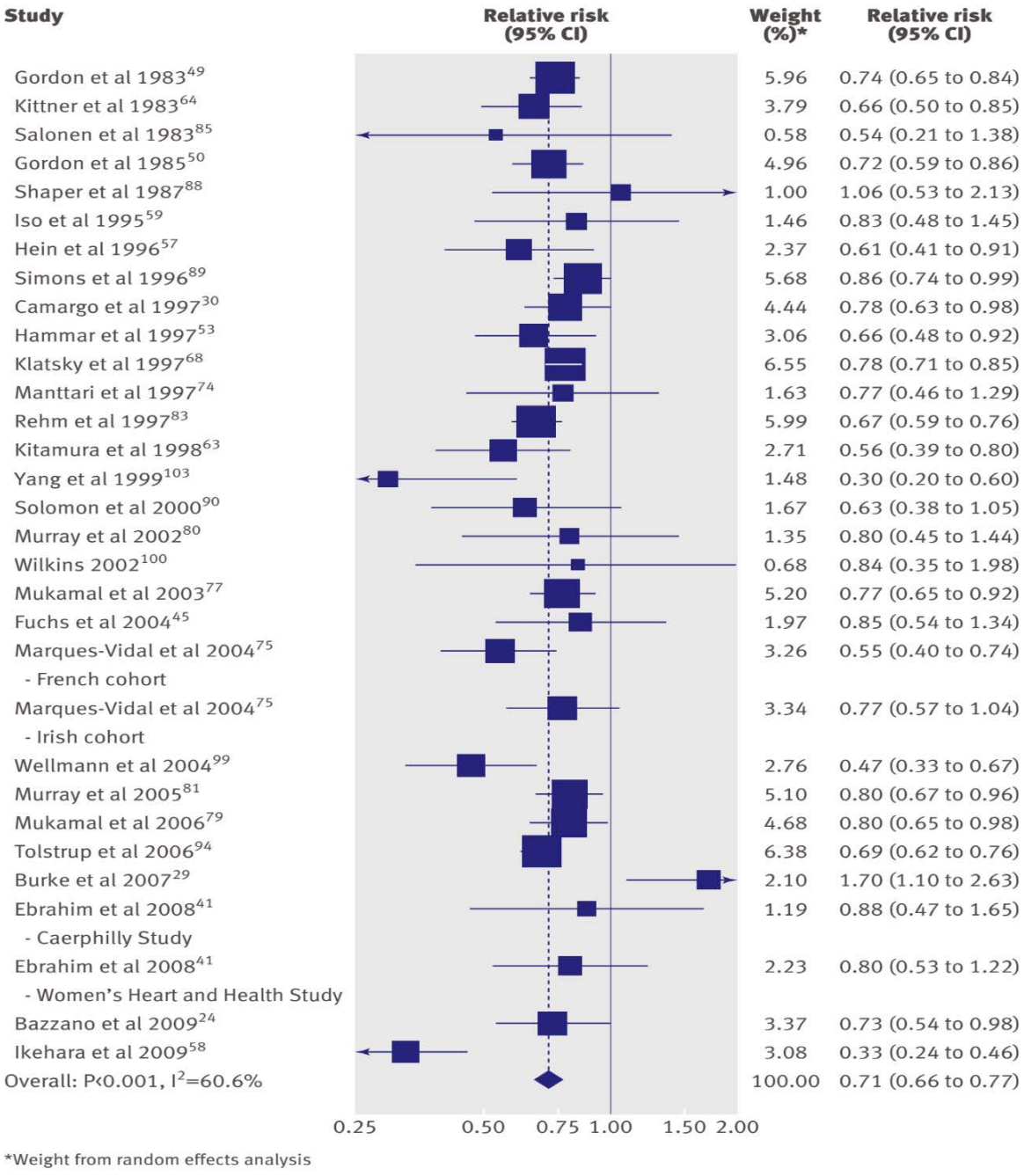

Figure 1-8. Association of ≤1drink/day (2.5 – 14.9 gram of alcohol/day) with incident coronary heart disease

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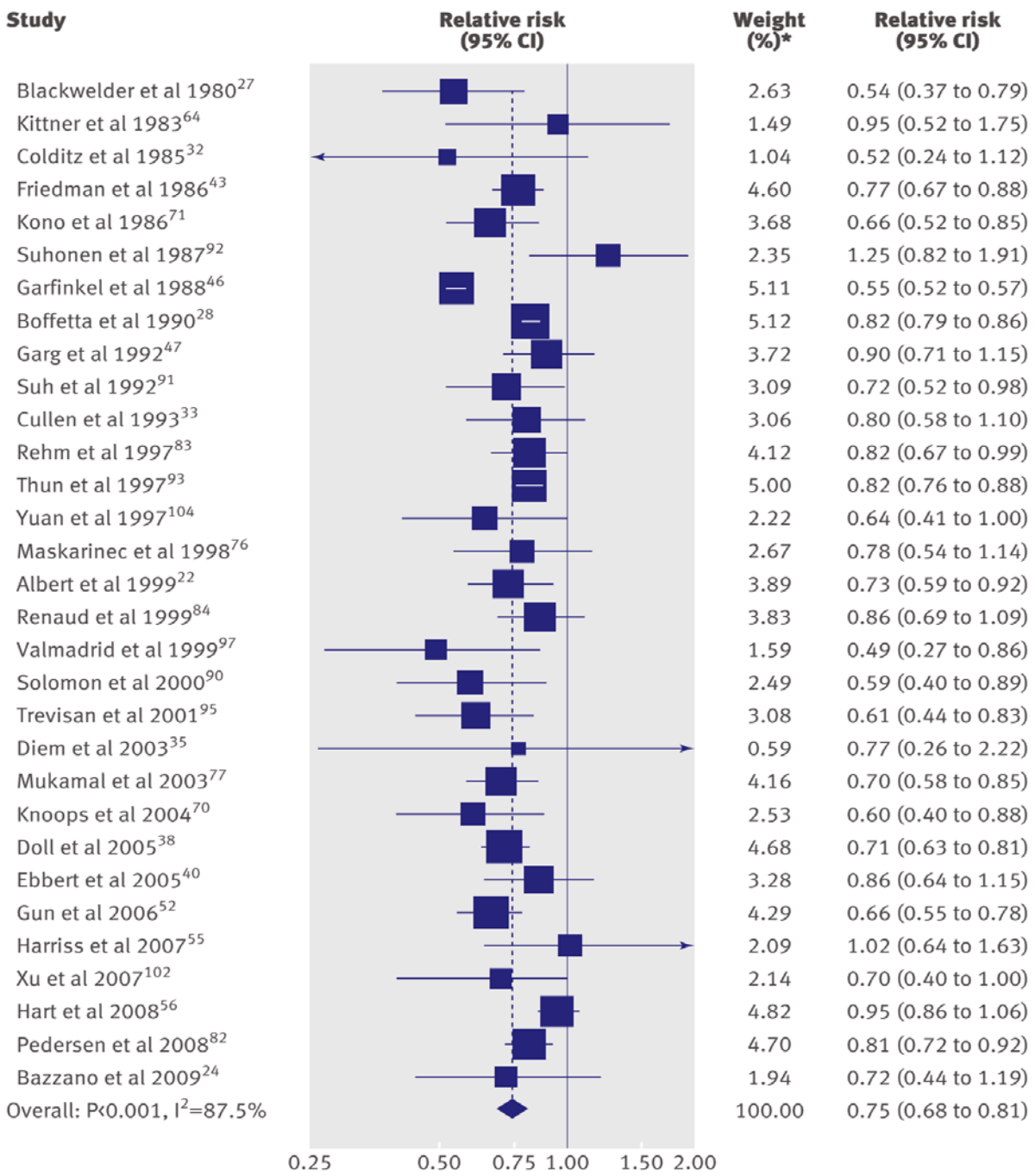

Figure 1-9. Association of ≤1drink/day (2.5 –14.9 grams of alcohol/day) with coronary heart disease mortality

1.6.2.1 Plausible mechanisms of action for the beneficial effect of light to moderate alcohol consumption with CVD outcomes

A limited number of studies have assessed the mechanism(s) by which alcohol can exert beneficial effects against the development of atherosclerosis/CHD/CVD. A meta-analysis of observational studies suggests that alcohol consumption mainly exerts a cardioprotective effect through its action on lipids and fibrinogen [122]. However, alcohol consumption also increases blood pressure and triglycerides, which are thought to be harmful to cardiovascular health [122].

i. Alcohol and HDL-C

Nearly, 40% to 60% of the cardioprotective effect of light-to-moderate alcohol consumption is thought to be mediated through an increase in HDL-C alone [123]. Large population-based studies have confirmed alcohol consumption to be related to beneficial levels of HDL-C [122, 124]. Rimm et al. in a meta-analysis of 42 observational studies reported that 30 g/day of alcohol consumption increased the concentrations of HDL-C by 3.99 mg/dl (95% CI = 3.25, 4.73) and of apolipoprotein A1 by 8.82 mg/dl (95% CI = 7.79, 9.86) [122]. In another meta-analysis of 63 studies, Brien et al. reported a significant dose-response between alcohol consumption and HDL-C levels: 12.5-29.9 g/day (1-2 drinks) of alcohol consumption increased HDL-C by 2.78 mg/dL (95% CI= 0.93, 4.60); 30-60 g/day (2-4 drinks), alcohol consumption increased HDL-C by 3.98 mg/dL (2.51, 5.45); and >60 g/day (≥5 drinks) of alcohol consumption increased HDL-C by 5.45 mg/dl (1.62, 9.28); (*p* for trend = 0.013) [125]. They also reported a significant positive association of alcohol consumption with apolipoprotein A1 [125]. In addition to large population-based studies, genetic association studies of the alcohol dehydrogenase type 3 (ADH3) polymorphism further strengthen the beneficial association of moderate alcohol consumption with CHD, which is mainly mediated by an increase in HDL-C [126]. Hines et al.

in a nested case–control study using data from the Physicians' Health Study reported that men who consumed at least one drink per day and were homozygous for the ADH3 genotype γ2 allele had the greatest reduction in the risk of MI [RR (95% CI) = 0.14 (0.04, 0.45)] [126]. These study participants also had the highest plasma HDL-C levels [126]. Although the potential mechanism by which alcohol increases HDL-C is not clear, plausible mechanisms are (i) an increased transport rate of lipoproteins; and (ii) increased lipoprotein lipase activity [127, 128].

ii. Alcohol and fibrinogen

Fibrinogen is commonly considered as a prothrombotic factor. It is also considered as an acute-phase reactant and often found with other inflammation-sensitive proteins [129]. Rimm et al. in a meta-analysis of 42 observational studies investigating the effects of alcohol consumption on blood lipids and hemostatic factors in people with no prior history of chronic disease and no history of alcohol dependence found that 30 g of ethanol per day was estimated to non-significantly decrease fibrinogen by 0.08 g/L (95% CI= -0.18, 0.33) [122]. In another meta-analysis of 63 studies, Brien et al. reported lower serum levels of fibrinogen among alcohol consumers compared to alcohol abstainers. The pooled mean difference (95% CI) in fibrinogen levels between alcohol consumers and no alcohol consumption was -0.20 (-0.29, -0.11) g/L [125]. The effect of alcohol consumption on fibrinogen is not very well understood. One of the potential mechanism is that alcohol appears to affect the conformation and stability of fibrinogen molecules [130].

iii. Effect of alcohol on blood pressure

In 1994, the International Study of Electrolyte Excretion and Blood Pressure (INTERSALT), a study designed to investigate the relationship between salt intake and blood pressure in 50

centers worldwide, presented data on alcohol and blood pressure [131]. Marmot and colleagues in the INTERSALT study reported that among men and women alcohol consumption >34 g/day was significantly related with higher systolic (SBP) as well as diastolic blood pressure (DBP). Among men, heavy drinkers compared with nondrinkers had 2.7/1.6 mmHg higher SBP/DBP. Among women, these numbers were 3.9/3.1 mmHg [131]. Although the effect of alcohol on blood pressure appears to be of small magnitude, a pooled analysis of 61 prospective cohort studies reported that among middle-aged study participants, an increase in SBP of 3.3 mmHg and of 2.0 mmHg in DBP was associated with nearly 12%, and 16% increased risk of fatal CHD [132].

Blood pressure differences were found to vary by the pattern of drinking. Compared to daily drinkers, episodic drinkers had higher blood pressure [131]. Similarly, Strangers and colleagues reported higher blood pressure [133] among binge drinkers compared to non-drinkers. Corrao and colleagues, in a meta-analysis of 156 observational studies showed that compared to non-drinkers the RR (95% CI) for essential hypertension for alcohol at doses of 25 g/day, 50 g/day, and 100 g/ day were 1.43 (1.33, 1.53), 2.04 (1.77, 2.35) and 4.15 (3.13, 5.52) respectively [119].

Suggested plausible mechanisms for an association between alcohol intake and blood pressure include (i) decreased endothelium-dependent nitric oxide production; (ii) increased secretion of intracellular calcium leading to increased vascular reactivity; (iii) enhanced sympathetic activity; (iv) increased cortisol level; and (v) increased activation of the renin-angiotensin-aldosterone system [134].

iv. Alcohol and triglycerides

Several epidemiological studies have reported a significant positive association between heavy alcohol consumption and elevated plasma triglycerides [135]. However, no significant increase in plasma triglycerides was seen in people consuming 1-3 standard drinks (a J-shaped relationship between alcohol consumption and plasma triglycerides) [135]. Suggested mechanisms [136] are (i) increased VLDL-P secretion; (ii) impaired lipolysis; and (iii) increased free fatty acid transport from adipose tissue to the liver.

Other less studied biomarkers associated with alcohol intake include LDL-C, CRP, tumor necrosis factor-α, interleukin 6, intracellular adhesion molecule 1, adiponectin, plasminogen, tissue type plasminogen activator antigen, von Willebrand factor, lipoprotein(a), etc., (Figure 1-10) [122, 124].

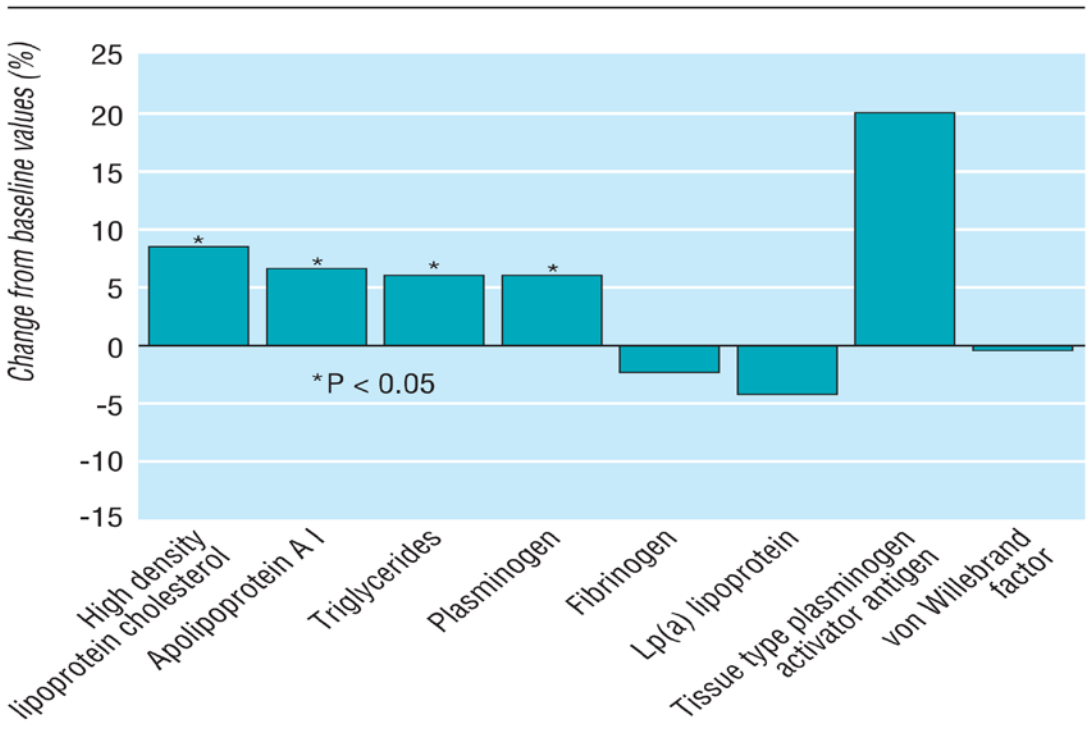

Figure 1-10. Percentage change in biomarkers associated with consumption of 30 grams of alcohol/day

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1.6.3 Alcohol and Atherosclerosis

Several observational studies have reported that light to moderate level of alcohol consumption lower the risk of CHD and other diseases associated with atherosclerosis [116]. Following the notion of a J-shaped association between alcohol consumption and risk of CHD, it was expected that light to moderate alcohol consumption would have an inverse association with several independent biomarkers of subclinical atherosclerosis: CIMT, CAC, calcified plaque, and aortic calcification. However, studies assessing the relationship between alcohol consumption and measures of sub-clinical atherosclerosis reported incongruous results (Table 1-7): no significant association [137, 138], a U or J-shaped association [139-142], and a dose-response association [143-145].

Mukamal and colleagues, in the Cardiovascular Health Study among 4247 study participants free of clinical CVD and aged ≥65 years, assessed the relationship between alcohol consumption and internal and common CIMT. One alcoholic drink was equivalent to 360 ml cans or bottles of beer or 180 ml glasses of wine, or 45 ml shot of liquor. Consumption of 1-6 drinks/week were associated with 0.07±0.04 mm lower composite IMT and alcohol consumption of ≥14 drinks/week was associated with 0.07±0.05 mm higher IMT than compared with abstainers (p for quadratic trend=0.02). This relationship was consistent across men and women, and internal and common carotid artery [140].

Pletcher and colleagues assessed the relationship between alcohol consumption and CAC among 3042 middle-aged men and women aged 33-45 years. One drink of alcohol was equivalent to 17.24 ml of ethanol. One drink of beer, wine, or liquor contained 16.7 ml, 17.0 ml, or 19.1 ml of ethanol respectively and was used in calculating total ethanol consumption per week. The total number of drinks/week was calculated as total ethanol consumption per week

divided by 17.24 ml. Consuming 7-13 [OR (95% CI) = 1.5 (1.0, 2.3)] and ≥14 alcoholic drinks/week [OR (95% CI) = 2.0 (1.3, 3.2)] as well as binge drinking [OR (95% CI) = 1.7 (1.2, 2.3)] was positively associated with CAC. Results of this study therefore suggest that the proatherogenic effects of alcohol in middle-aged study participants might counterbalance any possible benefits of alcohol consumption [145].

In the Netherlands, Vilegenthart and colleagues in the Rotterdam Coronary Calcification Study among 1795 asymptomatic men and women with mean age of 71 years, assessed the association between alcohol consumption and extensive CAC (CAC >400). One alcoholic drink was equivalent to "250 ml of beer (containing 12.5 ml of alcohol), 100 ml of wine (12 ml of alcohol), 75 ml of a moderately strong, sherry-type beverage (12 ml of alcohol), or 35 mL of liquor (12.3 ml of alcohol)". The OR (95% CI) for extensive CAC was 0.60 (0.44, 0.82) for ≤1drink/day, 0.51 (0.35, 0.76) for 1-2 drinks/day and 0.90 (0.62,1.29) for ≥2 drinks/day [141].

Janszky and colleagues in the Stockholm Female Coronary Risk Angiographic study in Sweden among hospital-based 93 females aged <66 years who have survived hospitalization for acute MI or unstable angina pectoris, assessed the relationship between alcohol consumption and atherosclerosis progression (measured by coronary artery luminal narrowing). Alcohol consumption categories were: nondrinkers (0 grams of alcohol per day), light drinkers (0.1-5 grams of alcohol/day) and moderate drinkers (>5 grams of alcohol per day). Over the period of approximately 3 years, coronary atherosclerosis progression among abstainers was 0.138 mm (95% CI = 0.027, 0.249), among light drinkers it was 0.137 mm (95% CI = 0.057, 0.217) and among moderate drinkers it was -0.054 mm (95% CI= -0.154, 0.047). Thus, moderate alcohol consumption may have slowed down the progression of atherosclerosis [146].

Tofferi and colleagues in the Prospective Army Coronary Calcium Project among 731 active-duty US Army personnel aged 39-45 years of age without known CAD, assessed the relationship between alcohol consumption and subclinical coronary atherosclerotic plaque burden. Alcohol consumption categories were: non-drinker (0 drink per day), light drinker (<1 drinks/day), moderate drinker (1-2 drinks/day), and heavy drinker (>2 drinks/day). There was no relationship between alcohol consumption and CAC plaque. The OR (95% CI) for light, moderate and heavy drinkers were [1.02 (0.64, 1.63)], [1.13 (0.59, 2.15)] and 1.26 (0.69, 2.59)] respectively [147].

Okamura and colleagues in the ERA-JUMP study of 245 men aged 40-49 without clinical CVD assessed the relationship between alcohol consumption and CAC. One drink of alcohol consumption was equivalent to 23g alcohol consumption/day. Investigators reported a J-shaped association between alcohol consumption and CAC. The OR (95% CI) for alcohol consumption of 1-22 g/day, 23-45 g/day, 46-68 g/day and >68 g/day compared to nondrinkers were 0.58 (0.23, 1.43), 1.21 (0.44, 3.31), 1.42 (0.47, 4.33) and 4.20 (1.16, 15.2) respectively. Whether a significant trend existed was not reported [148].

Schminke and colleagues, using data from a cross-sectional survey in northeastern Germany among 1230 men and 1190 women, assessed the relationship between alcohol consumption and CIMT. In men, CIMT and alcohol consumption had a J-shaped association, with the highest CIMT values among abstainers and the lowest at an alcohol consumption of 61 to 80 g/d. In women, there was no relationship between alcohol consumption and CIMT [139].

Ellison and colleagues in the NHLBI Family Heart Study among 3116 participants aged ≥30 years assessed the relationship between alcohol consumption and calcified plaque in the coronary artery and infrarenal abdominal aorta. One drink of alcohol was equivalent to a 360 ml

bottle or can of beer, a 120 ml glass of table wine, or a 30-45 ml shot of liquor or spirits. There was no significant association between alcohol consumption and calcified atherosclerotic plaque in the coronary arteries or in the aorta which may suggest that alcohol may affect the risk of cardiovascular events through mechanisms other than calcification of atherosclerotic plaque [138]. In men, compared to nondrinker, the ORs (95% CI) for CAC >100 in consumers of 1-3, 4-7, 8-14, and >14 drinks/week were 0.8 (0.4,1.3), 1.1 (0.6,1.9), 0.9 (0.5, 1.5), and 1.5 (0.9, 2.5), respectively; among women the ORs were 0.9 (0.5, 1.6), 1.3 (0.8, 2.3), 1.3 (0.7, 2.2), and 2.1 (0.8, 5.9) respectively [138].

McClelland and colleagues in the MESA study among 6814 study participants aged 45-84 years assessed the relation between alcohol consumption and the prevalence, incidence, and progression of CAC. They assumed that the alcoholic content of drink was assuming 9.3%, 3.6%, and 14.2% for wine, beer, and liquor, respectively. There was no evidence of any protective or a J-shaped association of alcohol with CAC prevalence, incidence, or CAC progression. The heavy alcohol consumption was positively associated with accumulation of CAC [β-coeff (95% CI) = 24.9 (3.5, 46.3)] and CAC progression [β-coeff (95% CI) = 14.2 (2.8, 25.6)]. Thus, the cardioprotective effect of moderate alcohol consumption may not be mediated through reduced CAC accumulation [137].

Rantakomi and colleagues in the FinDrink study in Finland among 751 middle aged men, assessed the relationship between ≥6 drinks of alcohol consumption at one session and the progression of mean change in maximum thickness of IMT, the mean change in thickness, and the change in plaque height. One drink of alcohol was equivalent to 12-14 grams of alcohol consumption. Over a follow-up period of 11 years, among binge drinkers vs no binge drinkers: the mean change in mean IMT was 0.228 mm vs. 0.206 mm (*p*-value >0.05); the mean change

in maximum CIMT was 0.387 mm vs. 0.332 (p-value <0.05); and the mean change in plaque height was 0.248 vs. 0.192 (p-value <0.05) [149].

Jiang and colleagues, using data from the Guangzhou Biobank Cohort Study among 19624 men and women in China, assessed the relationship between alcohol consumption and aortic calcification (presence and severity). Alcohol consumption categories among men were: never drinkers, occasional drinkers (<1 drink/week), moderate drinkers (1 drink /week with ≤210 gm of ethanol), and excessive drinkers >210 gm of ethanol/week; among women, the categories were: never drinkers, occasional drinkers (<1 drink/week), moderate drinkers (1 drink /week with ≤140 gm of ethanol), and excessive drinkers >140 gm of ethanol/week. Among men, the OR (95% CI) for the presence of aortic calcification for occasional drinkers it was 1.00 (0.88, 1.15), for moderate drinkers it was 1.13 (0.96, 1.32), and for excessive drinkers it was 1.49 (1.21, 1.83); (p for trend = 0.001). The OR (95% CI) for aortic calcification severity for occasional drinkers was 0.97 (0.85, 1.10), for moderate drinkers it was 1.05 (0.90, 1.22), and for excessive drinkers it was 1.33 (1.09, 1.62); (p for trend = 0.03). Among women, there was no significant association between alcohol consumption and aortic calcification [143].

Tanaka and colleagues in the Circulatory Risk in Communities Study among 404 males aged 30-79 years, assessed the relationship between alcohol consumption and % flow-mediated dilation (FMD) of brachial artery as a measure of endothelial function. One drink of alcohol was equivalent to 22 grams of alcohol per day. Heavy alcohol consumption was positively associated with endothelial dysfunction with no protective effect from moderate alcohol consumption. Compared with nondrinkers, the ORs (95% CI) of low %FMD (<5.3%) for former, light (one drink/day), moderate (two drinks/day ethanol), and heavy (≥two drinks/day) drinkers were 1.76 (0.69, 4.50), 0.86 (0.42, 1.76), 0.98 (0.45, 2.12), and 2.39 (1.15, 4.95) respectively [150].

Thus, in contrary to a J-shaped association of alcohol consumption with CHD/CVD, inconsistent relationships have been reported between alcohol consumption and several measures of subclinical atherosclerosis though the reason for this is not clear. Alcohol can affect different stages of atherosclerosis development. Heavy alcohol consumption can facilitate increased accumulation of cholesterol into the arterial wall, maturation of lesions by inflammatory cells, associated dysfunction of endothelial cells, and thrombosis at sites of arterial injury [151]. Researchers have been studying the association of alcohol consumption with lipids (HDL-C, LDL-C, and triglycerides), blood pressure, hemostatic factors, and inflammatory markers to gain insight into the underlying mechanism underlying the cardioprotective effect of light to moderate alcohol consumption on the risk of CHD/CVD. It is also important to investigate the relationship between alcohol consumption and different measures of subclinical atherosclerosis because it may help further clarify the mechanisms underlying the association between alcohol and CHD/CVD.

Table 1-7. Observational studies assessing the relationship between alcohol consumption and atherosclerosis

Author, year, name of the study	Study population	Predictor (alcohol consumption)	Point estimate (95% CI)	Conclusion/ interpretation
Mukamal 2003 US, The CHS [140]	Community based, N=4247 age≥65 years, ~55% men, ~85% Caucasians, ~15% Blacks	Drinks/week: None (reference), Former, < 1 drink, 1-6 drinks, 7-13 drinks, ≥14 drinks; Outcome- CIMT	Compared to abstainers: 1-6 drinks/week had 0.07 ± 0.04 mm lower composite IMT; ≥14 drinks/ week had 0.07 ± 0.05 mm higher IMT;	A J-shaped association between alcohol consumption and carotid atherosclerosis.
Pletcher 2004 USA, The CARDIA Study [145]	Community based, N=3042, age= 33 - 45 years, ~ 45% men, ~55% Caucasians, ~45% Blacks	Drinks /week: 0 (reference), 1-6, 7-13 or ≥14; Binge drinking= ≥5 drinks at single occasion Outcome: CAC= 0, >0	Compared to abstainers: 7-13 drinks/week: 1.5 (1.0, 2.3); ≥14 drinks/week: 2.0 (1.3, 3.2); Binge drinking: 1.7 (1.2, 2.3);	Among black men dose response relationship between alcohol consumption and CAC. Binge drinking was significantly associated with CAC.
Vilegenthart 2004 Netherland, The Rotterdam Coronary Calcification Study [141]	Community based, N=1795, mean age= 71±5.7 years, ~42.5% men	Drinks/day: 0 drink(reference), ≤1 drink, >1-2 drink, >2 drink Outcome: CAC >400	Compared to 0 drinks/day: ≤1 drink/day: 0.60 (0.44, 0.82); 1-2 drinks/day: 0.5 (0.35, 0.76); >2 drinks/day: 0.90 (0.62, 1.29);	Alcohol consumption of two drinks or fewer per day has a strong inverse association with the amount of coronary calcification.
Janszky et al. 2004, Sweden Stockholm Female Coronary Risk Angiographic study [146]	Hospital based, N=93, age<66 years, all women	Alcohol consumption (grams/day): 0 grams, light drinkers (0.1-5 grams), moderate drinkers (>5 grams); Outcome: Coronary artery luminal narrowing;	Coronary atherosclerosis progression - among abstainers was of 0.138 mm (0.027, 0.249); - among light drinkers was of 0.137 mm (0.057, 0.217); - among moderate drinkers was of -0.054 mm (- 0.154, 0.047);	Inverse association between moderate alcohol consumption and atherosclerosis progression
Tofferi 2004 US, The PACC study [147]	N=725 army personnel, mean age=42 ± 2.0 years, 83% men, ~71% Caucasians	Drinks/day: Non-drinker (reference), < 1 drinks, 1-2 drinks, > 2 drinks; Outcome: CAC=0, > 0;	Compared to non-drinker: - Light drinkers: 1.02 (0.64, 1.63); - Moderate drinkers: 1.13 (0.59, 2.15); - Heavy drinkers: 1.3 (0.7, 2.6);	No relationship between Alcohol consumption and CAC.
Okamura T 2005 Japan, The ERA-JUMP Study [148]	Community based, N=250, age= 40-49 years, all men	Alcohol consumption (grams/day): never (reference), 1-22 grams, 23-45 grams, 46-68 grams, ≥69 grams; Outcome: CAC=0, > 0; CAC<10, ≥ 10;	Compared to non-drinkers: - Light drinker: 0.58 (0.23, 1.43); - Moderate drinker: 1.21 (0.44, 3.31); - Heavy drinker: 1.42 (0.47, 4.33); - Extremely heavy drinker: 4.20 (1.16, 15.20);	Non-significant J-shaped association between alcohol consumption and CAC.
Schminke 2005 Germany, The SHIP study [139]	Community based, N=2420, age= 45-79 years, 50% men	Alcohol consumption (grams/day): Men- Never drinker (reference), former drinkers, 1-20 grams, 21-40 grams, 41-60 grams, 61-80 grams, >80 grams; Women- Never drinker (reference), former drinkers, 1-5 grams, 6-10 grams, 11-15 grams, 16-20	In men, 0.009 unit decrease in IMT (p value <0.05) with each increase in alcohol consumption by 20 grams/day till 80 grams/day;	A J-shaped relationship between alcohol consumption and CIMT in men. No association seen in women.

Table 1-7. Continued

		grams, >20 grams; Outcome: CIMT		
Ellison 2006 US, NHLBI Family Heart Study [138]	Community-based, N=3116, age ≥30 years, 41% males, 81% Caucasians, 19% Blacks	Drinks per week= 0 (reference), 1 to 3, 4 to 7, 8 to 14 or > 14 drinks per week. Outcome: CAC=0, >0, >100, ≥160, >300, >400) Aortic calcium: > 0, >1000, >3000	Men: compared to non-drinker, for CAC >100, Men: 1-3 alcohol drinks/week: 0.8 (0.4, 1.3); 4-7 drinks/week: 1.1 (0.6, 1.9); 8-14 drinks/week: 0.9 (0.5, 1.5); >14 drinks/week: 1.5 (0.9, 2.5); Women: 1-3 alcohol drinks/week: 0.9 (0.5, 1.6); 4-7 drinks/week: 1.3 (0.8, 2.3); 8-14 drinks/week: 1.3 (0.7, 2.2); >14 drinks/week: 2.1 (0.8, 5.9);	Alcohol consumption was not associated with calcified atherosclerotic plaque in the coronary arteries or in the aorta.
McClelland 2008 US, The MESA study [137]	Community based, N=6814, age=45-84 years, ~47% men, Prospective cohort study, FU= 2-4 years, ~38% Caucasians, ~27% Blacks, ~22% Hispanic, ~12% Chinese	Drinks per day- Never drinker (reference), former drinker, <1 drink, 1-2 drinks, >2 drinks; 1 drink= 10 g alcohol; Outcome: Prevalence, incidence and annual progression of CAC, CAC=0, >0	Heavy alcohol consumption: - Accumulation of CAC [β-coeff (95% CI): 24.9 (3.5, 46.3)]; - CAC progression [β-coeff (95% CI) = 14.2(2.8, 25.6)];	No evidence of a J-shaped association of alcohol with CAC prevalence, incidence, or progression. Consumption of >20g of hard liquor was significantly associated with higher CAC at baseline and CAC progression.
Rantakomi 2008 Finland, The FinDrink Study [149]	Community-based, N=751, age = ~50 years, all men, Prospective cohort study, FU=11 years	Drinks/day: <6 drinks (reference)vs ≥6 drinks; Outcome: Progression of mean change in maximum thickness of IMT, mean change in thickness, change in plaque height	For binge drinking: - Mean change in IMT was 0.228 mm; (p-value >0.05). - Mean change in maximum IMT was 0.387 mm; (p-value <0.05). - Mean change in plaque height was 0.248; (p-value <0.05).	Binge drinking was associated with an increased risk of atherosclerosis progression of mean change in maximum thickness of IMT, mean change in thickness, change in plaque height.
Jiang 2013 China, The Guangzhou Biobank Cohort Study [143]	Community-based, N=19624, age= 50-85 years, 21% men	Drink/week: Men- never drinkers (reference), occasional drinkers (less than one drink per week), moderate drinkers (once per week with less than or equal to 210 gm of ethanol), excessive drinkers- more than 210 gm of ethanol. Women - never drinkers (reference), occasional drinkers (less than one drink per week), moderate drinkers (once per week with less than or equal to 140 gm of ethanol), excessive drinkers- more than 140 gm of ethanol. Outcome: Aortic calcification (present or	Men: for aortic calcification presence - Occasional drinker: 1.00 (0.88, 1.15); - Moderate drinker:1.13 (0.96, 1.32); - Excessive drinker = 1.49 (1.21, 1.83), (p for trend= 0.001); for aortic calcification severity - Occasional drinker: 0.97 (0.85, 1.10); - Moderate drinker: 1.05 (0.90, 1.22); - Excessive drinker: 1.33 (1.09, 1.62); (p for trend= 0.03). Women: for aortic calcification presence - Occasional drinker: 1.11 (1.01, 1.23); - Moderate drinker: 0.96 (0.79,	Significant association between alcohol drinking and AAC in men with a clear dose-response relation between frequency or quantity of drinking and presence as well as severity of AAC.

Table 1-7. Continued

		absent), Severity of aortic calcification: Grade 0 - no aortic arch calcification, Grade 1- length of calcification plaque <10 mm and width of calcification plaque <4 mm, Grade 2- length of calcification plaque ≥10 mm or width of calcification plaque ≥4 mm	1.17); - Excessive drinker: 0.85 (0.41, 1.76); (p for trend= 0.35). for aortic calcification severity - Occasional drinker: 1.06 (0.96, 1.17); - Moderate drinker: 0.9 (0.8, 1.15); - Excessive drinker: 0.90 (0.45, 1.81); (p for trend= 0.74).	
Tanaka 2016 Japan, The Circulatory Risk in Communities study [150]	Community based, N= 404, age=30-79 years, all men	Alcohol consumption (grams/day): never, former, light (<23 gm), moderate (23.0-45.9 gm), heavy (≥46.0 gm); Outcome: %Flow mediated dilation (FMD) of brachial artery (measure of endothelial function)	Low %FMD=5.3%, compared to never drinker: - Light drinker: 0.9 (0.4, 1.8); - Moderate drinker: 0.9 (0.5, 2.1); - Heavy drinkers: 2.39 (1.15, 4.95);	Heavy alcohol consumption as an independent risk factor of endothelial dysfunction with no protective effect from moderate alcohol consumption.
Abbreviations: AU, Agatston' unit; CAC, coronary artery calcification; CABG, coronary artery bypass graft; CAD, coronary arterial disease; CHD, coronary heart disease; CI, confidence interval; CIMT, carotid intima-media thickness; CV, cardiovascular; CVD, cardiovascular disease; CRP, C-reactive protein; FMD, flow-mediated dilation; FU, follow-up; FRS, Framingham Risk Score; HDL-C, high density lipoprotein cholesterol; HDL-P, high density lipoprotein particle; MI, Myocardial Infarction; PCI, percutaneous coronary intervention; PTCA, percutaneous transluminal coronary angioplasty; SD, standard deviation;				

1.6.4 Potential sources of biases in the relationship between alcohol and atherosclerosis/CHD

Several methodological issues should be considered while interpreting the results of studies assessing the relationship between alcohol consumption and atherosclerosis/CHD (Table 1-8). Residual confounding, choice of reference category, the definition used to define different alcohol consumption categories, type, and pattern of drinking, and within-person variations in the alcohol consumption over time could distort the actual magnitude of the association between alcohol consumption and atherosclerosis/CHD [123].

Table 1-8. Potential sources of bias in epidemiological studies of the relationship between alcohol consumption and the risk of vascular disease

Source of bias	Description
Confounding by type of drink or pattern of drinking	If either the type of drink consumed (eg, beer, wine, or spirits) or the pattern of drinking (eg, with/without meals, regular/episodic) have effects on risk independently of amount consumed (and if these characteristics vary with amount consumed), then these factors will confound the observed relationship between amount of alcohol consumed and risk.
Confounding by socio-economic and lifestyle characteristics	Differences in socio-economic and lifestyle characteristics between different drinking groups causes confounding of the true relationship between alcohol consumption and vascular risk. Even if attempts are made to adjust for these characteristics, some residual confounding will still generally occur.
Choice of reference group	Use of nondrinkers as the reference group with which to compare different levels of active drinking could lead to misleading results if the group includes ex-drinkers, particularly those who gave up because of ill health (see also "reverse causality bias").
Reverse causality bias	A previous diagnosis of vascular disease might cause a change (typically a reduction) in an individual's alcohol consumption, leading to the subsequent high incidence rates among such people being incorrectly attributed to the new level of drinking.
Recall error/misclassification	Errors in the reporting of alcohol consumption can alter the magnitude and even direction of true risk-relationships with alcohol intake. For instance, cases in case-control studies might systematically under-report their previous alcohol intake.
Within-person variation	In prospective cohort studies, variations in an individual's alcohol intake over time can distort the risk-relationship between *average* alcohol intake during the study and risk, when baseline measures of alcohol intake are used in analyses.
Study design/publication bias	Case-control studies may be more susceptible to biases in exposure recall than cohort studies and also have the difficultly of finding an appropriate control group. Alcohol-disease association studies may also be more likely to be submitted for publication (and accepted) if it shows a striking result, as opposed to small studies with less striking results.

Derived from Emberson et al. Vascular Health and Risk Management 2006:2(3) 239–249 [123]. Copyright © 2006 Dove Medical Press Limited

1.7 N-3 POLYUNSATURATED FATTY ACIDS AND CHD/CVD

1.7.1 Structure of fatty acids

Fatty acids have a hydrocarbon chain with the absence (saturated fatty acids) or presence (unsaturated fatty acid) of a double bond between two carbon molecules of hydrocarbon chain [152]. This hydrocarbon chain has a methyl group at one end and a carboxyl group at the other

end. Unsaturated fatty acids have either one double bond (monounsaturated fatty acids) or two or more (polyunsaturated fatty acids (PUFAs)) double bonds within hydrocarbon chain [152]. Depending on the position of the double bond within hydrocarbon chain from methyl group carbon ("ω" or "n"), polyunsaturated fatty acids are further divided into n-3 and n-6 fatty acids with the first double bond between the third and fourth carbons, and sixth and seventh carbon from the methyl group carbon respectively. N-3 fatty acids playing an important role in the human body are α-linolenic acid (ALA; 18:3), eicosapentaenoic acids (EPA (20:5)) and docosahexaenoic acids (DHA (22:6)). Based on the length of the hydrocarbon chain, ALA is known as 'intermediate-chain n-3 polyunsaturated fatty acids, and EPA and DHA are known as long chain- n-3 polyunsaturated fatty acids (LCn-3PUFAs). ALA cannot be synthesized in the human body (known as 'essential fatty acid') and is mainly derived from plant sources such as canola oil, flaxseed oil, walnuts, etc. whereas, EPA and DHA can be synthesized in the human body from ALA (known as 'non-essential fatty acids') via series of biochemical reactions [152]. However, the primary sources of LCn-3PUFAs are fish, fish oils, and specialty egg/dairy products. Therefore, LCn-3PUFAs are also known as 'marine derived n-3 fatty acids'.

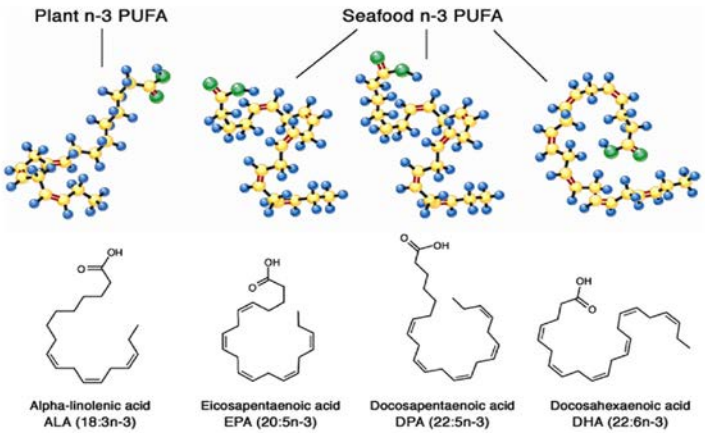

Figure 1-11. Structure of n-3 polyunsaturated fatty acids

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1.7.2 Properties of Long-Chain n-3 Polyunsaturated Fatty Acids

In vitro studies, animal experiments, observational studies, and RCTs in humans have demonstrated that LCn-3PUFAs have several cardioprotective effects including reduction in triglycerides [154], blood pressure [155], resting heart rate [156], systemic vascular resistance [157], and arrhythmias [158], inhibition of platelets [159] and inflammatory metabolites [160], improvement in endothelial dysfunction [161], myocardial efficiency [162], and left ventricular diastolic filling [162].

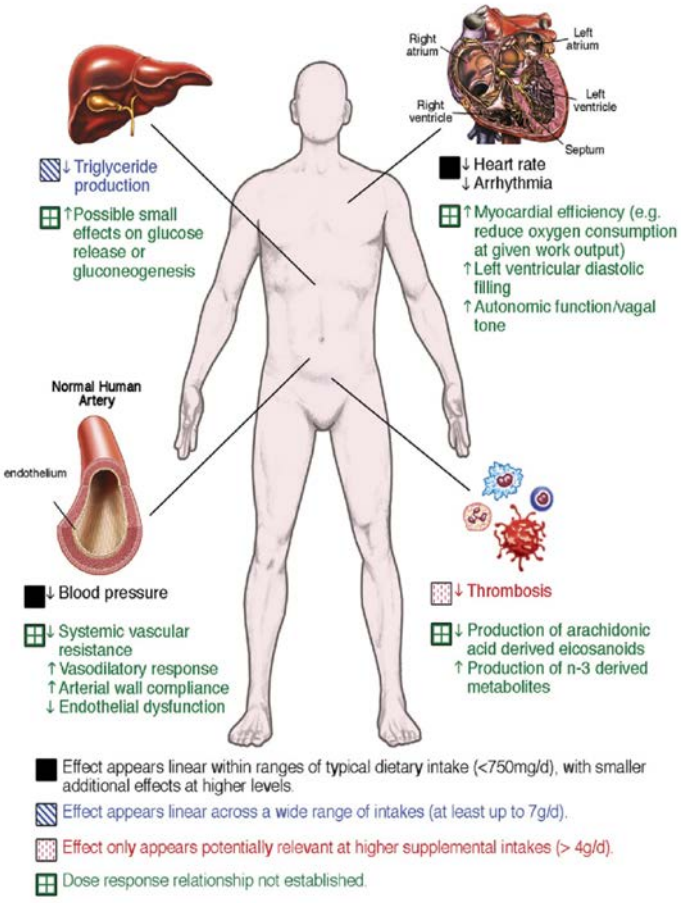

Figure 1-12. Physiological functions of n-3 polyunsaturated fatty acids in human body

1.7.3 N-3 Polyunsaturated Fatty Acids and CVD events

Several prospective observational studies (Table 1-10) and randomized controlled trials (RCTs) (Table 1-9) have been conducted to assess the effects of dietary fish or LCn-3PUFAs intake on CVD outcomes. The results from secondary prevention trials carried out in the earlier period during the 1980s through the early 2000s and meta-analyses of observational studies showed a cardioprotective effect of LCn-3PUFA. On the contrary, recent RCTs (except the Japan EPA Lipid Intervention Study) have failed to show any significant association of LCn-3PUFA with CHD/CVD (Table 1-9). Several possible explanations [163] for the inconsistent results may include:

i. Studies were of small samples and lower statistical power,

ii. Low event rates,

iii. High background fish/seafood intakes,

iv. Suboptimal omega-3 fatty acids dosage,

v. Shorter supplementation duration,

vi. Age at study enrollment,

vii. Short length of follow-up,

viii. Concurrent standard of care for CVD treatment

Selected systematic reviews and meta-analyses of RCTs conducted to synthesize the evidence assessing the effect of omega-3 fatty acids with different CVD outcomes are shown in Figure 1-12 [164, 165]. Rizos and colleagues in their systematic review and meta-analysis of 20 RCTs which mainly enrolled patient at high risk for CVD or patient with prevalent CHD, reported a significant inverse association between omega-3 fatty acid supplementation and CHD death [RR

(95% CI) = 0.91 (0.85, 0.98). This finding was mainly driven by the findings of RCTs conducted before 2002 compared to recent RCTs.

In the systematic review and meta-analysis of 18 RCTs, Dominic and colleagues reported a non-significant lowering of CHD risk with EPA+DHA supplementation [summary RR (95% CI) = 0.94 (0.85, 1.05)]. However, in subgroup analyses they reported a statistically significant inverse association between EPA+ DHA supplementation and CHD risk among participants with triglycerides >150 mg/dl levels [summary RR (95% CI) = 0.84 (0.72, 0.98)] and LDL-C >130 mg/dl [(summary RR (95% CI) = 0.86 (0.76, 0.98)] of data from RCTs [165].

Similarly, Dominic and colleagues in a meta-analysis of 16 prospective cohort studies showed a statistically significant association for higher consumption of EPA+DHA and risk of any CHD event [summary RR (95% CI) = 0.82 (0.74, 0.92)] [165]. Del Gobbo and colleagues, pooling data from19 studies from 16 countries with 45,637 participants and 7,973 total CHD, 2,781 fatal CHD, and 7,157 nonfatal MI events, reported that a 1-SD increase in the LCn-3PUFAs biomarkers [ALA, docosapentaenoic acid (DPA), and DHA] was associated with a reduced risk of fatal CHD: [RR (95% CI) = 0.91 (0.84, 0.98)] for ALA; [RR (95% CI) = 0.90 (0.85, 0.96)] for DPA; and [RR (95% CI) = 0.90 (0.84, 0.96) for DHA. Total LCn-3PUFAs (EPA+DPA+DHA) was associated with an 11% reduced risk of fatal CHD [RR (95% CI) = 0.89 (0.84, 0.95)] [166]. Similarly, a pooled analysis of two prospective cohort studies [the Nurses' Health Study (n = 83,349) and the Health Professionals Follow-up Study (n = 42,884)], demonstrated that consumption of total PUFAs was associated with lower total mortality when comparing the 5th with the 1st quintile of total PUFAs consumption [total PUFAs: HR (95% CI) = 0.95 (0.91, 0.99)], [LCn-3PUFAs: HR (95% CI) = 0.96 (0.93, 1.00)] [167]. He and colleagues in a meta-analysis of 13 cohort studies reported that each 20 g/d increase in fish consumption

was associated with a 7% lower risk of CHD mortality [RR (95% CI) = 0.93 (0.87, 0.99] [168]. Similarly, in a meta-analysis of 19 observational studies (14 cohort + 5 case-control studies), Whelton and colleagues reported a lower risk of fatal CHD [RR (95% CI)= 0.83 (0.76, 0.90)] and total CHD [RR (95% CI) = 0.86 (0.81, 0.92)] among fish consumers compared to no fish consumption [169].

Discrepancies noted in the results of several large prospective observational studies (table 1-10) and RCTs (table 1-9) could be attributed to high background levels of dietary fish and n-3 PUFA intakes among participants enrolled in RCTs [153]. Observational studies usually examine CVD events among individual with higher vs. lower levels of fish or n-3 PUFA. Whereas, RCTs generally enroll a study participants with comparatively high background levels of dietary fish and n-3 PUFA intakes in treatment and control groups, which basically lowers the ability of the study to detect a significant difference [153].

Table 1-9. Selected RCTs showing association of n-3 PUFAs and CHD

Author-Year-Location-Study name	Populations	Follow-Up, Intervention, and control group	Primary Outcome	Point estimate (95% CI)	Conclusion/ interpretation
Burr et al. 1989, UK, The Diet and Reinfarction Trial (DART) [170]	N= 2033; Men aged <70 years with recent MI; Exclusion criteria: DM, planned cardiac surgery or eating fish or whole grains	Intervention- fatty fish or fish oil capsules two servings per week Control: routine care; FU = 2 years	Total events= CHD death + nonfatal MI	Total events = 224: 0.84 (0.66, 1.07); CHD death events = 194: 0.71 (0.54, 0.93);	Fatty fish or fish capsule supplementation was significantly associated with reduced risk of CHD mortality.
Stone et al. 1999, Italy, The Gruppo Italiano per lo Studio della Sopravvivenza nell'infarto miocardio-Prevenzione trial (GISSI) [171]	N =11324, 85% men, with recent MI, flexible age limit Exclusion criteria: participants with cancer and overt heart failure	Intervention: the 875 mg fish oil capsules (850 to 882 mg EPA and DHA as ethyl esters; Control: routine care; FU= 3.5 years	All deaths, CVD deaths nonfatal MI or stroke	All death + nonfatal MI + stroke events = 1513: 0.85 (0.74, 0.98); CVD deaths + nonfatal MI + stroke =1187: 0.80 (0.68, 0.94);	Fish oil capsule supplementation was significantly lowered all deaths as well as CVD deaths.
Burr et al. 2003, US, The Diet and Reinfarction Trial 2 (DART-2) [172]	N= 3114, men with stable angina age <70 years	Intervention: two portions of oily fish/week or 3 g/day of fish oil capsules; Control- Sensible eating advice; FU= 3-9 years	Cardiac death	Cardiac death= 319 events: 1.26 (1.00, 1.58);	Oily fish supplementation was associated with a higher risk of cardiac death.
Yokoyama et al. 2007, Japan, the Japan EPA Lipid Intervention Study (JELIS) [173]	N= 18645, age= 40-75 years, 30% men, total cholesterol ≥6.5 with statin, with or without h/o of CVD	Intervention: 1800 mg/day of EPA; Control group: Routine care; FU= 4.6 years	Coronary events (Sudden cardiac death + MI + unstable angina + coronary revascularization)	Coronary events= 586: 0.81 (0.69, 0.95);	EPA supplementation reduced the risk of coronary events.
GISSI-HF investigators, 2008, USA, GISSI-Heart failure (GISSI-HF) [174]	N=6975, men and women with chronic heart failure irrespective of cause and LVEF, patients with ACS or revascularization within 1 month, Exclusion criteria: patients with significant liver disease were excluded	Intervention: 850–882 mg EPA + DHA; Control - Olive oil; FU= 3.9 years	All-cause mortality, CVD hospital admission+ CVD death	All-cause mortality events= 1969: 0.91 (0.83, 0.99); CVD hospital admissions or death events= 4034: 0.92 (0.85, 0.99);	Significant reduction of all-cause mortality or CVD hospitalization among intervention group.
Rauch et al. 2008, Germany, OMEGA [175]	N= 3804, 75% men, age ≥18years, with recent MI in last 2 weeks	Intervention: 460 mg EPA + 380 mg DHA; Control: Olive oil; FU= 1 years	Sudden cardiac death, cardiac arrest with death within 3 weeks	Total events= 57: 0.95 (0.56, 1.60);	No significant association between EPA + DHA supplementation and sudden cardiac deaths or

Table 1-9. Continued

					cardiac arrest
Kromhout et al. 2010, Netherlands, Alpha OMEGA [176]	N=4837, 78% men, age 60-80 years with h/o MI in last 10 years	Intervention: 226 mg of EPA + 150 mg of DHA, 1.9 g of ALA, or both, Control: margarine; FU = 3.4 years	Fatal and non-fatal CVD events + coronary revascularization	Total events= 671 For EPA + DHA: 1.01 (0.87, 1.17); ALA: 0.91 (0.78, 1.05);	No significant association between OM3 supplementation and CVD events.
Galan et al. 2010, France, Supplémentation en Folates et Omega-3 (SU.FOL.OM3) [177]	N=2501, 79% men, mean age 45-80 years, patients with recent coronary or cerebral ischemic events	Intervention: Daily 600 mg OM3 (EPA +DHA); Control: placebo; FU = 4.7 years	Nonfatal MI, stroke, CVD death	CVD events =157: 1.08 (0.79, 1.47);	No beneficial effect of OM3 supplementation compared to control group.
Bosch et al, 2012, multicountry, The Outcome Reduction with an Initial Glargine Intervention (ORIGIN) [178]	N=12536, DM patients, aged ≥50 years treated with ≤1oral agent and h/o CVD	Intervention: 1 g/day of fish oil (465 mg EPA + 375 mg DHA); Control: olive oil, FU = 6.2 years	Total CVD death	Total CVD death =1155: 0.98(0.87, 1.10);	No significant association between fish oil supplementation and total CVD death
Risk and Prevention study collaborative group 2013, Italy, (Risk and Prevention trial) [179]	N= 12513, 62% men, mean age 64 years, prior CVD patients with ≥4 risk factors or DM + 1 CVD risk factor, Exclusion criteria: patients with MI	Intervention: 1 gram/day n3 FA; Control: Olive oil; FU = 5 year;	CVD death or CVD hospitalization	Total events =1478: 0.98 (0.88, 1.08);	Compared to control group no significant reduction in CVD death or hospitalization among intervention group
Bonds et al. 2014, USA, the Age-Related Eye Disease Study 2 (AREDS2) [180]	N= 4203, 45% men, age 50-85 years with intermediate or advanced macular degeneration Exclusion criteria: patients with CVD in previous 12 months were excluded	Intervention: (350-mg DHA + 650-mg EPA), macular xanthophylls (10-mg lutein + 2-mg zeaxanthin) or combination of the two, Control: placebos; FU = 4.8 years	MI, hospitalized ACS; CABG; hospitalized CHF; unexpected sudden cardiac death; resuscitated cardiac arrest; cardiac angioplasty or stent; implantable cardioverter-defibrillator; TIA; ischemic stroke; hemorrhagic stroke; and carotid artery stent, angioplasty, or endarterectomy	Total events =370: 0.9 (0.8, 1.2);	No reduction cardiovascular events with the supplementation of DHA + EPA
Ongoing trials					
A Study of Cardiovascular Events in Diabetes (ASCEND) PI- Jane Armitage, UK, 2004- ongoing [181]	N= 15480, >40 years, with diabetes (type 1 or 2), patients with h/o vascular disease were excluded	Intervention- one g/day omega-3 fatty acids (400 mg EPA+300 DHA as ethyl esters); Control- Placebo (olive oil); FU- 5-7	non-fatal MI, non-fatal stroke, transient ischemic attack, vascular death excluding cerebral hemorrhage		

Table 1-9. Continued

		years			
(The Vitamin D and Omega-3 Trial) (VITAL) PI - JoAnn Manson, US, 2010-ongoing [182]	N= 25,874 US men >50 years and women >55 years, general population without cancer or CVD at baseline;	Intervention- 1 g/day Omacor fish oil capsule (465 mg EPA + 375 mg DHA); Control- Placebo; FU- 5 years	MI, stroke, cardiovascular mortality		
The Reduction of Cardiovascular Events with EPA-Intervention Trial (REDUCE-IT) PI- Deepak Bhatt, US, 2011 [183]	N= 8000, aged ≥45 years with hypertriglyceridemia, established CVD or high risk for CVD	Intervention- 4 g/day of Vascepa (EPA ethyl ester) with statin therapy; Control- statin therapy alone; FU - 4-6 years	Cardiovascular events (composite of cardiovascular death, MI, stroke, coronary revascularization, and hospitalization for angina		
Statin Residual Risk Reduction with Epanova in High-Risk Patients with Hypertriglyceridemia (STRENGTH) PI- Steven Nissen, US, 2014 [184]	N = 13,000, aged 18–99 years with optimal LDL-C levels, hypertriglyceridemia, low HDL-C, and high CVD risk	Intervention - 4 g/day Epanova (omega-3 fatty acids) plus statin; Control - corn oil placebo plus statin; FU- 3-5 years	Composite of cardiovascular death, non-fatal MI or stroke, coronary revascularization, or angina hospitalization		
Abbreviations: ACS, Acute coronary syndrome AU, Agatston' unit; CAC, Coronary artery calcification; CABG, Coronary artery bypass graft; CAD, Coronary arterial disease; CHD, Coronary heart disease; CI, confidence interval; CIMT, carotid intima-media thickness; CV, Cardiovascular; CVD, Cardiovascular disease; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FMD, flow-mediated dilation; FU, follow-up; FRS, Framingham Risk Score; HDL-C, high density lipoprotein cholesterol; HDL-P, high density lipoprotein particle; LC n-3 PUFAs, long-chanin omega 3 polyunsaturated fatty acids; LDL-C, low density lipoprotein cholesterol; MI, Myocardial Infarction; PCI, percutaneous coronary intervention; PTCA, Percutaneous transluminal coronary angioplasty; SD, Standard deviation;					

Table 1- 10. Observational studies showing association of n-3 PUFAs and CHD

Author-Year-Location-Study name	Populations	Type of study	CVD events	Point estimate	Results/ Interpretation
Ascherio et al. 1995, US, 'The Health Professionals Follow-up Study' [185]	N= 44,895 male health professionals, aged 40-75 years, without clinical CVD	Prospective cohort; FU = 6 years;	deaths from coronary disease =264, nonfatal myocardial infarctions=547, coronary-artery bypass or angioplasty procedures=732	Risk of CHD in 5th quintile of n-3 fatty acids intake vs. 1st (0.58 vs 0.07 median g/d): 1.12 (0.96, 1.31);	No significant associations between dietary intake of n-3 fatty acids or fish intake and the risk of coronary disease.
Pietinen et al. 1997, Finland, 'The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study' [186]	N= 21,930 smoking males aged 50-69 years initially free of diagnosed CVD	Prospective cohort; FU= 6.1 years;	Major coronary events = 1,399; Coronary deaths= 635;	Risk of death for men in 5th quintile of n-3 fatty acids intake vs. 1st (0.8 vs. 0.2 median g/d): 1.30, (1.01, 1.67);	The intake of omega-3 fatty acids from fish was positively associated with the risk of coronary death.
Albert et al. 1998, US 'The US Physicians' Health Study' [187]	N= 20551 male physicians aged 40-84 years free of MI, CBVD, and cancer	Prospective cohort; FU= 11 years	Sudden cardiac death=133, MI= 737	Risk of sudden death - fish at least once/week vs. fish less than monthly: 0.48(0.24, 0.96); n-3 intake >2.7 grams/month vs. <0.3 grams/month: 0.34 (0.15, 0.75);	Dietary fish intake was indirectly associated with sudden cardiac death but not with MI, non-sudden cardiac death or total CVD mortality.
Yuan et al. 2001, China, Shanghai Cohort Study [188]	N= 18,244 men aged 45–64 years with no h/o cancer	Prospective cohort; FU= 12 years	Deaths from acute MI= 113; Total mortality= 2134	Participants with ≥200 g of fish/shellfish/week vs. with men consuming <50 g per week: - Fatal acute MI: 0.41(0.22, 0.78); - Total mortality: 0.79 (0.69, 0.91);	Eating fish and shellfish weekly was indirectly associated with the risk of fatal MI.
Hu et al. 2003 US, 'Nurses' Health Study' [189]	N=5103 female nurses, aged 30-55 years, diagnosed type 2 diabetes but free of CVD or cancer at baseline	Prospective cohort; FU=16 years	CHD= 362 (CHD deaths=141, nonfatal MI= 221), total deaths= 468	CHD: for fish consumption <1 serving/ month vs. - 1-3 times/month: 0.70 (0.48, 1.03); - for once/week: 0.60 (0.42, 0.85); - for 2-4 times/ week: 0.64(0.42, 0.99); - ≥5/week: 0.36 (0.20, 0.66); Total mortality: for fish consumption ≥5/week vs. <1serving/month: 0.48 (0.29, 0.80);	Higher consumption of fish and LC n-3 PUFA indirectly related with a lower CHD incidence and total mortality.
Mozaffarian et al. 2005, US 'The Health Professionals Follow-up Study' [190]	N=5722 men free of known CVD, aged 40-75 years	Prospective cohort; FU=14 years	Sudden cardiac deaths= 218 events, Nonfatal MI = 1521, Total CHD events (combined sudden death, other CHD deaths, and nonfatal MI) =	Sudden cardiac deaths: 0.52 (0.34, 0.79); Nonfatal MI: 1.16 (0.99, 1.36); Total CHD events: 1.05 (0.92, 1.19);	Long-chain and intermediate-chain n-3 PUFA consumption was associated with reduced CHD risk.

Table 1-10. Continued

			2306		
Iso et al. 2006 Japan, Japan Public Health Center Based Study [191]	N= 41,578 Japanese men and women aged 40-59 years free of prior diagnosis of CVD and cancer, ~50% males	Prospective cohort; FU= 11 years	CHD = 258 events (198 definite MI + 23 probable MI + 37 sudden cardiac deaths)	5th quintile of n-3 PUFA Vs 1st quintile: CHD: 0.58 (0.35, 0.97) Total MI: 0.43 (0.24, 0.78); Sudden cardiac death: 1.24 (0.39, 3.98); Fatal coronary events: 1.54 (0.60, 3.99); Nonfatal coronary events: 0.33 (0.17, 0.63);	Higher intake of n-3 PUFA (median of 2.1 g/day) was associated with reduced risk of cardiac events
Jarvinen et al. 2006, Finland, The Finnish Mobile Clinic [192]	N= 5220 ~52% males, aged 30-79 years free of CHD	Prospective cohort; FU= 21.5 years	Coronary death = 498 events (335 men +163 women)	Men: Coronary death: 0.96 (0.68,1.38); Women: Coronary death: 0.73 (0.44, 1.19);	No association between intake of n-3 fatty acids and the risk of CHD in either men or women
Streppel et al. 2008, Netherland, the Zutphen Study [193]	N= 1373 men age=40-90 years	Prospective cohort; FU= 40 years	CHD death, sudden coronary deaths	EPA+DHA 1-250 mg vs. 0 mg/day: - CHD death: 0.76 (0.49, 1.18); - Sudden cardiac death: 0.96 (0.36, 2.52); - Fatty fish consumption: CHD death: 0.88 (0.65, 1.19) - Sudden cardiac death: 0.46 (0.27, 0.78);	Fatty-fish consumption was associated with reduced sudden cardiac death risk.
de Goede et al. 2010, Netherland, 'The Monitoring Project on Risk Factors for Chronic Diseases' (MORGAN) [194]	N=21,342 participants 45% men aged 20–65 years with no h/o MI or stroke	Prospective cohort; FU= 11.3 years	Fatal CHD=82, Fatal MI=64 events, Nonfatal MI=252 events	4th quartile of EPA+DHA vs. 1st quartile: - Fatal CHD =0.51 (0.27, 0.94); - Fatal MI = 0.38 (0.19, 0.77); - Nonfatal MI =1.07 (0.74, 1.54); 4th quartile of fish intake Vs 1st quartile: - Fatal CHD = 0.52 (0.28, 0. 95); - Fatal MI=0.40 (0.19, 0.86); - Nonfatal MI =1.01 (0.71, 1.45);	EPA+DHA and fish intake was associated with reduced fatal CHD and MI risk in a dose-responsive manner.
Joensen et al. 2009 Denmark, Danish Diet, Cancer and Health Cohort Study [195]	N= 57053 ~50% males aged 50-64 years	Prospective cohort; FU= 7.6 years	Acute coronary syndrome	5th highest quantiles of n-3 PUFA intake vs. 1st quintile: - Men - 0.81(0.67, 1.03); - Women - 0.97(0.62, 1.52);	Men: Borderline significant inverse relationship between the intake of n-3 PUFA and ACS. Women: No significant association
Manger et al. 2010, US, the Western Norway B Vitamin Intervention Trial [196]	N= 2412 81% males aged >18 years diagnosed with CAD	Prospective cohort; FU= 57 months	Coronary event = 292, Coronary death= 76, Acute MI = 210	4th quartile vs. 1st quartile of LC n-3 PUFA intake: - Coronary event = 0.95 (0.69, 1.31); - Coronary death = 1.33	No significant trends toward a lowered risk of cardiac events with increasing consumption of LC

Table 1-10. Continued

				(0.67, 2.62); - Acute MI = 1.05 (0.72, 1.52);	n-3 PUFAs
Chiuve et al. 2012, US, the Nurses' Health Study [197]	N=91,981 women aged 34–59 year	Prospective cohort; FU= 30 years	Sudden cardiac deaths (SCD)	SCD: for a 5% increment of dietary PUFAs 0.79 (0.69, 0.90); 5th quintile vs. 1st of PUFA: 0.50 (0.35, 0.70);	PUFAs was negatively associated with SCD risk.
Takata et al. 2012, China, the Shanghai Women's Health Study and the Shanghai Men's Health Study [198]	N= 134,296 ~50% men aged 40–70 years	Prospective cohort; FU= 13 years	Ischemic heart disease (IHD) death: Men=225, Women=251	5th quintile vs. 1st quintile LCn-3PUFAS: - Men: 0.84 (0.60, 1.16); - Women: 0.79(0.57, 1.09); - Combined: 0.79 (0.57, 1.09); 5th quintile vs. 1st quintile of total fish consumption: - Men: 1.10 (0.70, 1.73); - Women: 0.94 (0.59, 1.49); - Combined: 1.02 (0.74, 1.41);	No association of either fish consumption or LC n-3 PUFAs with IHD mortality.
Koh et al. 2013 China, The Singapore Chinese Health Study [199]	N= 63,257 45% men, aged 45–74 years	Prospective cohort; FU= 19 years	Cardiovascular deaths = 4780 events (CHD=2697 deaths + stroke deaths=1298)	4th quartile vs. 1st quartile of EPA+ DHA Intake: - CHD mortality: 0.86 (0.74, 0.99); - Cardiovascular mortality: 0.84 (0.74, 0.95);	High dietary consumption of both LC n-3 PUFAs was associated with lowered risk of cardiovascular death.
Miyagawa et al. 2014, Japan, The National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged (NIPPON-DATA) [200]	N= 9190 47% men, mean aged 50 years	Prospective cohort; FU= 24 years	Cardiovascular deaths= 879 CHD deaths=131	4th quartile vs. 1st quartile intake of LCn3FA: - CVD deaths: 0.80 (0.66-0.96); - CHD deaths: 0.82(0.53, 1.29);	LC n-3 PUFAs consumption was associated with lower long-term risk of total CVD mortality but not with CHD mortality.
Bergkvist et al. 2015, Sweden, Swedish Mammography Cohort [201]	N=33,446 women, free from CVD, cancer, and diabetes, mean age ~62 years	Prospective cohort; FU= 12 years	MI= 1368 events	- 3rd quartile vs. 1st quartile of EPA+ DHA Intake: 0.74 (0.56, 0.98); - 4th quartile vs. 1st quartile of EPA+ DHA intake: 0.74 (0.52, 1.06);	No significant trend with increase in intake of EPA+ DHA with MI

Abbreviations: ACS, Acute coronary syndrome; AU, Agatston' unit; CAC, Coronary artery calcification; CABG, Coronary artery bypass graft; CAD, Coronary arterial disease; CHD, Coronary heart disease; CI, confidence interval; CIMT, carotid intima-media thickness; CV, Cardiovascular; CVD, Cardiovascular disease; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FMD, flow-mediated dilation; FU, follow-up; FRS, Framingham Risk Score; HDL-C, high density lipoprotein cholesterol; HDL-P, high density lipoprotein particle; LC n-3 PUFAs, long-chanin omega 3 polyunsaturated fatty acids; LDL-C, low density lipoprotein cholesterol; MI, Myocardial Infarction; PCI, percutaneous coronary intervention; PTCA, Percutaneous transluminal coronary angioplasty; SD, Standard deviation;

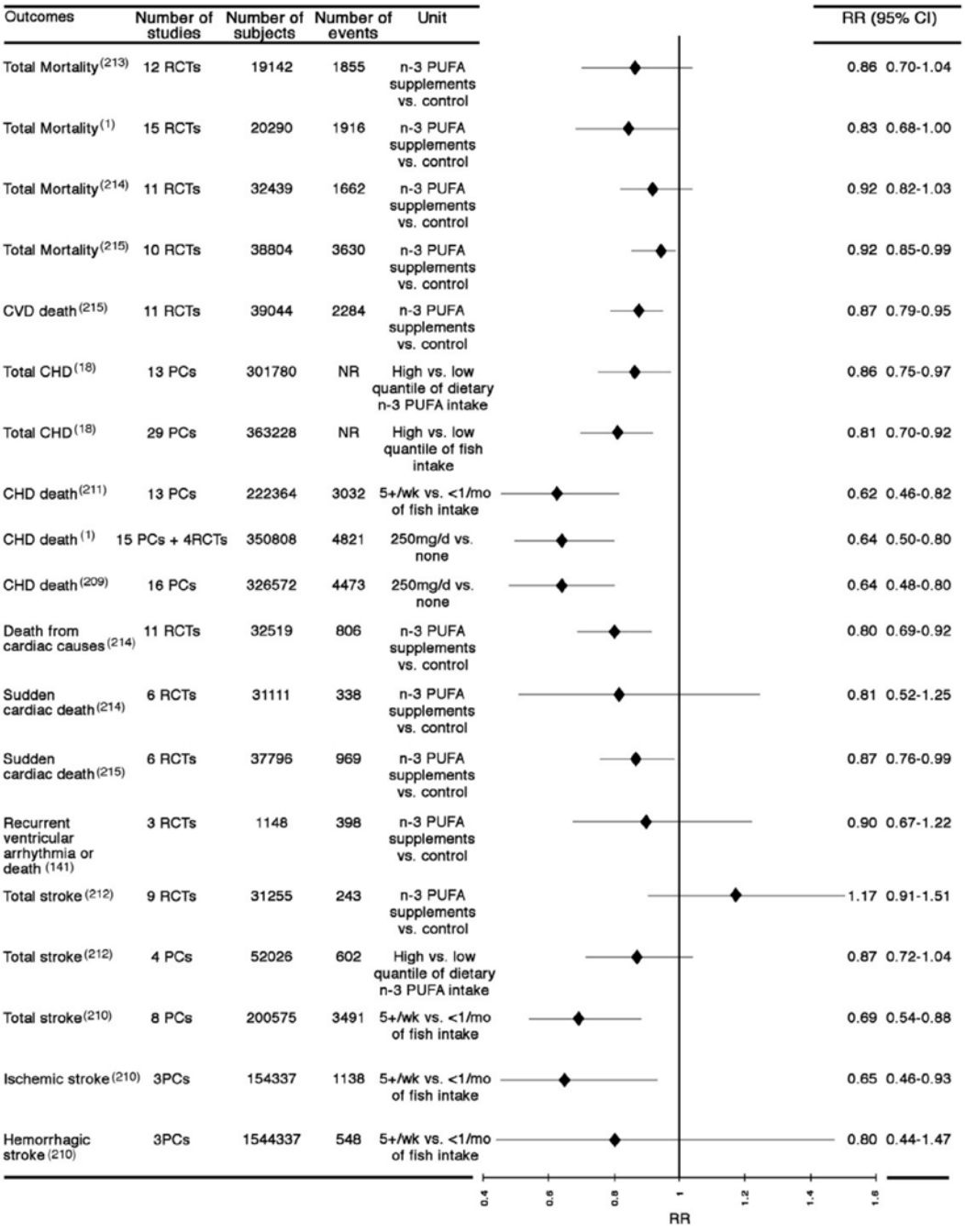

Figure 1-13. Meta-analysis of RCTs and prospective observational studies assessing the effect of n-3 polyunsaturated fatty acids and CHD/CVD

1.7.3.1 AHA recommendations for the use of n-3 PUFAs in CHD/CVD

Differences in the indications (primary vs. secondary prevention setting), studied participants, interventions used and studied outcomes hinder the synthesis of evidence assessing the effect of LCn-3PUFAs on clinical CVD. Based on the results from several RCTs in different population settings, the AHA advisory committee has recommended the use LCn-3PUFAs for the secondary prevention of CHD and SCD among patients with prevalent CHD [1]. The beneficial effect of LCn-3PUFAs in the secondary prevention of CHD and SCD was mainly attributed to its anti-arrhythmic effects ("stabilization of ischemic-induced myocyte membrane resting depolarization") [1].

1.7.4 N-3 PUFAs and Atherosclerosis

Despite several documented beneficial effects of LCn-3PUFAs in the human population (Figure 1-11), little amount of research has been conducted to assess the anti-atherosclerotic effect of LCn-3PUFAs. Animal studies [202-204] and basic research experiments [205] support the anti-atherosclerotic effect of LCn-3PUFAs, but human population studies reported inconsistent results [206-213] (Table 1-12). Moreover, none of the human studies have assessed the relationship between serum biomarkers of LCn-3PUFAs and aortic calcification. It is important to study the relationship between LCn-3PUFAs and atherosclerosis to gain further insight into the relationship of LCn-3PUFAs with CHD.

Recent observational studies and RCTs (Table 1-12) examining the relationship between LCn-3PUFAs and atherosclerosis in Japan showed that LCn-3PUFAs are anti-atherogenic [206-208]. Mita and colleagues conducted an RCT in Japan assigning 60 type 2 DM patients to 1.8 grams of EPA daily or placebo and following them for 2.1±0.2 years. Patients in the treatment

group had significant annual decreases in mean and maximum CIMT compared to the control group. The treatment group also had significantly improved brachial pulse wave velocity. Thus, EPA administration significantly delayed the progression of atherosclerosis [207]. In the ERA-JUMP study, Akira and colleagues assessed the relationship between DHA and EPA with CIMT among 608 men aged 40-49 years from the US and Japan [208]. Among the Japanese population, study participants with higher tertiles of DHA had significantly lower CIMT compared to study participants with lower tertile of DHA. Among US White there was no significant differnces in CIMT across different DHA tertiles. In both populations, EPA was not significantly associated with CIMT [208]. In another study among 172 Japanese men and women with a mean age of 68.2 years, Urabe and colleagues reported a significant inverse association between EPA and EPA+DHA with presence and extent of noncalcified plaques [206].

Studies conducted among western populations (Table 1-12) have reported discrepent findings compared to the above-mentioned Japanese studies. In the MESA Study, He and colleagues cross-sectionally assessed the association of the dietary intake of LCn–3PUFAs with common CIMT, internal CIMT, CAC, and ABI among 5488 individuals aged 45-84 years. The dietary intake of LCn–3PUFAs was inversely associated with common CIMT [OR (95% CI) = 0.69(0.55, 0.88)] but not with internal CIMT, CAC, and ABI [212]. Similarly, Lindqvist and colleagues in Sweden cross-sectionally assessed the association of EPA and DHA with carotid artery plaque, femoral artery IMT, and femoral artery plaque among 513 men aged >61 years. There was no significant association between EPA or DHA with carotid artery plaque, femoral artery IMT, femoral artery plaque [210]. In the Rotterdam study, Heine-Broring and colleagues cross-sectionally assessed the association of EPA+DHA with CAC among 1570 individuals with a mean age of 64 years. Investigators reported a non-significant association between dietary

intake of EPA+DHA and CAC [211]. Shang and colleagues in the Melbourne Collaborative Cohort study among 312 men and women aged 45-64 years, did not find any significant association between dietary intake of EPA+DHA and abdominal aortic calcification [209]. Several potential explanations for discrepent findings among various studies may include differences in the age distribution, subclinical atherosclerosis assessment techniques, examined vascular bed, and the use of blood biomarkers of LCn-3PUFAs as opposed to self-reported dietary assessment of fatty acids.

Table 1- 11. Studies showing the association of LC n-3 PUFAs with measures of atherosclerosis

Author-Year-Location-Study name	Populations	Study design	Endpoints/ primary outcomes	Point estimates	Results
Mita et al. 2007, Japan [207]	N= 81, ~60% Japanese men with type 2 diabetes, mean age=60 years, Japanese	Randomized controlled trial; Intervention = 1800 mg of EPA; Control= Placebo; FU=2.1±0.2 years	Carotid IMT and brachial-ankle pulse wave velocity (baPWV)	Max IMT (mm/year) annual change: Intervention group: −0.084 ± 0.113; Control group: −0.005 ± 0.108; baPWV (cm/s/year) annual change: Intervention group: −22.1 ± 127.9; Control group: 62.3 ± 223.0;	EPA delays the progression of atherosclerosis indicated by IMT and baPWV.
He et al. 2008, US, MESA study [212]	N= 5488 aged 45-84 years, free of clinical CVD, ~47% men, ~38% Caucasians, ~27% Blacks, ~22% Hispanic, ~12% Chinese	Cross-sectional	cCIMT, iCIMT, CAC, ABI	OR (95% CI) for the highest to the lowest quartile of dietary n-3 PUFA intake: - cCIMT: 0.69 (0.55, 0.86); - iCIMT: 0.97 (0.78, 1.22); - CAC: 1.14 (0.94, 1.38); - ABI: 1.28 (0.81, 2.02);	The dietary intake of LC n–3 PUFAs was inversely associated with cCIMT but not with iCIMT, CAC and ABI.
Lindqvist et al. 2009, Sweden [210]	N= 513 men aged 61 years	Cross-sectional	Right and left carotid artery IMT, plaque, femoral artery IMT, plaque	Point estimate not provided.	No association between EPA or DHA with any of the atherosclerotic measure.
Heine-Broring et al. 2010, Netherland, Rotterdam study [211]	N=1570 asymptomatic cardiac subjects, mean age of 64 years, 44% men	Cross-sectional	CAC: mild/moderate CAC (11-400); Severe CAC (>400)	The 3rd vs. 1st tertile of EPA+DHA intake: Compared to no CAC, mild/moderate CAC (11-400): 0.93 (0.84, 1.04); - Severe CAC (>400): 0.97(0.83, 1.13);	No association between EPA+DHA intake and CAC.
Akira et al. 2011, US, the ERA-JUMP Study [208]	N=608 Japanese and US white men aged 40-49, ~50% Caucasian, ~50% Japanese	Cross-sectional	IMT of common and internal carotid artery	Among Japanese: mean IMT among [DHA (T1)= 631, DHA(T2)= 619, DHA(T3)= 604; (p trend = 0.014)] [EPA (T1)= 626, EPA(T2)= 623, EPA(T3)= 606; (p trend = 0.064). Among US white: mean IMT among [DHA (T1)= 678, DHA(T2)= 678, DHA(T3)= 658; (p trend = 0.129)]	Differential significant association of DHA with IMT independent of risk factors among Japanese but not in US White.

Table 1-11. Continued

				[EPA (T1)= 668, EPA(T2)= 672, EPA(T3)= 674; (p trend = 0.651).	
Urabe et al. 2013, Japan [206]	N= 172, mean age = 68.2±7.8 years, ~63% men, all Japanese	Cross-sectional	Coronary plaque- calcified (CP) and noncalcified (NCP); Extent of the Plaque - Extensive plaque (when present in 2 or more segments of coronary artery), focal plaque (when present in 1 or part of 1 segment); High risk plaque= low density plaque + positive remodeling	For EPA ≤median (61.3μg/ml) vs. >median 3-vessel plaque involvement: 2.12 (1.14, 4.03); NCP: 2.36 (1.18, 4.83); Extensive NCP ≥2 segments: 2.15 (1.13, 4.15); High risk: 2.47 (1.27, 4.92; Serum EPA+DHA ≤median (198.9μg/ml) vs. >median: 3-vessel plaque involvement: 1.83 (0.97, 3.48); NCP: 3.51 (1.70, 7.59); Extensive NCP ≥2 segments: 1.93 (1.02, 3.67); High risk: 2.68 (1.38, 5.29);	EPA and EPA+ DHA were associated with the presence and extent of NCP, and high-risk plaques.
Shang et al. 2015, Australia, the Melbourne Collaborative Cohort Study [209]	N= 312 participants aged 45–64 years old at baseline, ~42% men	Cross-sectional	Abdominal aortic calcification (AAC) measured by lateral radiography and DXA scan of lumbar spine	Men: EPA+ DHA - Radiography: 1.28 (0.51, 3.20); - DXA scan: 1.20 (0.46, 3.10); Women: EPA+ DHA - Radiography: 0.54 (0.25, 1.13); - DXA scan: 0.59 (0.27, 1.31);	Baseline EPA+DHA or change in EPA+ DHA over the period of 18 years were not significantly associated with AAC severity.
Nosaka et al. 2017, Japan [214]	N=238 participants with acute coronary syndrome, all Japanese	Randomized controlled trial; Intervention = 1800 mg of EPA + Pitavastatin; Control= Pitavastatin; FU= 12 months	Cardiovascular cause, nonfatal stroke, nonfatal MI and revascularization	Cardiovascular events: HR (95% CI) for the EPA + Pitavastatin to Pitavastatin group: 0.42 (0.21–087);	Treatment with EPA + statin after successful primary PCI reduced CV events.
Watanabe et al. 2017, Japan, The CHERRY Study [215]	N= 193 CHD patients aged >20 years, all Japanese	Randomized controlled trial; Intervention = 1800 mg of EPA + Pitavastatin; Control= Pitavastatin;	Coronary plaque volume measured by integrated backscatter IVUS	Significant reduction in coronary plaque volume and increases plaque stabilization in EPA + Pitavastatin group compared to Pitavastatin group. (numbers were not available);	The addition of EPA is a promising option to reduce residual CHD risk under intensive statin therapy.

Table 1-11. Continued

		FU= 6-8 months			
Niki et al. 2016, Japan [216]	N= 95 dyslipidemic patients with stable angina pectoris aged ~69 years, all Japanese	Randomized controlled trial; Intervention = 1800 mg of EPA + Statin; Control= Statin; FU= 6 months;	Characteristic of coronary plaque measured by integrated backscatter IVUS and plasma levels of inflammatory cytokines	From baseline to 6 months: Change in lipid volume (mm^3), EPA + Statin group= 18.5±1.3 to 15.0±1.5; Statin group: 17.8±1.3 to 19.3±2.1; Change in fibrous volume, EPA + Statin group: 22.9±0.8 to 25.6±1.1; Statin group: 24.0±1.0 to 21.8±1.9; Change in CS levels Pentraxin 3 (ng/ml): EPA + Statin group: 3.3±2.1 to 2.6±1.2; Statin group: 2.8±2.6 to 2.5±1.4; Change in CS levels monocyte chemoattractant protein-1 (pg/ml): 120.4±26.2 to 110.2±26.8; Statin group: 111.5±32.8 to 103.8±30.8;	The addition of EPA to statin further stabilizes coronary plaques in patients with stable angina.
Ongoing Trial					
Effect of Vascepa on improving coronary atherosclerosis in people with high triglycerides taking statin therapy (EVAPORATE) PI- Budoff M, US, 2016 [217]	N = 80, aged 30–85 years with optimal LDL-C levels, hypertriglyceridemia	Intervention - 4 g/day Vascepa (omega-3 fatty acids) plus statin; Control – placebo+ statin; FU= 18 months;	Progression rates of low attenuation plaque, The morphology of non-calcified coronary atherosclerotic plaque, markers of inflammation	-	-

Abbreviations: AU, Agatston' unit; CAC, Coronary artery calcification; CABG, Coronary artery bypass graft; CAD, Coronary arterial disease; CHD, coronary heart disease; CI, confidence interval; CIMT, carotid intima-media thickness; CV, Cardiovascular; CVD, cardiovascular disease; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FMD, flow-mediated dilation; FU, follow-up; FRS, Framingham Risk Score; HDL-C, high density lipoprotein cholesterol; HDL-P, high density lipoprotein particle; LCn-3PUFAs, long-chanin omega 3 polyunsaturated fatty acids; LDL-C, low density lipoprotein cholesterol; MI, myocardial infarction; PCI, percutaneous coronary intervention; PTCA, percutaneous transluminal coronary angioplasty; SD, Standard deviation;

1.7.4.1 LCn-3PUFAs and atherosclerosis: plausible mechanisms

Atherosclerosis is a systemic chronic inflammatory disease of the vessel walls and inflammation resulting from the interaction of modifed atherogenic lipoproteins, inflammatory cells, and smooth muscle cells of the vessel wall play a major role in the initiation and progression of the

atherosclerotic plaque [11]. Available evidence from short term experimental studies suggest that LCn–3PUFAs may exert antiatherosclerotic effects through several anti-inflammatory properties [218, 219]. First, increased dietary intake of LC n–3 PUFAs is associated with a change in the fatty acid composition of cell membranes of inflammatory cells leading to altered cell signaling, gene expression, and synthesis of lipid mediators [220]. Second, LCn–3PUFAs lower pro-inflammatory eicosanoids from arachidonic acids, and therefore lead to a reduction in mediators and regulators of inflammation including leukotrienes, prostaglandins, thromboxane, etc., thus decreasing the synthesis of leukocyte chemoattractant protein and the recruitment of inflammatory cells at the site of endothelial damage in the vessel wall [221, 222]. Third, LCn–3PUFAs reduce activation of nuclear factor kappa B [223], lower the plasma concentrations of soluble adhesion molecules, and the intercellular adhesion molecule expression on the surface of leukocytes [224], which further alters the leukocyte-endothelial adhesion interaction [225]. Fourth, major LCn–3PUFAs, EPA and DHA, give rise to E1 resolvin and D1 resolvin respectively which are thought to resolve the ongoing inflammation further limiting tissue damage [221]. Fifth, LC n–3 PUFAs prevent thinning and weakening of the fibrous cap of atherosclerotic plaque by decreasing the number of inflammatory cells in its necrotic core, increase plaque stability and therefore prevention of plaque rupture [226, 227]. Thus, either by inhibiting atherosclerotic plaque development or promotion of plaque stabilization, LCn-3PUFAs could lower ischemic CHD/CVD.

1.8 SPECIFIC RESEARCH QUESTIONS (RQ)

This dissertation focuses on the following three specific research questions:

RQ1: Do differences in the distribution of NMR-measured lipoproteins account for differences in the prevalence of coronary artery calcification between US White and Japanese participants of the ERA JUMP study?

Alternate hypothesis (H1): Differences in the distribution of NMR-measured lipoproteins partially account for differences in the prevalence of CAC between middle aged healthy US White and Japanese men in the ERA-JUMP study.

RQ2: Is alcohol consumption associated with aortic calcification among middle-aged men in the ERA JUMP Study?

Alternate hypothesis (H1): Compared to no alcohol consumption, heavy alcohol consumption is positively and light to moderate alcohol consumption is negatively associated with aortic calcification in healthy middle-aged men in the ERA-JUMP Study.

RQ3: Are serum levels of long chain n-3 polyunsaturated fatty acids inversely related to aortic calcification among middle-aged men in the ERA JUMP study?

Alternate hypothesis (H1): Total LCn-3PUFAs, EPA or DHA are inversely associated with aortic calcification in healthy middle-aged men in the ERA-JUMP Study.

2.0 MANUSCRIPT I: NMR-MEASURED LIPOPROTEIN PARTICLE DISTRIBUTIONS AND CORONARY ARTERY CALCIFICATION IN US WHITE AND JAPANESE MEN AGED 40-49 YEARS

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Authors names and academic degrees

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2.1 ABSTRACT

Background: In a Post-World War II birth cohort of middle-aged men, the prevalence of coronary artery calcification (CAC) is significantly higher in US White (the US residing Caucasian) compared to Japanese (Japan-residing Japanese), despite Japanese having a worse profile for smoking and hypertension.

Objective: The present study was conducted to examine whether contrasting distributions of lipoproteins contribute to differences in the prevalence of CAC between the two groups of middle-aged males: the US White and the Japanese.

Methods: We examined the research question using data from the population-based ERA-JUMP Study, comprising of 570 randomly selected asymptomatic men aged 40-49 years (270 US White and 300 Japanese). NMR-spectroscopy was used to determine lipoprotein particle concentrations and their average sizes. We used multivariable logistic regression to assess the relationship between race/ethnicity and CAC (measured by Electron Beam Computed Tomography and quantified using the Agatston method), after adjustment for traditional and novel risk factors for coronary heart disease (CHD).

Results: US White compared to Japanese had significantly different NMR-measured lipoprotein particle distributions. US White had a significantly higher prevalence of CAC ≥10 compared to Japanese after adjustment for CHD risk factors [OR = 3.25; 95% CI = 1.55, 6.84], and this difference was partially attenuated with further adjustment for lipoprotein levels [OR = 2.58; 95% CI = 1.16, 5.77]. There was no reclassification improvement with further addition of lipoproteins particle concentration/size to a model that already included traditionally measured lipids, cardiovascular risk factors, and inflammatory markers (net reclassification index = -3% to

1%), nor did the addition of NMR-measured lipoproteins result in a statistically significant improvement in the area under the receiver operating characteristic curve for CAC ≥10.

Conclusions: Variations in the distribution of lipoprotein particles partially accounted for differences in the prevalence of CAC between middle-aged US White and Japanese men.

2.2 INTRODUCTION

Coronary artery calcification (CAC) is a well-established biomarker of coronary atherosclerosis - the major underlying cause of coronary heart disease (CHD), and is strongly associated with atheromatous burden found in the coronary arteries [228]. Both baseline CAC score [4] and its progression [229] predict future CHD among men and women of all ages and of various ethnicities. Cardiovascular disease (CVD) mortality studies [230, 231] and autopsy studies of atherosclerosis [232, 233] have reported a much higher burden of coronary atherosclerosis among US residing white men (US White) compared to Japanese men residing in Japan (Japanese). Furthermore, we have reported a much higher prevalence of CAC among US White compared to Japanese despite Japanese having higher rates of smoking and hypertension, and similar levels of serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and diabetes [234].

LDL-C and high-density lipoprotein cholesterol (HDL-C) are well-established risk factors for CHD [235]. These enzymatically measured lipid concentrations (LDL-C, HDL-C, and triglycerides) are not virtually equal to lipoprotein particle concentrations measured by nuclear magnetic resonance (NMR) spectroscopy, and residual risk of CHD remains even after achieving recommended levels of LDL-C and HDL-C with medications. Therefore, to account for this

limitation, researchers have shifted their focus to NMR-measured lipoprotein particle concentrations, which have been suggested as alternative biomarkers for improved risk assessment of atherosclerosis and CHD [66, 67, 236]. Several studies have reported that NMR-measured small low-density lipoprotein particles (LDL-P), large LDL-P, total LDL-P, large very low-density lipoprotein particles (VLDL-P), total VLDL-P, and total high-density lipoprotein particles (HDL-P) are significantly associated with subclinical atherosclerosis and CHD/CVD [56, 65, 66, 70, 71, 103, 104, 237-241]. In fact, some studies have shown that NMR-measured lipoprotein particle numbers (total LDL-P and total HDL-P) are independent and more robust predictors of atherosclerosis and CHD/CVD events than their cholesterol counterparts (LDL-C and HDL-C) [65, 71, 103, 104].

We have previously reported differences in the distributions of NMR-based lipoprotein profiles in US White and Japanese [56]. To the best of our knowledge, however, no previous study has examined whether these results account for the difference in the prevalence of CAC between US White and Japanese. Therefore, we aimed first, to evaluate the association between NMR-measured lipoproteins and CAC in healthy US White and Japanese men aged 40-49 years, and secondly, to assess the role of NMR lipoproteins in determining differences in CAC prevalence between the two populations. We hypothesized that differences in the distributions of NMR lipoproteins would partially account for differences in the prevalence of CAC. We tested the stated hypothesis using the electron beam computed tomography (EBCT), risk factor assessment among Japanese and US men in the post-World-War-II birth cohort (the ERA-JUMP) study, a population-based study of 623 men aged 40–49 years comprising US White and Japanese.

2.3 MATERIALS AND METHODS

2.3.1 Study Population

We have previously described the details of the study protocol [234]. Briefly, we randomly selected 623 healthy men aged 40-49 years, without clinical CVD or other severe illnesses residing in Allegheny County, Pennsylvania, US (n=310 from the voter registration list) or Kusatsu City, Shiga, Japan (n=313 from the Basic Residents' Register). Recruitment was conducted between 2002 and 2006. All participants gave informed consent. The study protocol followed 'the 1975 Declaration of Helsinki ethical guidelines'. The Institutional Review Boards of University of Pittsburgh, Pittsburgh, US and Shiga University of Medical Science, Otsu, Japan approved the study. Of 623 participants, we excluded 53 participants from the present study: 4 participants with missing data for CAC and 49 participants were taking lipid lowering medications. We excluded participants taking lipid-lowering medications because lipid-lowering medications could distort the relationship between NMR-measured lipoproteins and CAC [242]. Our final sample size was 570 study participants: 270 US White and 300 Japanese.

2.3.2 Measurement of Coronary Artery Calcification (CAC)

As described earlier [55], EBCT was performed using a GE-Imatron C150 EBCT scanner, GE Medical Systems, South San Francisco, US. From the level of the aortic root to the apex of the heart, scanning was performed using a standardized protocol to obtain 30–40 contiguous 3-mm-thick transverse images. All scan data were saved to optical disc. Centrally in the Cardiovascular Institute, University of Pittsburgh, readings of the scanning were done using a DICOM (Digital

Imaging and Communications in Medicine) workstation and software by AccuImage (AccuImage Diagnostic Cooperation, San Francisco, US). Quantification of CAC was done using software program which implements the widely accepted Agatston scoring method [16]. A trained radiology technician who was blinded to each participant's characteristics and the study centers evaluated the readings. The intra-reader reproducibility of non-zero Agatston Coronary Calcium Score (CCS) had an intra-class correlation of 0.99 [55].

2.3.3 Risk Factor Assessment

As explained previously, all participants underwent a physical examination, a laboratory assessment, and completed a self-administered questionnaire [55]. We measured body weight and height while the participant was wearing light clothing without shoes and calculated body mass index (BMI) as weight (kg)/height squared (m^2). Blood pressure was measured, using an automated sphygmomanometer (BP-8800; Colin Medical Technology, Komaki, Japan) and an appropriately sized cuff on the right arm of the seated participants after they emptied their bladder and sat quietly for 5 minutes. The average of two measurements was used in the analyses. We defined hypertension as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or use of antihypertensive medications. Participants were considered smokers if they reported current use of cigarettes or had stopped smoking within the past 30 days. Pack-years of smoking were calculated as years of smoking multiplied by the number of cigarettes per day divided by 20. Those drinking alcohol ≥2 days per week were considered alcohol drinkers.

Venipuncture was performed early in the clinic visit after a 12-hour fast. Serum and plasma samples were stored at -70ºC and shipped to the University of Pittsburgh. As mentioned

previously [55], we assayed glucose, insulin, lipids [including TC, triglycerides, LDL-C, and HDL-C], fibrinogen, and C-reactive protein (CRP) using serum/plasma samples. Serum lipids were measured with standardized enzymatic methods according to the Centers for Disease Control and Prevention. LDL-C was estimated by the Friedewald equation [243]. We measured LDL-C directly when the value of triglycerides exceeded 4.52 mmol/L (400 mg/dL). Hexokinase-glucose-6-phosphate-dehydrogenase enzymatic assay was used to measure serum glucose. We defined diabetes as individuals with fasting glucose ≥7.0 mmol/L or use of medications for diabetes.

2.3.4 Measurement of Lipoprotein Subclasses

Using serum samples stored at −70C°, NMR spectroscopy (LipoScience, Raleigh, NC) was used to measure lipoprotein subclass particle concentrations and average VLDL, LDL, and HDL particle diameters. As the basis for the quantification, the NMR method uses the characteristic signals broadcast by lipoprotein subclasses of different size. The NMR method measured the particle concentrations of the following lipoprotein species: 3 VLDL subclasses (large, >60 nm; medium, 35-60 nm; and small, 27-35 nm), 3 LDL subclasses (intermediate-density lipoprotein [IDL], 23-27 nm; large, 21.3-23 nm; small, 18.3-21.2 nm), and 3 HDL subclasses (large, 8.8-13 nm; medium, 8.2- 8.8 nm; and small, 7.3-8.2 nm). Weighted average particle sizes (average diameters) of VLDL-P, LDL-P, and HDL-P were calculated from the subclass levels.

2.3.5 Statistical Analysis

The distribution of continuous variables was assessed. CRP, triglycerides, ethanol intake, large VLDL-P, and medium VLDL-P were highly skewed, and therefore log transformation was applied to these measures after the addition of one unit. All other continuous variables with nearly normal distribution were standardized. Agatston CCS was categorized into two groups (<10 and ≥10). A CCS cutoff point of 10 was selected due to the possibility that scores ranging from 1-9 may be an imaging artifact from a spurious noise and have a low reliability [55, 244]. Socio-demographic characteristics of study participants, standard lipids, and NMR-measured lipoprotein distributions were analyzed per race/ethnicity. Normally distributed continuous variables were expressed as means±standard deviations (SD) and compared using a two-sample t-test. Highly skewed continuous variables were expressed as a median and interquartile range and compared using the Mann-Whitney U test. Categorical variables were expressed in percentages and compared using the Chi-square statistic.

We used age-adjusted logistic regression for US White and Japanese separately to determine the association of traditional lipids and lipoproteins (particle concentrations and their sizes) with CCS ≥10. Furthermore, we calculated the age-adjusted odds ratio (OR) for race/ethnicity (reference group= Japanese) with CCS ≥10 as an outcome. In addition to age, we further adjusted for individual lipids and lipoproteins in separate logistic regression models to assess the change from the age-adjusted OR for race/ethnicity. To determine the independent effect of race/ethnicity, we used multivariable logistic regression after adjustment for traditional cardiovascular risk factors (age, smoking, BMI, hypertension, diabetes, HDL-C, LDL-C, and triglycerides), alcohol consumption, inflammatory markers (CRP and fibrinogen), and lipoproteins which have shown major changes in OR for race/ethnicity in age-adjusted logistic

regression models. In multivariable models, we also assessed for the presence of an interaction between race/ethnicity and individual lipoproteins on CAC.

For 'lipoprotein models' [models with traditional cardiovascular risk factors, alcohol consumption, inflammatory markers, and lipoproteins], compared to the 'referent model' [traditional cardiovascular risk factors, alcohol consumption, and inflammatory markers], the area under the receiver operator characteristic curve (AUC) was used to assess the concordance of predictive value. The significance of the difference between the two AUC was tested using the Wilcoxon test. To assess reclassification improvement by lipoproteins, we used the Net Reclassification Improvement (NRI) index and the Integrated Discrimination Improvement (IDI) index to compare our models (lipoprotein models vs. referent model). For NRI, we defined four categories of risk <5.0%, 5.0% to <10.0%, 10.0% to <20.0%, and ≥20%. The NRI distinguished the movements in reclassified categories by the observed outcome (CCS ≥10). The IDI measured the mean difference in the predicted probabilities between CCS ≥10 and CCS <10. All *p*-values were two-tailed and *p*-values <0.05 were considered significant. SAS version 9.4 (SAS Institute, Cary, North Carolina) was used for all statistical analyses.

2.4 RESULTS

The prevalence of CCS ≥10 was 23.0% in US White and 11.0% in Japanese (Table 2-1). US White compared to Japanese had higher levels of inflammatory markers (CRP and fibrinogen), fasting insulin, and were more obese. Japanese compared with US White had less favorable profiles for the presence of hypertension, fasting glucose, smoking status, drinking status, and average alcohol consumption per day.

The two populations had similar levels of total LDL-P, small LDL-P, large LDL-P, LDL particle size, and total VLDL-P (*p-value* >0.05) (Table 2-2). US White had significantly higher small HDL-P, large VLDL-P, and larger VLDL particle size. In addition, from the total VLDL-P, US White had a higher proportion of large VLDL-P compared to Japanese (6.9% vs. 4.2%) (data not shown). Japanese had significantly higher HDL-C, IDL-P, total HDL-P, medium HDL-P, large HDL-P and small VLDL-P, and larger HDL particle size compared to US White.

Within both populations, total LDL-P was significantly associated with CCS ≥10 (Figures 2-1 and 2-2). Among US White, triglycerides, small LDL-P, LDL particle size, IDL, VLDL particle size, and large VLDL-P were significantly associated with CCS ≥10 (Figure 2-1). Among Japanese, LDL-C, HDL particle size, small HDL-P, and large HDL-P were significantly associated with CCS ≥10 (Figure 2-2).

The age-adjusted odds of CCS ≥10 for US White were 2.58 times higher compared with Japanese (95% CI = 1.61, 4.12) (Table 2-3). Major changes in the odds of CCS ≥10 for US White were seen with further adjustment for HDL-C, HDL particle size, VLDL particle size, medium HDL-P, large HDL-P, and large VLDL-P. In a model containing age and large HDL-P, odds of CCS ≥10 for US Whites were 1.85 times higher compared to Japanese (95% CI = 1.10, 3.11). There was an approximately 29% reduction in the age-adjusted OR for US White after adjustment for large HDL-P. There was no significant interaction between lipids or lipoproteins and CCS ≥10 by race/ethnicity.

US White were 2.72 times more likely to have CCS ≥10 compared to Japanese after adjustment for traditional cardiovascular risk factors (Model I, OR = 2.72, 95% CI = 1.35, 5.48) (Table 2-4). With further adjustment for alcohol consumption and inflammatory markers, the odds of CCS ≥10 for US White increased nearly to 3.25 (Model II,OR = 3.25, 95% CI = 1.55,

6.84). Major changes in the odds of CCS ≥10 for US White were noticed with further adjustment for large HDL-P (Model II-A, OR = 2.90, 95% CI = 1.33, 6.34) or VLDL particle size (Model II-C, OR = 2.98, 95% CI = 1.40, 6.35) or large VLDL-P (Model II-D, OR = 3.07, 95% CI = 1.42, 6.68). Major attenuation (16 to 19%) in the odds of CCS ≥10 for US White was seen after including large HDL-P and VLDL-P/ VLDL particle size (models III-A and III-B) together in a model including CVD risk factors. In a fully adjusted model (model IV-A) including CVD risk factors, total LDL-P large HDL-P, and VLDL particle size, US White had 2.58 times greater odds of CCS ≥10 compared to Japanese men (OR = 2.58, 95% CI = 1.16, 5.77, *p*-value <0.001).

The AUC for the 'lipoprotein models' [Model IV-A/IV-B= traditional cardiovascular risk factors + alcohol consumption + inflammatory markers + total LDL-P + large HDl-P + VLDL-P/ VLDL particle size], compared to the 'referent model' [Model II = traditional cardiovascular risk factors + alcohol consuption + inflammatory markers], was not significantly different. Based on NRI and IDI, no significant reclassification improvement was found with the addition of total LDL-P + large HDL-P + VLDL-P/ VLDL particle size to the referent model (Table 2-4).

2.5 DISCUSSION

In a community-based sample of healthy middle-aged men, US White compared with Japanese had significant differences in lipoprotein particle distributions. US White had higher concentrations of small HDL-P, large VLDL-P; lower concentrations of IDL-P, total HDL-P, medium HDL-P, large HDL-P, small VLDL-P; larger VLDL particle size and smaller HDL particle size. US White had 3.25 times higher odds of CCS ≥10 compared to Japanese adjusting for traditional cardiovascular risk factors, alcohol consumption, and inflammatory markers. This

difference was partially attenuated with further adjustment for large HDL-P and large VLDL-P/VLDL particle size. Several studies have reported an inverse association of large HDL-P with atherosclerosis and CVD [245]. On the other hand, large VLDL-P or larger VLDL particle size, have shown the positive associations with CVD [73, 237]. In our study, US White compared with Japanese had significantly lower large HDL-P, higher large VLDL-P, and larger VLDL particle size which could explain the partial attenuation in the odds of having CCS ≥10 among US White after adjustment for large HDL-P and higher large VLDL-P or larger VLDL particle size.

The finding of significantly higher large HDL-P and larger HDL particle size among Japanese compared to US White is compatible with previous findings mentioned on differences between the two populations in certain lifestyle factors such as alcohol consumption, eating of fish, and lean BMI. Increased alcohol consumption, higher fish intake, and lean BMI are reported to be directly associated with the activity of cholesterol ester transfer protein (CETP [246, 247]), which is associated with higher total HDL-P, large size HDL particles, and a higher concentration of total HDL-C [246-249]. Since, Japanese eat more fish [250], drink more alcohol, and have lower BMI than their US counterparts, as shown in this study [55] and other studies [248, 249], these specific features of Japanese men may have increased their HDL size, large HDL-P concentration as well as total HDL-C.

Similarly, the finding of larger VLDL particle size and higher large VLDL-P among US White compared to Japanese is consistent with reports from the literature on plasma concentrations of lipoproteins and obesity [251]. The prevalence of obesity is likely to be substantially increased in US White compared with Japanese, not only during the time of the survey but also in the past [231, 252], and therefore the lifetime burden of (exposure to) obesity

differes in these two populations. One potential mechanism for an association between obesity and atherosclerosis is increased plasma concentrations of triglyceride-rich large VLDL-P and larger VLDL size [253]. Triglyceride-rich large VLDL-P are found in the human intima and have been isolated from atherosclerotic lesions [254]. Large VLDL-P have high-affinity for LDL receptors and bind to a unique triglyceride-rich lipoproteins/apoB48 receptors expressed specifically on monocytes, macrophages, and endothelial cells [255]. Large VLDL-P cause rapid, receptor-mediated macrophage lipid accumulation and are related to the the progression of atherosclerosis in humans [256]. Increased secretion of large VLDL-P also favors the transfer of triglyceride from triglyceride-rich lipoprotein to LDL-P and HDL-P through the action of CETP [257]. Subsequently, increased hepatic lipase activity converts the triglyceride-rich LDL-P and HDL-P to small LDL-P and small HDL-P respectively [257]. Further, this process is also related to the lowered HDL-C which was noted to have a relatively dose-response relationship to reduced levels of large HDL-P [258]. Consistent with the lipoprotein metabolism theory mentioned above, in our study, US White had higher small HDL-P and lower total HDL-P, large HDL-P, and HDL-C compared to Japanese.

Differences in the prevalence of CAC between the two race/ethnicities cannot be attributed to their lifetime exposure to traditional risk factors or different genetic make-up or genetic responses to various risk factors. Differences in the prevalence of CAC because of lifetime exposure to traditional risk factors is unlikely because available data from national or population-based surveys indicates that US White and Japanese have very similar levels of total cholesterol and blood pressure from childhood to adulthood [230, 259]. Furthermore, US White have much lower rates of cigarette smoking than Japanese [230, 260]. Similarly, differences in the prevalence of CAC between US White and Japanese because of genetic factors is very

unlikely because a study of Japanese migrants to the US showed that Japanese residing in America have similar or higher levels of subclinical atherosclerosis compared to US White [234]. In a supplementary analysis, Japanese Americans compared to Japanese in Japan had significantly different NMR-measured lipoprotein particle distributions. Japanese American had a significantly higher prevalence of CCS ≥10 compared to Japanese after adjustment for CHD risk factors [OR = 4.15; 95% CI = 2.05, 8.38], and this difference was partially attenuated (16% to 23%) with further adjustment large HDL-P and large VLDL-P (Tables 2-5 and 2-6).

One possible mechanism for the difference in the prevalence of CAC between two populations could be increased insulin resistance among US White indicated by higher BMI. Insulin resistance is independently associated with prevalence of CAC in both US White [261] and Japanese [262]. US White are expected to be more insulin resistant because they had significantly higher BMI compared to Japanese. Their lipid and lipoprotein profile (higher large VLDL-P, small HDL-P, larger VLDL particle size, and lower total HDL-P, medium HDL-P, large HDL-P, small VLDL-P, and smaller HDL particle size) is also consistent with the lipid and lipoprotein profile seen in insulin resistant individuals [251]. However, in this study with further adjustment for fasting insulin or HOMA-IR [Homeostasis model assessment of insulin resistance (calculated as: insulin (IU/l)×(glucose [mg/dl])/22.5)]) above other CHD risk factors, attenuation in the difference in CAC prevalence between the two population was very minimal (data not shown). Also, the magnitude of reduction in the difference in the CAC prevalence between the two populations after adjusting for large HDL-P and large VLDL-P/VLDL particle size above CHD risk factors was very similar with or without adjustment for fasting insulin or HOMA-IR (data not shown).

Findings of the present study should be considered in light of important limitations. First, the study is cross-sectional in design, and we cannot confirm any causality between CAC and lipoproteins. Second, our study examined apparently healthy men aged 40-49 years in the US or Japan only; therefore, the results of the study cannot be generalized to other populations and age groups. Third, CAC may not detect some atherosclerosis plaques, because it is not a direct measure of coronary atherosclerosis. Despite this limitation, CAC is a reliable biomarker of coronary atherosclerosis and independently predicts CHD [228]. Fourth, we analyzed blood samples obtained at one-time point only. Fifth, although, we adjusted for several covariates in multivariable logistic regression, we cannot exclude the possibility of residual confounding because of unmeasured variables.

This study has several strengths. This was the first community-based study trying to explore the association of NMR lipoprotein distributions and difference in prevalence of CAC between US White and Japanese. All laboratory analyses and CAC measurements were conducted at the same laboratory. Although the population sizes for both US White and Japanese were not large, the age range was narrow 40-49 years, possibly providing greater precision for examining the stated hypothesis in an apparently healthy population. We focused on male gender and age group 40-49 years, because population levels of total cholesterol and blood pressure have been similar in these US White and Japanese populations throughout their lifetime [230, 259].

2.6 CONCLUSION

In a community-based sample of asymptomatic US White and Japanese men aged 40-49 years, US White had significantly different lipoprotein particle distributions compared to the Japanese. Despite having an adverse profile for major independent risk factors among Japanese, US White had significantly higher prevalence of CAC compared to Japanese, and this difference could not be entirely attributed to variations in the distribution of lipoproteins. Our findings support the notion that there is a common source exposure in the diet among the Japanese which may account for their lower rates of atherosclerosis and CHD. Further investigations are needed.

2.7 TABLES

Table 2-1. Demographic and clinical characteristics of US White men in Allegheny County, US, and Japanese men in Kusatsu, Japan

Variables	US White Men (n=270)		Japanese Men (n=300)		*p*-value
	Mean	SD	Mean	SD	
Age[a], years	44.9	2.8	45.1	2.8	0.442
Waist circumference[a], cm	98.0	11.3	85.1	8.2	<0.001
Body mass index[a], kg/m^2	27.7	4.0	23.7	3.1	<0.001
Pack-years of smoking[b]	0.0	(0.0, 2.0)	18.75	(3.0, 29.0)	<0.001
Systolic blood pressure[a], mmHg	122.8	11.4	124.8	15.7	0.092
Hypertension[c]	13.3	-	25.7	-	<0.001
Current smoker[c]	7.8	-	49.3	-	<0.001
Current drinker[c]	45.6	-	67.2	-	<0.001
Alcohol consumption[b], gm/day	4.9	(1.0, 16.5)	14.3	(2.4, 42.0)	<0.001
Fasting glucose[a], mg/dL	101.2	15.4	106.7	18.6	<0.001
Diabetes mellitus[c]	3.3	-	5.7	-	0.182
Fibrinogen[a], µmol/L	8.5	2.1	7.5	1.9	<0.001
Fasting insulin[a], (µIU/ml)	14.7	7.6	10.2	4.4	<0.001
C-reactive protein[b], nmol/L	8.6	(4.8, 17.1)	2.9	(1.9, 6.7)	<0.001
Agatston coronary calcium score $\geq$10[c]	23.0	-	11.0	-	<0.001
Calcium score percentiles (50^{th}, 75^{th}, 90^{th}, 95th)	(0, 8.9, 45.6, 119.8)		(0, 1.5, 13, 37.2)		-

[a]Continuous normally distributed variables expressed in mean (standard deviation (SD)) and compared using a two-sample t-test;

[b]Continuous non-normally distributed variables expressed in median (interquartile range) and compared using Mann-Whitney U test;

[c]Categorical variable expressed as percentages and compared using Pearson's chi-squared test;

SI conversion factors: To convert glucose to mmol/L, multiply values by 0.0555

Table 2-2. Lipoprotein subfractions by chemical analysis and NMR spectroscopy in US White men in Allegheny County, US, and Japanese men in Kusatsu, Japan, 2002-2006

Lipids and Lipoprotein Profile	US White Men (n=270)		Japanese Men (n=300)		*p*-value
	Mean	SD	Mean	SD	
Standard Lipids					
Total cholesterol**,** mmol/L	5.6	1.0	5.6	0.9	0.554
LDL-C**,** mmol/L	3.6	0.9	3.4	0.9	0.070
HDL-C, mmol/L	1.2	0.3	1.4	0.4	0.011
Triglycerides**,** mmol/L	1.7	1.2	1.7	0.9	0.734
NMR-measured Lipoprotein Particles					
LDL particles					
Total LDL-P, nmol/L	1480.6	339.6	1469.4	400.9	0.721
Small LDL-P, nmol/L	686.6	358.9	663.1	358.0	0.432
Large LDL-P, nmol/L	661.1	314.1	654.0	254.2	0.774
IDL-P, nmol/L	132.9	100.1	152.3	112.0	0.031
LDL Size, nm	20.9	0.7	20.9	0.7	0.192
HDL particles					
Total HDL-P, umol/L	31.2	5.8	35 .6	6.8	<0.001
Small HDL-P, umol/L	20.5	4.4	17.2	5.6	<0.001
Medium HDL-P, umol/L	7.7	4.0	12.1	6.3	<0.001
Large HDL-P, umol/L	3.1	2.7	6.3	3.6	<0.001
HDL Size, nm	8.5	0.6	9.1	0.6	<0.001
VLDL particles					
Total VLDL-P, nmol/L	83.5	43.2	87.5	47.5	0.285
Small VLDL-P, nmol/L	40.3	24.0	44.9	28.0	0.031
Medium VLDL-P[a]**,** nmol/L	32.0	(14.0, 53.0)	32.0	(15.0, 53.0)	0.813
Large VLDL-P[a], nmol/L	3.0	(1.0, 7.0)	1.0	(0, 4.0)	<0.001
VLDL Size, nm	47.0	7.8	44.8	7.1	<0.001

NMR, nuclear magnetic resonance; LDL, low-density lipoprotein; IDL, intermediate density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; SD, standard deviation; All continuous nearly normally distributed variables expressed in mean (SD) and compared using a two-sample t-test;

[a]Continuous variables with skewed distribution were expressed in median (interquartile range) and compared using Mann-Whitney U test;

Table 2-3. Age-adjusted odds ratio for CCS ≥10 in US White men in Allegheny County US compared to Japanese men in Kusatsu, Japan (Reference group for race/ethnicity = Japanese)

Logistic Regression Models	Race/Ethnicity OR (95% CI)	Percent change in OR[a]	Lipids and lipoproteins OR (95% CI)
Age	2.58 (1.61, 4.12)	-	-
Age and Standard Lipids			
Age + LDL-C	2.56 (1.60, 4.12)	-0.77	1.36 (1.07, 1.72)
Age + HDL-C	2.36 (1.46, 3.82)	-8.53	0.81 (0.63, 1.04)
Age + Triglycerides	2.66 (1.66, 4.26)	+3.10	1.60 (1.02, 2.51)
Age and NMR-measured Lipoprotein Particles			
Age and LDL particles			
Age + Total LDL-P	2.66 (1.65, 4.29)	+3.10	1.44 (1.13, 1.82)
Age + Small LDL-P	2.57 (1.60, 4.13)	0.00	1.40 (1.13, 1.75)
Age + Large LDL-P	2.57 (1.61, 4.11)	0.00	0.88 (0.71, 1.10)
Age + IDL-P	2.76 (1.71, 4.45)	+6.98	1.35 (1.09, 1.68)
Age + LDL Size	2.66 (1.66, 4.27)	+3.10	0.80 (0.64, 0.99)
Age and HDL particles			
Age + Total HDL-P	2.65 (1.61, 4.36)	+2.71	1.04 (0.81, 1.34)
Age + Small HDL-P	2.43 (1.49, 3.97)	- 5.81	1.11 (0.86, 1.43)
Age + Med HDL-P	**2.90 (1.73, 4.86)**	**+12.40**	1.16 (0.91, 1.48)
Age + Large HDL-P	**1.85 (1.10, 3.11)**	**- 28.29**	0.66 (0.49, 0.89)
Age + HDL Size	**1.99 (1.17, 3.37)**	**- 22.87**	0.75 (0.57, 0.98)
Age and VLDL particles			
Age + Total VLDL-P	2.63 (1.64, 4.22)	+1.94	1.18 (0.95, 1.46)
Age + Small VLDL-P	2.61 (1.63, 4.18)	+1.16	1.06 (0.84, 1.33)
Age + Med VLDL-P[b]	2.61 (1.63, 4.17)	+0.78	1.20 (0.95, 1.52)
Age +Large VLDL-P[b]	**2.32 (1.43, 3.75)**	**- 10.08**	1.27 (1.00, 1.61)
Age + VLDL Size	**2.38 (1.48, 3.83)**	**- 7.75**	1.22 (0.98, 1.53)

OR, odds ratio; Each lipid or lipoprotein was modeled separately in a model adjusted for age;

[a]Percent change in OR for race compared to age-adjusted model,

[a]Percent change in OR compared to age-adjusted model was calculated as: *[(OR for race in an age-adjusted model) – (OR for race in a given respective model)]*100 / [OR for race in an age-adjusted model]*

Bold font in above table indicates major change in OR for race

[b]Medium VLDL-P and large VLDL-P were log transformed after addition of one unit. All other continuous variables were standardized.

Table 2-4. Multivariable-adjusted OR of race/ethnicity (US White), change in OR, AUC, change in AUC, IDI, and NRI for CCS ≥10 when NMR-measured lipoproteins were added to referent model

Logistic Regression Models	OR (95% CI)	% change in OR compared to model II[a]	AUC	AUC change (*p*-value)	IDI (*p*-value)	NRI (*p*-value)
Model I	2.72 (1.35, 5.48)	-	76.6 (71.6, 82.3)	-	-	-
Model II[b]	3.25 (1.55, 6.84)	-	78.0 (72.8, 83.3)	-	-	-
Model II-A	2.90 (1.33, 6.34)	-10.76	78.0 (72.8, 83.2)	0.00 (0.88)	0.000 (0.95)	-0.019 (0.48)
Model II-B	3.23 (1.51, 6.93)	0.00	78.0 (72.7, 83.3)	0.00 (0.37)	-0.000 (0.72)	0.000 (1.00)
Model II-C	2.98 (1.40, 6.35)	-8.31	78.2 (73.0, 83.4)	0.20 (0.70)	0.003 (0.22)	0.008 (0.78)
Model II-D	3.07 (1.42, 6.68)	-5.54	78.0 (72.8, 83.2)	0.00 (0.83)	0.001 (0.54)	-0.008 (0.61)
Model III-A	2.62 (1.18, 5.82)	-19.39	78.2 (73.0, 83.4)	0.20 (0.70)	0.004 (0.28)	0.011 (0.75)
Model III-B	2.74 (1.21, 6.19)	-15.69	78.0 (72.8, 83.2)	0.00 (0.83)	0.001 (0.71)	-0.019 (0.48)
Model IV-A	2.58 (1.16, 5.77)	-20.61	78.2 (73.0, 83.4)	0.20 (0.65)	0.004 (0.26)	0.008 (0.79)
Model IV-B	2.73 (1.21, 6.17)	-16.00	78.1 (72.8, 83.2)	0.10 (0.75)	0.003 (0.70)	-0.034 (0.18)

OR, odds ratio; AUC, area under the receiver operating characteristic curve; IDI, Integrated Discrimination Improvement; NRI, Net Reclassification Improvement; Reclassification categories for NRI: <5.0%, 5.0-9.9%, 10.0-19.9%, and high ≥20%;
Model I: race, age, BMI, pack -year of smoking, hypertension, diabetes, triglyceride, LDL-C, HDL-C
[b]Model II (*Referent Model*): model I + alcohol intake + CRP + fibrinogen
Model II-A: model II + large HDL-P
Model II-B: model II + HDL particle size
Model II-C: model II + VLDL particle size
Model II-D: model II + large VLDL-P
Model III-A: model II + large HDL-P + VLDL size
Model III-B: model II + large HDL-P + large VLDL-P
Model IV-A: model II + total LDL-P + large HDL-P + VLDL size
Model IV-B: model II + total LDL-P + large HDL-P + large VLDL-P
[a]Percent change in OR for race compared to model II was calculated as: *[(OR for race in model II) – (OR for race in each model (model II-A to IV-B))]*100/ [OR for race in model II]*

Table 2-5. Age-adjusted odds ratio for CCS ≥10 in Japanese American men in Honululu, US compared to Japanese men in Kusatsu, Japan (Reference group for race/ethnicity = Japanese)

Logistic Regression Models	Race/Ethnicity OR (95% CI)	Percent change in OR[a]	Lipids and lipoproteins OR (95% CI)
Age	2.87 (1.79, 4.59)	-	-
Age and Standard Lipids			
Age + LDL-C	3.06 (1.90, 4.94)	-0.77	1.33 (1.05, 1.67)
Age + HDL-C	2.79 (1.73, 4.49)	-8.53	1.00 (0.79, 1.25)
Age + Triglycerides	2.79 (1.74, 4.48)	+3.10	1.47 (0.98, 2.23)
Age and NMR-measured Lipoprotein Particles			
Age and LDL particles			
Age + Total LDL-P	3.26 (2.00, 5.32)	+3.10	1.37 (1.08, 1.73)
Age + Small LDL-P	2.70 (1.68, 4.35)	0.00	1.37 (1.10, 1.70)
Age + Large LDL-P	2.65 (1.58, 4.43)	0.00	0.92 (0.71, 1.18)
Age + IDL-P	2.96 (1.84, 4.76)	+6.98	1.22 (0.98, 1.53)
Age + LDL Size	2.76 (1.71, 4.46)	+3.10	0.93 (0.74, 1.17)
Age and HDL particles			
Age + Total HDL-P	2.79 (1.74, 4.47)	+2.71	1.20 (0.94, 1.52)
Age + Small HDL-P	2.20 (1.32, 3.65)	- 5.81	1.44 (1.09, 1.91)
Age + Med HDL-P	**2.90 (1.80, 4.67)**	**+12.40**	1.07 (0.84, 1.37)
Age + Large HDL-P	**2.50 (1.54, 4.07)**	**- 28.29**	0.78 (0.60, 1.01)
Age + HDL Size	**2.70 (1.66, 4.38)**	**- 22.87**	0.95 (0.75, 1.21)
Age and VLDL particles			
Age + Total VLDL-P	2.76 (1.72, 4.43)	+1.94	1.19 (0.95, 1.48)
Age + Small VLDL-P	2.84 (1.78, 4.56)	+1.16	1.14 (0.92, 1.41)
Age + Med VLDL-P[b]	2.73 (1.70, 4.38)	+0.78	1.31 (1.00, 1.70)
Age +Large VLDL-P[b]	**2.61 (1.60, 4.28)**	**- 10.08**	1.14 (0.90, 1.45)
Age + VLDL Size	**2.81 (1.74, 4.55)**	**- 7.75**	0.98 (0.78, 1.24)

OR, odds ratio; Each lipid or lipoprotein was modeled separately in a model adjusted for age;

[a]Percent change in OR for race compared to age-adjusted model,

[a]Percent change in OR compared to age-adjusted model was calculated as: *[(OR for race in an age-adjusted model) – (OR for race in a given respective model)]*100 / [OR for race in an age-adjusted model]*

Bold font in above table indicates major change in OR for race

[b]Variables Medium VLDL-P and large VLDL-P were log transformed after addition of one unit. All other continuous variables were standardized.

Table 2-6. Multivariable-adjusted OR of race/ethnicity (Japanese American), change in OR, AUC, change in AUC, IDI, and NRI for CCS ≥10 when NMR-measured lipoproteins were added to referent model

Logistic Regression Models	OR (95% CI)	% change in OR compared to model II[a]	AUC	AUC change (*p*-value)	IDI (*p*-value)	NRI (*p*-value)
Model I	3.84 (1.97, 7.48)	-	75.5 (70.5, 80.6)	-	-	-
Model II[b]	4.15 (2.05, 8.38)	-	77.1 (72.3, 82.0)	-	-	-
Model II-A	3.43 (1.67, 7.03)	-17.59	77.4 (72.6, 82.1)	0.03 (0.56)	0.008 (0.18)	0.064 (0.15)
Model II-B	4.16 (2.05, 8.44)	0.00	77.1 (72.2, 82.1)	0.00 (0.27)	0.000 (0.97)	0.015 (0.26)
Model II-C	4.39 (2.14, 9.01)	5.78	76.7 (71.8, 81.6)	-0.40 (0.93)	0.003 (0.46)	0.077 (0.02)
Model II-D	4.03 (1.94, 8.37)	-2.89	76.9 (72.0, 81.8)	-0.20 (0.76)	0.001 (0.33)	0.014 (0.41)
Model III-A	3.70 (1.78, 7.70)	-10.84	77.4 (72.6, 82.1)	0.30 (0.52)	0.012 (0.07)	0.056 (0.24)
Model III-B	3.46 (1.65, 7.27)	-16.62	77.4 (72.7, 82.2)	0.30 (0.53)	0.014 (0.04)	0.071 (0.13)
Model IV-A	3.46 (1.62, 7.38)	-16.62	77.6 (72.8, 82.2)	0.50 (0.41)	0.013 (0.06)	0.070 (0.14)
Model IV-B	3.20 (1.50, 6.83)	-22.89	77.5 (72.0, 81.8)	0.40 (0.46)	0.016 (0.02)	0.111 (0.03)

OR, odds ratio; AUC, area under the receiver operating characteristic curve; IDI, Integrated Discrimination Improvement; NRI, Net Reclassification Improvement; Reclassification categories for NRI: <5.0%, 5.0-9.9%, 10.0-19.9%, and high ≥20%;
Model I: race, age, BMI, pack -year of smoking, hypertension, diabetes, triglyceride, LDL-C, HDL-C
[b]Model II (*Referent Model*): model I + alcohol intake + CRP + fibrinogen
Model II-A: model II + large HDL-P
Model II-B: model II + HDL particle size
Model II-C: model II + VLDL particle size
Model II-D: model II + large VLDL-P
Model III-A: model II + large HDL-P + VLDL size
Model III-B: model II + large HDL-P + large VLDL-P
Model IV-A: model II + total LDL-P + large HDL-P + VLDL size
Model IV-B: model II + total LDL-P + large HDL-P + large VLDL-P
[a]Percent change in OR for race compared to model II was calculated as: *[(OR for race in model II) – (OR for race in each model (model II-A to IV-B))]*100/ [OR for race in model II]*

2.8 FIGURES

Figure 2-1. Age-adjusted association of lipids and lipoproteins sub-fractions with the CCS ≥10 in US White men in Allegheny County, US, 2002-2006

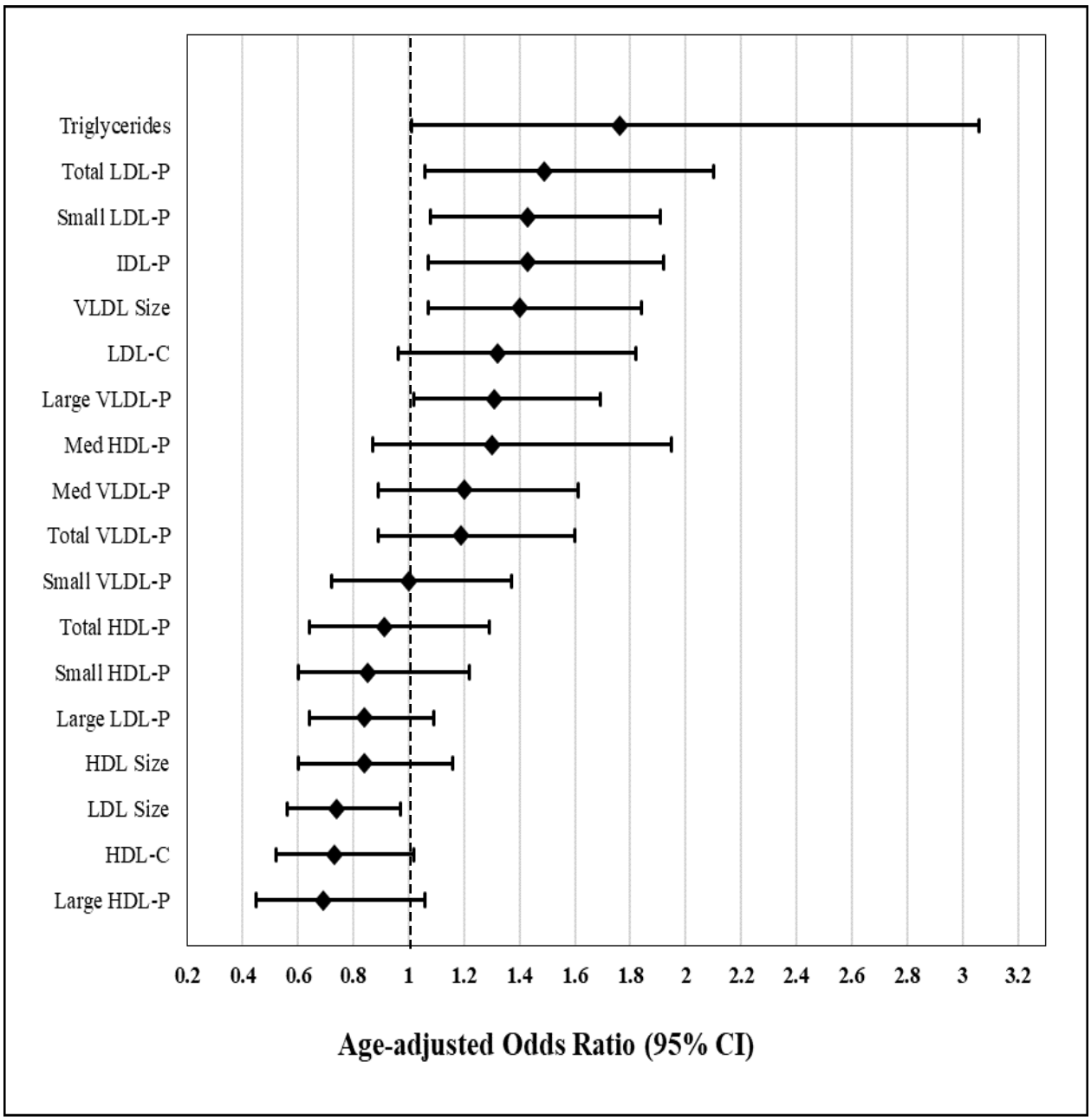
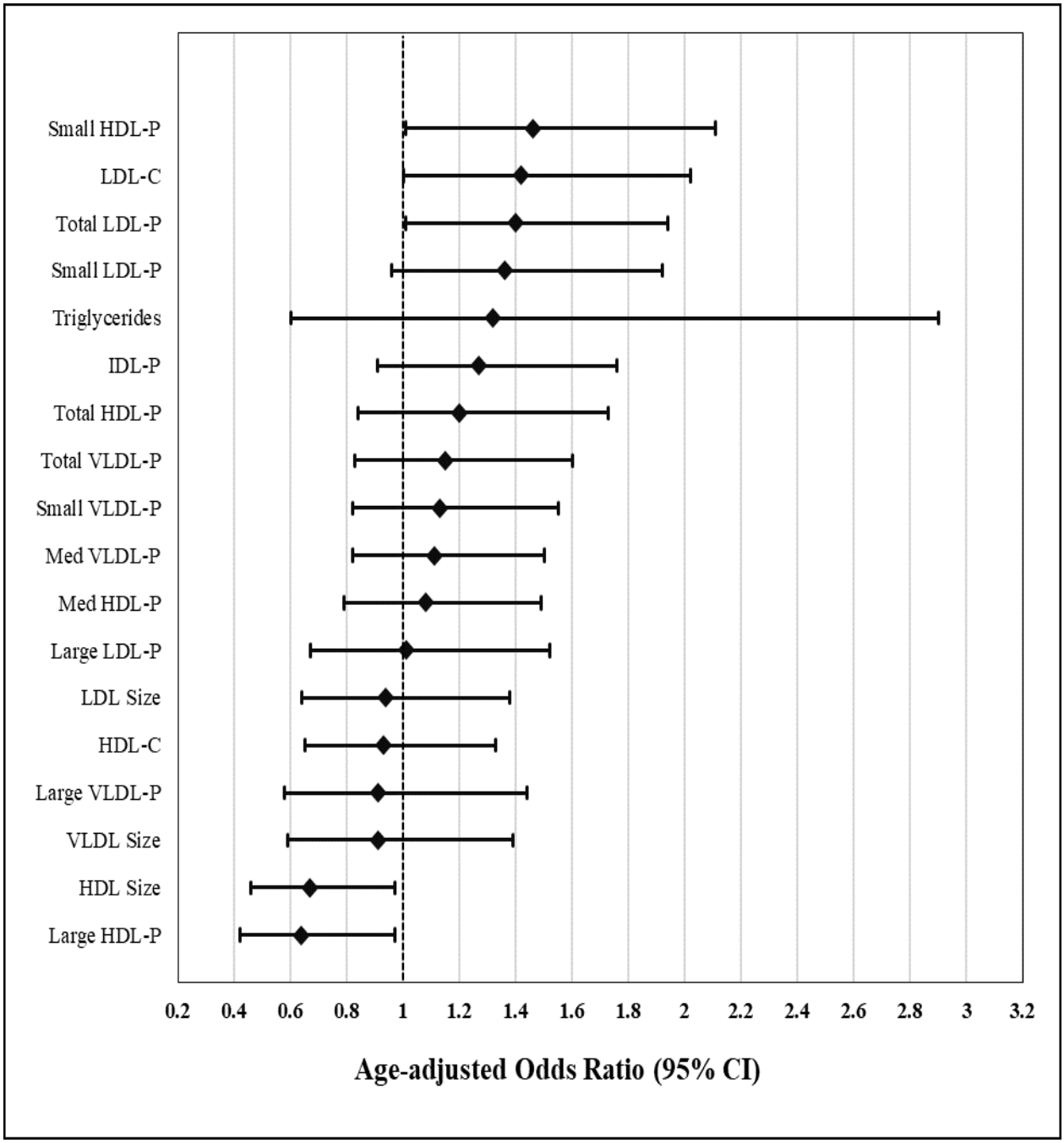


Figure 2-2. Age-adjusted association of lipids and lipoproteins sub-fractions with the CCS ≥10 in Japanese men in Kusatsu, Japan, 2002-2006



3.0 MANUSCRIPT II: ASSOCIATION OF ALCOHOL CONSUMPTION AND AORTIC CALCIFICATION IN HEALTHY MEN AGED 40-49 YEARS FOR THE ERA-JUMP STUDY

A manuscript in preparation for publication

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3.1 ABSTRACT

Background and aims: Several studies have reported a significant inverse association of light to moderate alcohol consumption with coronary heart disease (CHD). However, studies assessing the relationship between alcohol consumption and atherosclerosis have reported inconsistent results. The current study was conducted to determine the relationship between alcohol consumption and aortic calcification**.**

Methods: We examined the research question using data from the population-based ERA-JUMP Study, comprising of 1006 healthy men aged 40-49 years without clinical cardiovascular diseases from four race/ethnicities: 301 White, 103 African American, 292 Japanese American, and 310 Japanese in Japan. Aortic calcification was assessed by electron-beam computed tomography and quantified using the Agatston method. Alcohol consumption was categorized into four groups: 0 (nondrinkers), ≤1 (light drinkers), >1 to ≤3 (moderate drinkers) and >3 drinks per day (heavy drinkers) (1 drink = 12.5 grams of ethanol). Tobit conditional regression and ordinal logistic regression were used to investigate the association of alcohol consumption with aortic calcification after adjsusting for cardiovascular risk factors and potential confounders.

Results: The study participants consisted of 25.6% nondrinkers, 35.3% light drinkers, 23.5% moderate drinkers, and 15.6% heavy drinkers. Heavy drinkers [Tobit ratio (95% CI) = 2.34 (1.10, 4.97); Odds ratio (95% CI) = 1.67 (1.11, 2.52)] had significantly higher expected aortic calcification score compared to nondrinkers after adjusting for socio-demographic and confounding variables. There was no significant interaction between alcohol consumption and race/ethnicity on aortic calcification.

Conclusion: Our findings suggest that the heavy alcohol consumption may be an independent risk factor for atherosclerosis.

Keywords: Alcohol, Aorta, Atherosclerosis, Calcification, Men

3.2 INTRODUCTION

Although a J-shaped association has been established between alcohol consumption and coronary heart disease (CHD) [116], with light to moderate drinkers showing a reduced risk compared with both heavy drinkers and nondrinkers, the underlying pathophysiological mechanisms remain to be elucidated. Plausible mechanisms for the protective effect of moderate alcohol consumption on CHD include: increased in high-density lipoprotein cholesterol (HDL-C), lower inflammation, anticoagulant effect (inhibition of the fibrinolytic system), improve endothelial function, and a reduced risk of type 2 diabetes mellitus [122, 151, 263, 264]. Studies assessing the relationship between alcohol use and atherosclerosis - the major underlying cause of CHD [265], reported conflicting results: no significant association [138, 147], a U or J-shaped association [139-142], and a dose-response association [143-145]. The reason for these inconsistent results is not clear. It is important to investigate the relationship between alcohol and atherosclerosis because it may help clarify the mechanisms underlying the association between alcohol and CHD.

Aortic calcification, a surrogate biomarker of generalized atherosclerosis, is independently associated with cardiovascular morbidity and mortality [27, 266] and has a high specificity for detection of severe coronary atherosclerosis [267]. Aortic calcification is a less commonly used measure of atherosclerosis compared to coronary artery calcification (CAC). Some studies have reported that aortic calcification may be a better measure of atherosclerosis than CAC - a well established biomarker of atherosclerosis, because it is more prevalent, has an

earlier onset [28], has a better association with cardiovascular risk factors [29, 266, 268], and seems to add prognostic information of atherosclerotic burden beyond CAC [29, 266]. However, unlike CAC, studies examining the relationship between alcohol consumption and aortic calcification are scarce [28, 140, 143]. Moreover, the available results are inconsistent partly because of variability in studied populations, aortic segment examined, imaging modalities, and scoring method used, all of which avert unbiased comparisons [28, 137, 143].

Our objective was to determine the relationship between alcohol consumption and aortic calcification measured in asymptomatic men aged 40-49 years using data from the ERA-JUMP Study (the Electron Beam Computed Tomography (**E**BCT), **r**isk factor **a**ssessment among **J**apanese and the United States (**U**S) **m**en in the **p**ost-World-War-II birth cohort). Based on our previous finding of a J-shaped association between alcohol consumption and CAC among Japanese in Japan [148] as well as following the notion of a J-shaped association between alcohol consumption and CHD, we hypothesized that light to moderate alcohol consumption would have an inverse association, and heavy alcohol consumption would have a positive association with aortic calcification. To our knowledge, this is the first population-based study exploring the association of alcohol consumption and aortic calcification among asymptomatic middle-aged men across different races/ethnicities from various countries in a standardized manner.

3.3 METHODS AND MATERIALS

3.3.1 Study Population

The details of the study protocol have been described previously [269]. Briefly, during 2002–2006, a population-based sample of 1033 men aged 40–49 years, with no clinical cardiovascular diseases (CVD) or other severe illnesses, was obtained from 3 centers: 310 White and 107 Black from Pittsburgh, Pennsylvania, US; 303 Japanese American from Honolulu, Hawaii, US; 313 Japanese from Kusatsu City, Shiga, Japan [269, 270]. The study protocol followed 'the 1975 Declaration of Helsinki ethical guidelines'. The Institutional Review Boards of University of Pittsburgh, Pittsburgh, US; Kuakini Medical Center, Honolulu, Hawaii, US; Shiga University of Medical Science, Otsu, Japan approved the study. Written informed consent was obtained from all participants. We excluded participants with missing data for aortic calcification (n=27). Our final sample size was 1006 with 301 US White, 103 US Black, 292 Japanese American, and 310 Japanese in Japan.

3.3.2 Risk Factor Assessment

All participants underwent a physical examination, completed a lifestyle questionnaire, and a laboratory assessment as described previously [269, 270]. Data collection procedures were standardized across all centers. Body weight and height were measured while the participant was wearing light clothing without shoes. Body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m^2). Blood pressure and heart rate were measured after the participant emptied his bladder and sat quietly for 5 minutes. Blood pressure was measured twice

on the right arm with an automated sphygmomanometer (BP-8800, Colin Medical Technology, Komaki, Japan) using an appropriately sized cuff; the average of the two measurements was used. Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg or use of antihypertensive medications [271]. Participants were considered smokers if they reported current use of cigarettes or having stopped smoking within the past 30 days. Pack-years of smoking were calculated as years of smoking multiplied by the number of cigarettes per day divided by 20. Use of medications (antihypertensive, antidiabetic, and lipid-lowering) was reported as 'yes/no'. Meat intake was defined as intake of beef, pork, or sausage ≥2 times per week. Physical activity related to the current job was self-reported and categorized into sedentary, light, medium, and heavy physical activity [272].

Venipuncture was performed early in the clinic visit after a 12-hour fast. Blood samples were stored at -70 °C and shipped on dry ice from all the centers to the University of Pittsburgh. Serum lipids were determined using the protocol standardized by the Centers for Disease Control and Prevention including total cholesterol, HDL-C, and triglycerides [273]. Low-density lipoprotein cholesterol (LDL-C) was estimated by the Friedewald equation [243]. When the value of triglycerides exceeded 4.52 mmol/L (400 mg/dl), LDL-C was measured directly using an automated spectrophotometric assay [LDL Direct Liquid Select (Equal Diagnostics, Exton US)]. Serum glucose was determined by using hexokinase-glucose-6-phosphate-dehydrogenase enzymatic assay. Diabetes was defined as a fasting glucose ≥7.0 mmol/L or use of medications for diabetes [274]. C-reactive protein (CRP) was assessed using a calorimetric-competitive-enzyme-linked-immuno-sorbent assay, and fibrinogen was determined using an automated-clot-rate assay (Diagnostica Stago, Parsippany, U.S.)

3.3.3 Alcohol Consumption Assessment

The drinking habits of each subject were assessed by a validated self-administered questionnaires [275]. Alcohol consumption was assessed by asking whether the participant drank beer, wine, liquor, sake (Japanese rice wine), or other alcoholic beverages. The alcohol intake status of the study participants was classified as never drinker (lifetime abstainers), former drinkers, and current drinkers. Among current drinkers, alcohol consumption per day was estimated assuming that the concentration of alcohol was 5% for beer, 12% for wine, 40% for liquor, and 16% for sake. Current alcohol drinkers were further categorized into three groups: 'light drinkers' (≤1 drink), 'moderate drinkers' (>1 to ≤3 drinks), and 'heavy drinkers' (>3 drinks per day), with one drink, equal to 12.5 grams of alcohol [111] [which is approximately equivalent to 350 ml (12 oz) of regular beer, 150 ml (one glass) of wine, 45 ml of distilled spirits, and 110 ml of sake]. Former alcohol drinkers were combined with never drinkers (lifetime abstainers) and were together considered as 'nondrinkers.'

3.3.4 Aortic Calcification Assessment

EBCT was performed using a GE-Imatron C150 Electron Beam Tomography scanner (GE Medical Systems, South San Francisco, US) at the three center sites using standardized methods as described previously [55, 57]. The scanner was set to acquire 6 mm images from the aortic arch to the iliac bifurcation to evaluate aortic calcification. Technicians regularly calibrated scanners following a standardized protocol. All scan data were saved to optical disc. Readings of the scans were done centrally in the Cardiovascular Institute, University of Pittsburgh, using a DICOM (Digital Imaging and Communications in Medicine) workstation and software by

AccuImage (AccuImage Diagnostic Cooperation, San Francisco, US). The software program implemented the widely accepted Agatston scoring method [16]. One trained radiology technician evaluated the readings. The reader was blinded to each participant's characteristics and the study centers. The intra-reader reproducibility of non-zero Agatston Aortic Calcification Score (AoCaS) had an intra-class correlation of 0.98.

3.3.5 Statistical Analysis

Distributions of triglycerides, years of education, and pack-years of smoking were highly skewed and were therefore logarithmically transformed. Continuous variables with approximately normal distributions i.e., age, BMI, LDL-C, and HDL-C were standardized. Across the different categories of alcohol consumption as well as AoCaS (i) Age and race/ethnicity adjusted BMI, LDL-C, and HDL-C were expressed as means$\pm$standard error (SE); (ii) Age and race/ethnicity adjusted triglycerides, years of education, and pack-years of smoking were expressed as median and interquartile range; (iii) Age and race/ethnicity adjusted categorical variables were expressed in percentages. A *p*-value for trend across the different categories of alcohol consumption as well as AoCaS were determined using: linear regression when a response variable was continuous with normal distribution; quartile regression when a continuous variable with skewed distribution; and logistic regression when a response variable was categorical.

We used Tobit conditional regression and ordinal logistic regression to model the association of alcohol consumption and aortic calcification adjusting for potential confounders and intermediary variables. We considered Tobit conditional regression because it is suited to the uncommon distribution of AoCaS data (right sided skewness and many participants with AoCaS = 0) [276, 277]. For Tobit conditional regression, the outcome variable (i.e., aortic calcification)

was logarithmically transformed after adding of one unit to the original variable [ln (AoCaS + 1)]. “Tobit conditional regression is a combination of two regression approaches: a logistic regression of the presence of aortic calcification (AoCaS = 0 vs. AoCaS >0) and a linear regression of log-transformed aortic calcification when AoCaS >0 [278]. The combination of two regression approaches provides a single point estimate for the relationship of predictors with aortic calcification.” Secondarily, we also performed ordinal logistic regression to assess the likelihood of study participants being in a higher category of AoCaS. Four AoCaS categories were used: 0, 1-99, 100-299 and ≥300. To assess the relationship between alcohol consumption and aortic calcification, three models were constructed. Model I was adjusted for socio-demographic variables (age, race/ethnicity, and years of education); Model II was further adjusted for potential confounders (pack-years of smoking, BMI, diabetes, lipid-lowering medications, physical activity related to current job, meat intake, LDL-C, and CRP); Model III was additionally adjusted for intermediary variables (hypertension, HDL-C, triglycerides, and fibrinogen). In model III, we tested for an interaction between race/ethnicity and alcohol consumption on aortic calcification. To minimize the possibility of residual confounding, the inclusion of variables in regression models was based on the published literature on alcohol and atherosclerosis/CHD. In Tobit regression as well as in ordinal regression a *p*-value for linear and quadratic trend across the different categories of alcohol consumption was calculated using contrast.

We further conducted analyses stratifying by race/ethnicity because including the term ‘race’ in a multivariable model may not have provided adequate adjustment for race/ethnicity differences. In addition, because the inclusion of former drinkers and lifelong abstainers into the nondrinker group may have substantially increased the adverse effect of alcohol consumption

among nondrinkers, we repeated analysis by excluding former drinkers from the nondrinker category. We also evaluated the relationship of alcohol consumption and coronary artery calcification (CAC) by repeating the same statistical techniques and regression models used for alcohol consumption and aortic calcification. In the analysis examining the association between alcohol consumption and CAC, in Tobit conditional regression the outcome variable CAC was logarithmically transformed after addition of one unit [ln(CAC + 1)] and in ordinal logistic regression we used four CAC score categories: <10, 10-99, 100-299, and ≥300.

All *p*-values were two-tailed and *p*-value <0.05 was considered as significant. SAS version 9.4 (SAS Institute, Cary, North Carolina) and STATA version 14.0 (StataCorp LP, College Station, TX, US) were used for all statistical analyses.

3.4 RESULTS

Baseline characteristics of the total cohort as well as by race/ethnicity are presented in Table 3-1. We included 1006 study participants in our final analyses. Overall, mean (SD) age of the study participants was 45.3 (2.8) years. Study participants consisted of 25.6% nondrinkers [16.5% never drinkers + 9.1% former drinkers], 35.3% light drinkers, 23.5% moderate drinkers, and 15.6% heavy drinkers. Overall 56.9% study participants had AoCaS >0.

Table 3-2 describes the age and race/ethnicity adjusted demographic and clinical characteristics of participants across alcohol consumption categories. Compared to nondrinkers, alcohol consumers were younger, had a lower BMI, CRP, and fibrinogen, were less likely to use anti-lipid medications, and to have diabetes; and more likely to have higher AoCaS, HDL-C, and pack-years of smoking.

As shown in Table 3-3, except for HDL-C, after adjusting for age and race/ethnicity, compared to the no AoCaS category, participants with AoCaS >0 were more likely to have higher BMI, pack-years of smoking, LDL-C, triglycerides, CRP, and fibrinogen, diabetes, hypertension, and use lipid-lowering medications.

The Tobit regression analysis showed that in model II, heavy drinkers had a significantly higher expected AoCaS [TR (95% CI) = 2.34 (1.10, 4.97)] compared to nondrinkers (never drinkers + former drinkers) (Table 3-4). In model II, moderate drinkers appeared to have an inverse association with aortic calcification [TR (95% CI) = 0.86 (0.45, 1.66)], but this association was statistically nonsignificant. In model III, with further adjustment for potential mediators in the relationship between alcohol and atherosclerosis/CHD, the significant association of heavy drinking with aortic calcification was attenuated and beccme nonsignificant [TR (95% CI) = 1.90 (0.84, 4.28)].

Results of the ordinal regression analysis were very similar to Tobit regression analysis (Table 3-5). In model II, heavy drinkers had significantly higher odds of being in a higher category of AoCaS [OR (95% CI) = 1.67 (1.11, 2.52)]. In model III, with further adjustment for potential mediators, the significant association of heavy drinking with aortic calcification was attenuated and became nonsignificant [OR (95% CI) = 1.54 (0.99, 2.40)]. In Tobit regression as well as in ordinal regression, in model III, attenuation in significance was mainly due to adjustment for hypertension and HDL-C. When we repeated the analysis excluding hypertensive patients (n=257) from the main analysis (n=1006), there was no significant association between heavy alcohol consumption and aortic calcification after adjusting for socio-demographic variables and potential confounders [Model II: TR (95% CI) = 2.37 (0.88, 6.35), OR (95% CI) = 1.05 (0.58, 1.89)] (data not shown).

In Tobit regression as well as in ordinal regression, there was no significant interaction between alcohol consumption and race/ethnicity on aortic calcification. In a race/ethnicity stratified analysis, either in model II or model III, none of the alcohol consumption categories were significantly associated with aortic calcification (Table 3-4 and Table 3-5). After excluding former drinkers from the nondrinker category (reference category = never drinker), heavy drinkers [TR (95% CI) = 2.68 (1.15, 6.24); OR (95% CI) =1.80 (1.15, 2.81)] had significantly higher expected AoCaS compared with never drinkers after adjusting for socio-demographic and confounding variables (Table 3-7 and 3-8).

When CAC score was the outcome variable and with nondrinkers as a reference category - In model II, after adjustment for potential confounders, heavy alcohol consumption was associated with significantly higher expected CAC scores [TR (95% CI) = 2.75 (1.36, 5.56)]. In model III, with further adjustment for intermediary variables, there was no attenuation in the significant association of heavy drinking with CAC score [TR (95% CI) = 2.37 (1.11, 5.08)] (Table 3-9). These results were consistent when assessed using ordinal logistic regression (Table 3-10). When never drinkers were used as the reference category, results were unchanged compared to using nondrinkers as the reference category for both Tobit regression and ordinal logistic regression analyses (Table 3-11 and 3-12).

3.5 DISCUSSION

In this community-based sample of asymptomatic middle-aged men (US White, US Black, Japanese American, and Japanese in Japan), heavy alcohol consumption was significantly associated with higher AoCaS independent of potential confounders in the relation between

alcohol consumption and atherosclerois/CHD. The available literature describes various patterns of association between alcohol consumption and atherosclerosis: no significant association [138, 147], a U or J-shaped association [139-142], and a dose-response association [143-145]. Our results are consistent with several prospective reports of harmful effects of heavy drinking on atherosclerosis and no significant beneficial effect of light to moderate drinking [137, 140, 144]. McClelland et al. in the Multi-Ethnic Study of Atherosclerosis study among White, African American, Hispanic, or Chinese men and women aged 45-84 years from six different US communities, reported a significant positive association of alcohol consumption (≥2 drinks/day) with both baseline CAC and CAC progression [137]. Similarly, Tanaka et al., in the Circulatory Risk in Communities Study of men aged 30-79 years, reported a significant positive association of heavy alcohol consumption with endothelial dysfunction [150] which is hypothesized to contribute to the development of atherosclerosis and CHD [279]. Jiang et al., in a population-based cohort study with men and women aged 50-85 years showed a significant association between heavy alcohol consumption and both the presence as well as the severity of aortic arch calcification in men. There was no beneficial effect of moderate drinking of total alcohol or any types of alcoholic beverages on aortic arch calcification [143]. Pletcher and colleagues, in the CARDIA Study of US White middle-aged men and women aged 33-45 years, found a direct association between higher levels of alcohol consumption and CAC [145].

Our study findings imply that heavy alcohol consumption (>37.5 grams of alcohol/day) may have a detrimental effect on atherosclerosis indicated by aortic calcification among healthy middle-aged men. Although not tested in this study, the positive relationship between heavy alcohol consumption and aortic calcification could be explained by the deleterious effect of heavy alcohol consumption on endothelial function, platelet aggregation, the activation of the

clotting cascade, and the promotion of LDL oxidation by acetaldehyde [150, 280]. Several lines of evidence suggest that endothelial dysfunction is the initial step of atherosclerosis development [281]. Heavy alcohol consumption reduces nitric oxide (NO) production by reducing endothelial NO synthase activity, increases endothelial permeability to lipoproteins and other plasma components, and causes inflammatory/oxidative injury to the endothelium [282]. In response to the altered endothelial functions following various humoral and hemodynamic insults, as a part of the reparative mechanism, the systemic vasculature can respond by depositing calcium at the site of injury [283, 284].

In our study, light to moderate alcohol consumption was nonsignificantly associated with AoCaS. In contrast to our results, several studies have reported either a J-shaped association [140-142] with light to moderate alcohol consumption showing a protective effect on atherosclerosis or no significant association [138, 147, 285] between alcohol consumption and atherosclerosis. Mukamal and colleagues in the Cardiovascular Health Study of men and women aged ≥65 years and free of clinical CVD found that alcohol consumption of 1-6 drinks/week had 0.07±0.04 mm significantly lower composite carotid intima-media thickness (CIMT) than abstainers. This relationship was consistent across genders for internal and common carotid artery [140]. Vliegenthart et al., in the Rotterdam Coronary Calcification Study of men and women aged ≥55 years, reported a J-shaped association between alcohol consumption and CAC, with light and moderate drinkers having significantly lower odds of extensive CAC compared to nondrinkers [141]. Ellison et al. in the NHLBI Family Heart Study of men and women with an average age of 55 years reported no association between alcohol consumption and CAC [138]. Yang et al., in the South Bay Heart Watch study [285] of men and women aged ≥45 years and intermediate risk for CHD, reported no association between alcohol drinking and CAC. A J-

shaped association in the Rotterdam Study or a null association in the NHLBI Family Heart Study and the South Bay Heart Watch study could be because of 'abstainer error' (classifying people who had reduced or stopped drinking as lifetime abstainers). The potential for abstainer error is very high in all three studies because of inclusion of former drinkers, who might have stopped drinking because of age, ill health, or taking drugs that may interact with alcohol ('Sick Quitters'), in the never drinker group. The inclusion of former drinkers and lifelong abstainers into the non-drinker group could have substantially increased the risk of CAC among non-drinkers. In our study, the potential for abstainer error is very minimal because it is unlikely that healthy middle-aged men would stop drinking because of ill-health. Also, results were unchanged in a sensitivity analysis excluding former drinkers from the nondrinker category.

Our study has several limitations. First, we did not examine the relation of different drinking patterns (regular vs. episodic) and various types of alcohol beverages. Several lines of evidence suggest that binge drinking (episodic drinking of ≥5 drinks on any given occasion) is associated with atherosclerosis, and cardiovascular morbidity and mortality [145, 149, 286]. Second, alcohol consumption was assessed using self-administered standardized questionnaires, and we expect that participants might have underreported their alcohol consumption to avoid social embarrassment [287]. Under-reported alcohol consumption would most likely have attenuated the strength of association between alcohol and aortic calcification. Third, we mainly examined healthy men aged 40-49 years in Japan and the US; therefore, the results of the study cannot be generalized to females, other populations, or age groups. Fourth, although we have controlled for a variety of sociodemographic and clinical characteristics, the possibility of residual confounding cannot be excluded. However, any remaining potential confounder would need to be strongly associated with both alcohol consumption and atherosclerosis and not related

to other covariates included in regression models. Fifth, we cannot establish causality between alcohol consumption and aortic calcification based on our cross-sectional analyses.

The strengths of the current study include (i) The community-based nature of the study design with participants from four different races/ethnicities from two countries; (ii) All variable measurements were standardized across all centers; (iii) A considerable proportion of daily drinkers and subjects with aortic calcification to evaluate their association; (iv) Use of EBCT to detect aortic calcification which allowed a detailed examination of subclinical disease in arterial beds with accurate visualization of small calcific deposits in the arteries compared to X-ray; and (v) Availability of data on several potential confounders and intermediary variables in the relationship between alcohol consumption and atherosclerosis/CHD.

Our study findings have public health as well as clinical significance. All over the world, alcohol is one of the most commonly used recreational substances. Evidence concerning alcohol consumption and atherosclerosis is limited. Nevertheless, available evidence suggests the detrimental effect of heavy alcohol consumption on atherosclerosis measured by CAC [145] or aortic calcification [143] or CIMT [140]. Heavy alcohol consumption is associated with a risk of developing communicable diseases, non-communicable diseases, mental and behavioral disorders [113]. Evidence generated from this study further adds to the evidence on the serious health hazards of heavy alcohol consumption among healthy middle-aged men. Although results generated from a cross-sectional study like ours should be extrapolated to clinical care with caution, our study does support the 2015-2020 US dietary guidelines for Americans [288] which recommends ‘if alcohol is consumed, it should be consumed in moderation—up to two drinks per day for men.’ Mechanistically, the non-significant association of light to moderate alcohol consumption with aortic calcification may imply that a major part of the cardiovascular benefits

of light to moderate alcohol consumption is mediated through mechanisms other than the deposition of calcium in an arterial wall. These mechanisms may include the favorable effect of moderate alcohol consumption within the coagulation system or on endothelial function or an antioxidant effect or increase resistance of myocyte to ischemic injury [122, 151, 263, 264].

3.6 CONCLUSION

Our study showed a null association of light to moderate drinking and a positive association of heavy alcohol consumption with aortic calcification. Thus, the heavy alcohol consumption may be an independent risk factor for atherosclerosis and light to moderate alcohol consumption may decrease cardiovascular risk through mechanisms other than those associated with the reduced deposition of calcium in the atherosclerotic lesions. Prospective data are needed to further clarify the association between alcohol consumption and incidence and progression of atherosclerosis.

3.7 TABLES

Table 3-1. Descriptive characteristics of study participants for the ERA-JUMP Study, 2002-2006

Race/Ethnicity	Overall	US White	US Black	Japanese in Japan	Japanese American
Total number (%)	1006 (100)	301 (29.9)	103 (10.2)	310 (30.8)	292 (29.0)
Age[a], years	45.3 (2.8)	45.0 (2.8)	45.0 (2.8)	45.1 (2.8)	46.1 (2.8)
BMI[a], kg/m^2	26.8 (4.6)	27.8 (4.2)	29.7 (5.8)	23.7 (3.1)	27.9 (4.3)
Pack-years of smoking[b]	0.0 (0.0, 15.0)	0.0 (0.0, 1.5)	0.0 (0.0, 9.5)	18.9 (3.3, 29.0)	0.0 (0.0, 3.6)
LDL-C[a], mg/dL	129.5 (35.5)	134.9 (33.6)	128.1 (42.0)	132.3 (36.0)	121.4 (33.0)
HDL-C[a], mg/dL	51.0 (13.5)	47.8 (12.8)	51.4 (16.0)	54.1 (13.7)	50.8 (12.3)
Triglycerides[b], mg/dL	133.0 (95.0 188.0)	128.0 (93.0, 186.0)	108.0 (78.0, 166.0)	137.0 (104.0, 182.0)	141.5 (93.0, 225.5)
Hypertension[c]	257 (25.6)	44 (14.6)	33 (32.0)	83 (26.8)	97 (33.2)
Diabetes[c]	77 (7.7)	10 (3.3)	9 (8.8)	19 (6.1)	39 (13.4)
Anti-lipid med[c]	124 (12.3)	36 (12.0)	9 (8.7)	11 (3.6)	68 (23.3)
Meat intake[c]	761 (75.7)	232 (77.1)	75 (72.8)	207 (66.8)	247 (84.6)
Years of education[b]	16.0 (14.0, 16.0)	16.0 (16.0, 18.0)	14.0 (12.0, 16.0)	16.0 (12.0, 16.0)	16.0 (14.0, 16.0)
CRP[b], mg/dL	0.7 (0.3,1.4)	0.9 (0.5, 1.8)	1.5 (0.9, 3.1)	0.3 (0.2, 0.7)	0.7 (0.3, 1.3)
Fibrinogen[a], mg/dL	289.7 (74.4)	291.0 (70.2)	314.2 (73.7)	255.8 (65.8)	315.9 (73.4)
AoCaS[b]	4.9 (0.0, 50.0)	9.0 (0.0, 45.0)	14.0 (0.0, 46.0)	0.0 (0.0, 41.0)	5.0 (0.0, 77.0)
Alcohol intake[b], gm/day	7.4 (0.0, 25.9)	4.6 (1.0, 16.1)	3.1 (0.0, 24.7)	16.5 (2.5, 42.4)	1.0 (0.0, 25.9)

AoCaS, aortic calcification score; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein;

[a]Continuous normally distributed variables expressed as mean (standard deviation);

[b]Continuous non-normally distributed variables expressed as median (inter-quartile range);

[c]Categorical variables expressed as numbers (%);

SI conversion factors: To convert LDL-C and HDL-C to mmol/L, multiply values by 0.0259. To convert triglycerides to mmol/L, multiply values by 0.01129. To convert fibrinogen to µmol/L, multiply values by 0.0294. To convert CRP to nmol/L, multiply values by 9.524.

Table 3-2. Demographic and clinical characteristics by alcohol consumption categories for the ERA-JUMP Study, 2002-2006 (n=1006)

Alcohol Categories	Nondrinker	Light Drinker	Moderate Drinker	Heavy Drinker	*p*-trend[d]
Total number (%)	258 (25.6)	355 (35.3)	236 (23.5)	157 (15.6)	-
Age[a], years	45.7 (0.2)	45.1 (0.2)	45.1 (0.2)	45.6 (0.2)	0.61/0.01
BMI[a], kg/m^2	28.3 (0.4)	27.9 (0.3)	27.4 (0.3)	27.6 (0.4)	0.07/0.30
Pack-years of smoking[b]	0.0 (0.0, 3.1)	0.0 (0.0, 3.1)	0.0 (0.0, 3.1)	3.8 (0.0, 11.1)	0.01/0.01
LDL-C[a], mg/dL	134.2 (3.0)	134.8 (2.3)	137.8 (2.9)	124.8 (3.7)	0.03/0.01
HDL-C[a], mg/dL	44.0 (1.1)	46.9 (0.8)	51.5 (1.0)	57.7 (1.3)	0.01/0.06
Triglycerides[b], mg/dL	132.8 (90.1, 189.3)	129.3 (91.4, 186.3)	117.8 (93.5, 168.5)	123.8 (98.1, 201.5)	0.14/0.39
Hypertension[c]	42 (16.4)	44 (12.3)	34 (14.4)	60 (38.1)	0.01/0.01
Diabetes[c]	15 (5.7)	10 (2.6)	7 (3.0)	6 (3.5)	0.21/0.07
Anti-lipid med[c]	31 (14.9)	34 (12.2)	17 (9.2)	17 (13.5)	0.52/0.14
Meat intake[c]	187 (72.7)	269 (75.8)	195 (81.7)	130 (82.9)	0.01/0.63
Years of education[b]	16.0 (16.0, 18.0)	16.0 (16.0, 18.0)	16.0 (16.0, 18.0)	16.0 (16.0, 18.0)	1.00/1.00
CRP[b], mg/dL	1.1 (0.6, 1.8)	0.9 (0.5, 1.9)	0.8 (0.5, 1.6)	0.9 (0.5, 1.9)	0.02/0.23
Fibrinogen[a], mg/dL	301.6 (6.0)	291.2 (4.7)	285.3 (5.8)	292.1 (7.4)	0.13/0.07
AoCaS[b]	0.8 (0.0, 9.9)	0.6 (0.0, 11.3)	0.4 (0.0, 11.0)	0.7 (0.0, 16.0)	0.84/0.51

AoCaS, aortic calcification score; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein;
Values for all variables except age were adjusted for 'age' and 'race/ethnicity': value of age was fixed at 45.3 years and race was fixed as 'US White'.
[a]Continuous normally distributed variables were expressed as mean (standard error);
[b]Continuous non-normally distributed variables were expressed as median (inter-quartile range);
[c]Categorical variables were expressed as numbers (%);
[d]*p*-trend shows *p*-values for linear and quadratic trend across the alcohol consumption categories;
SI conversion factors: To convert LDL-C and HDL-C to mmol/L, multiply values by 0.0259. To convert triglycerides to mmol/L, multiply values by 0.01129. To convert fibrinogen to µmol/L, multiply values by 0.0294. To convert CRP to nmol/L, multiply values by 9.524.

Table 3-3. Demographic and clinical characteristics by aortic calcification score categories for the ERA-JUMP Study, 2002-2006 (n=1006)

AoCaS Categories	AoCaS=0	AoCaS 1-99	AoCaS 100-299	AoCaS ≥300	*p*-trend[d]
Total number (%)	434 (43.1)	372 (37.0)	91 (9.1)	109 (10.8)	-
Age[a], years	44.9 (0.1)	45.4 (0.2)	46.2 (0.3)	46.3 (0.3)	0.01/0.27
BMI[a], kg/m^2	26.2 (0.3)	28.8 (0.3)	28.9 (0.5)	27.4 (0.4)	0.01/0.01
Pack-years of smoking[b]	0.0 (0.0, 0.9)	0.0 (0.0, 1.2)	0.0 (0.0, 13.6)	11 (0.0, 19.7)	0.01/0.01
Alcohol[b], gm/day	5.0 (1.0, 15.9)	3.7 (1.0, 13.6)	3.4 (1.0, 11.5)	22.9 (2.1, 35.0)	0.01/0.01
LDL-C[a], mg/dL	130.5 (2.6)	137.8 (2.3)	131.8 (4.1)	137.9 (3.8)	0.18/0.83
HDL-C[a], mg/dL	50.3 (1.0)	46.4 (0.9)	46.0 (1.5)	49.6 (1.4)	0.62/0.01
Triglycerides[b], mg/dL	115.1 (80.7, 156.8)	130.1 (95.7, 185.5)	149.4 (104.7, 209.0)	134.3 (94.7, 239.0)	0.01/0.03
Hypertension[c]	43 (9.9)	60 (16.1)	19 (20.7)	18 (16.0)	0.01/0.02
Diabetes[c]	10 (2.3)	11 (2.9)	7 (7.3)	6 (5.3)	0.01/0.25
Anti-lipid med[c]	39 (9.0)	40 (10.8)	24 (26.1)	16 (14.3)	0.01/0.03
Meat intake[c]	325 (74.9)	290 (78.0)	74 (80.6)	83 (75.9)	0.70/0.25
Years of education[b]	17.0 (16.0, 18.0)	17.0 (16.0, 18.0)	16.0 (16.0, 18.0)	16.0 (16.0, 18.0)	0.01/1.00
CRP[b], mg/dL	0.8 (0.4, 1.6)	1.1 (0.6, 1.8)	1.0 (0.5, 2.2)	1.0 (0.5, 1.9)	0.04/0.17
Fibrinogen[a], mg/dL	284.7 (5.2)	293.0 (4.8)	300.4 (8.1)	299.1 (7.6)	0.04/0.40

AoCaS, aortic calcification score; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein;
Values for all variables except age were adjusted for 'age' and 'race/ethnicity': Value of age was fixed at 45.3 years and race was fixed as 'US White';
[a]Continuous normally distributed variables were expressed as mean (standard error);
[b]Continuous non-normally distributed variables were expressed as median (inter-quartile range);
[c]Categorical variables were expressed as numbers (%);
[d]*p*-trend shows *p*-values for linear and quadratic trends across the aortic calcification score categories;
SI conversion factors: To convert LDL-C and HDL-C to mmol/L, multiply values by 0.0259. To convert triglycerides to mmol/L, multiply values by 0.01129. To convert fibrinogen to µmol/L, multiply values by 0.0294. To convert CRP to nmol/L, multiply values by 9.524.

Table 3-4. Tobit conditional regression describing the association between alcohol consumption and aortic calcification score for the ERA-JUMP Study, 2002-2006

Alcohol Categories	Non-drinkers	Light Drinkers	Moderate Drinkers	Heavy Drinkers	-
All Participants (n = 1006)					
n (%)	258 (25.6)	355 (35.3)	236 (23.5)	157 (15.6)	-
Mean AoCaS	81.2	107.7	112.5	283.7	-
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**
Unadjusted	1.00	0.92 (0.49, 1.75)	0.48 (0.24, 0.98)	1.15 (0.52, 2.55)	0.85/0.07
Model I	1.00	1.09 (0.58, 2.05)	0.67 (0.33, 1.33)	2.63 (1.19, 5.81)	0.06/0.02
Model II	1.00	1.25 (0.69, 2.27)	0.86 (0.45, 1.66)	2.34 (1.10, 4.97)	0.06/0.13
Model III	1.00	1.23 (0.67, 2.23)	0.82 (0.42, 1.60)	1.90 (0.84, 4.28)	0.22/0.22
Race/Ethnicity Stratified Analysis					
US White (n = 301)					
n (%)	57 (18.9)	162 (53.8)	71(23.6)	11(3.7)	-
Mean AoCaS	66.7	105.7	90.7	381.0	-
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**
Model II	1.00	1.43 (0.61, 3.34)	1.25 (0.46, 3.37)	1.34 (0.20, 9.11)	0.85/0.77
Model III	1.00	1.41 (0.60, 3.34)	1.35 (0.48, 3.75)	1.89 (0.26, 13.59)	0.61/0.98
Japanese in Japan (n = 310)					
n (%)	53 (17.1)	82 (26.5)	81 (26.1)	94 (30.3)	-
Mean AoCaS	121.0	76.5	67.7	251.3	-
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**
Model II	1.00	1.13 (0.15, 8.27)	0.78 (0.11, 5.83)	2.68 (0.39, 18.36)	0.42/0.31
Model III	1.00	0.85 (0.11, 6.34)	0.79 (0.10, 6.04)	1.54 (0.20, 11.95)	0.67/0.42
Japanese American (n = 292)					
n (%)	113 (38.7)	75 (25.7)	59 (20.2)	45 (15.4)	-
Mean AoCaS	82.1	157.8	224.9	232.5	-
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**
Model II	1.00	1.25 (0.46, 3.38)	0.86 (0.30, 2.52)	2.02 (0.59, 6.07)	0.36/0.53
Model III	1.00	1.33 (0.50, 3.59)	0.95 (0.33, 2.77)	1.60 (0.41, 5.17)	0.59/0.84

TR, Tobit ratio; CI, confidence interval; AoCaS, aortic calcification score;

Model I: Alcohol consumption, age, race, years of education;

Model II: Model I + pack-years of smoking, BMI, diabetes, anti-lipid medication, job physical activity, meat intake, LDL-C, and CRP;

Model III: Model II + HDL-C, triglycerides, hypertension, fibrinogen;

[a]*p-trend* shows *p*-values for linear and quadratic trends across the alcohol consumption categories calculated using contrast.

Table 3-5. Ordinal logistic regression describing the association between alcohol consumption and aortic calcification score for the ERA-JUMP Study, 2002-2006

Alcohol Categories	Non-drinkers	Light Drinkers	Moderate Drinkers	Heavy Drinkers	-
All Participants (n = 1006)					
n (%)	258 (25.6)	355 (35.3)	236 (23.5)	157 (15.6)	-
Mean AoCaS	81.2	107.7	112.5	283.7	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**
Unadjusted	1.00	0.92 (0.68, 1.23)	0.68 (0.49, 0.94)	1.05 (0.73, 1.52)	0.15/0.30
Model I	1.00	1.02 (0.74, 1.39)	0.79 (0.56, 1.12)	1.67 (1.13, 2.47)	0.19/0.31
Model II	1.00	1.10 (0.79, 1.52)	0.86 (0.60, 1.23)	1.67 (1.11, 2.52)	0.48/0.32
Model III	1.00	1.07 (0.77, 1.50)	0.83 (0.58, 1.21)	1.54 (0.99, 2.40)	0.47/0.55
Race/Ethnicity Stratified Analysis					
US White (n = 301)					
n (%)	57 (18.9)	162 (53.8)	71 (23.6)	11 (3.7)	-
Mean AoCaS	66.7	105.7	90.7	381.0	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**
Model II	1.00	1.26 (0.69, 2.31)	1.10 (0.55, 2.23)	1.30 (0.35, 4.84)	0.65/0.85
Model III	1.00	1.21 (0.65, 2.25)	1.10 (0.53, 2.29)	1.62 (0.42, 6.35)	0.87/0.61
Japanese in Japan (n = 310)					
n (%)	53 (17.1)	82 (26.5)	81 (26.1)	94 (30.3)	-
Mean AoCaS	121.0	76.5	67.7	251.3	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**
Model II	1.00	1.02 (0.46, 2.25)	0.96 (0.44, 2.12)	1.68 (0.79, 3.55)	0.69/0.39
Model III	1.00	0.89 (0.39, 2.01)	1.00 (0.44, 2.27)	1.40 (0.62, 3.14)	0.60/0.43
Japanese American (n = 292)					
n (%)	113 (38.7)	75 (25.7)	59 (20.2)	45 (15.4)	-
Mean AoCaS	82.1	157.8	224.9	232.5	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**
Model II	1.00	1.16 (0.66, 2.05)	0.78 (0.42, 1.44)	1.47 (0.75, 2.87)	0.83/0.97
Model III	1.00	1.21 (0.68, 2.17)	0.81 (0.43, 1.53)	1.38 (0.66, 2.91)	0.96/0.88

OR, odds ratio; CI, confidence interval; AoCaS, aortic calcification score;

Model I: Alcohol consumption, age, race, years of education;

Model II: Model I + pack-years of smoking, BMI, diabetes, anti-lipid medication, job physical activity, meat intake, LDL-C, and CRP;

Model III: Model II + HDL-C, triglycerides, hypertension, and fibrinogen;

[a]*p-trend* shows *p*-values for linear and quadratic trends across the alcohol consumption categories calculated using contrast.

3.8 SUPPLEMENTARY TABLES

Table 3-6. Demographic and clinical characteristics by alcohol consumption categories for the ERA-JUMP Study, 2002-2006 (n=914)

Alcohol Consumption	Never drinkers	Current Drinkers			*p*-trend[d]
		Light	Moderate	Heavy	
Total number (%)	166 (16.5)	355 (35.3)	236 (23.5)	157 (15.6)	-
Age[a], years	45.9 (0.2)	45.1 (0.2)	45.1 (0.2)	45.6 (0.2)	0.29/0.01
AoCaS[b]	0.0 (0.0, 9.6)	0.0 (0.0, 10.5)	0.0 (0.0, 10.5)	0.0 (0.0, 15.9)	0.42/0.65
BMI[a], kg/m^2	28.5 (0.5)	27.9 (0.3)	27.4 (0.4)	27.6 (0.5)	0.030/0.14
Pack-years of smoking[b]	0.0 (0.0, 0.9)	0.0 (0.0, 1.9)	0.0 (0.00, 1.9)	3.8 (0.0, 1.0)	0.01/0.01
LDL-C[a], mg/dL	133.9 (3.7)	135.0 (2.4)	138.0 (2.9)	124.8 (3.8)	0.05/0.01
HDL-C[a], mg/dL	43.3 (1.3)	46.5 (0.9)	51.2 (1.1)	57.4 (1.4)	0.01/0.14
Triglycerides[b], mg/dL	134.7 (94.9, 189.7)	128.7 (92.9, 185.9)	116.7 (94.5, 168.7)	125.7 (98.5, 202.2)	0.17/0.20
Hypertension[c]	31 (18.5)	46 (12.8)	36 (15.0)	63 (39.7)	0.01/0.00
Diabetes[c]	6 (3.6)	8 (2.3)	6 (2.6)	5 (2.8)	0.61/0.30
Anti-lipid med[c]	22 (16.3)	36 (12.3)	18 (9.2)	18 (13.6)	0.38/0.10
Meat intake[c]	119 (71.6)	268 (75.4)	195 (82.6)	130 (82.7)	0.01/0.59
Years of education[b]	17.0 (16.0, 18.0)	17.0 (16.0, 18.0)	17.0 (16.0, 18.0)	17.0 (16.0, 18.0)	1.0/1.0
CRP[b], mg/dL	1.0 (0.6, 1.9)	0.9 (0.5, 1.9)	0.8 (0.5, 1.7)	0.9 (0.5, 2.0)	0.08/0.28
Fibrinogen[a], mg/dL	307.3 (7.2)	292.8 (4.7)	287.0 (5.9)	294.4 (7.6)	0.07/0.03

AoCaS, aortic calcification score; BMI, body mass index; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; CRP, C-reactive protein;

Values for all variables except age were adjusted for 'age' and 'race/ethnicity': value of age was fixed at 45.3 years and race was fixed as 'US White'.

[a]Continuous normally distributed variables expressed as mean (standard error);

[b]Continuous non-normally distributed variables expressed as median (inter-quartile range);

[c]Categorical variables expressed as numbers (%);

[d]*p*-trend shows *p*-values for linear and quadratic trends across the alcohol consumption categories;

SI conversion factors: To convert LDL-C and HDL-C to mmol/L, multiply values by 0.0259. To convert triglycerides to mmol/L, multiply values by 0.01129. To convert fibrinogen to µmol/L, multiply values by 0.0294. To convert CRP to nmol/L, multiply values by 9.524.

Table 3-7. Tobit regression describing the association between alcohol consumption and aortic calcification for the ERA-JUMP Study, 2002-2006 [Reference category= never drinkers]

Alcohol Categories	Never drinkers	Light Drinkers	Moderate Drinkers	Heavy Drinkers	
		All Participants (n = 914)			
n (%)	166 (18.2)	355 (38.8)	236 (25.8)	157 (17.2)	-
Mean AoCaS	77.5	107.7	112.5	283.7	-
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p-trend*[a]**
Unadjusted	1.00	1.29 (0.60, 2.77)	0.66 (0.29, 1.52)	1.59 (0.64, 3.95)	0.61/0.29
Model I	1.00	1.21 (0.57, 2.58)	0.74 (0.33, 1.65)	3.02 (1.24, 7.34)	0.05/0.04
Model II	1.00	1.38 (0.68, 2.81)	0.95 (0.44, 2.04)	2.68 (1.15, 6.24)	0.06/0.18
Model III	1.00	1.38 (0.67, 2.82)	0.91 (0.42, 2.00)	2.12 (0.86, 5.28)	0.19/0.30
		Race/Ethnicity Stratified Analysis			
US White (n = 276)					
n (%)	32 (11.6)	162 (58.7)	71 (25.7)	11 (4.0)	-
Mean AoCaS	62.4	105.7	90.7	381.0	-
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p-trend*[a]**
Model II	1.00	1.38 (0.47, 4.10)	1.22 (0.37, 4.07)	1.23 (0.16, 9.64)	0.89/0.79
Model III	1.00	1.43 (0.46, 4.47)	1.39 (0.39, 5.00)	1.87 (0.22, 16.17)	0.19/0.31
Japanese in Japan (n = 305)					
n (%)	48 (15.7)	82 (26.9)	81 (26.6)	94 (30.8)	-
Mean AoCaS	129.1	76.5	67.7	251.3	-
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p-trend*[a]**
Model II	1.00	1.30 (0.17, 10.29)	0.91 (0.11, 7.26)	3.05 (0.41, 22.46)	0.39/0.37
Model III	1.00	0.95 (0.12, 7.67)	0.88 (0.11, 7.21)	1.73 (0.21, 14.27)	0.64/0.45
Japanese American (n = 250)					
n (%)	71 (28.4)	75 (30.0)	59 (23.6)	45 (18.0)	-
Mean AoCaS	60.3	157.8	224.9	232.5	-
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p-trend*[a]**
Model II	1.00	1.77 (0.56, 5.65)	1.26 (0.36, 4.37)	2.87 (0.75, 11.06)	0.17/0.81
Model III	1.00	1.88 (0.59, 5.96)	1.38 (0.40, 4.77)	2.27 (0.53, 9.82)	0.29/0.93

TR, Tobit ratio; CI, confidence interval; AoCaS, aortic calcification score;

Model I: Alcohol consumption, age, race, years of education;

Model II: Model I + pack-years of smoking, BMI, diabetes, anti-lipid medication, job physical activity, meat intake, LDL-C, and CRP;

Model III: Model II + HDL-C, triglycerides, hypertension, fibrinogen;

[a]*p-trend* shows *p*-values for linear and quadratic trends across the alcohol consumption categories calculated using contrast.

Table 3-8. Ordinal logistic regression describing the association between alcohol consumption and aortic calcification for the ERA-JUMP Study, 2002-2006 [Reference category= never drinkers]

Alcohol Categories	Never drinkers	Light Drinkers	Moderate Drinkers	Heavy Drinkers	
All Participants (n = 914)					
n (%)	166 (18.2)	355 (38.8)	236 (25.8)	157 (17.2)	-
Mean AoCaS	77.5	107.7	112.5	283.7	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p-trend*[a]**
Unadjusted	1.00	1.08 (0.76, 1.52)	0.80 (0.55, 1.16)	1.23 (0.82, 1.85)	0.70/0.73
Model I	1.00	1.09 (0.76, 1.57)	0.86 (0.58, 1.27)	1.80 (1.18, 2.75)	0.42/0.21
Model II	1.00	1.16 (0.80, 1.70)	0.92 (0.62, 1.39)	1.80 (1.15, 2.81)	0.73/0.22
Model III	1.00	1.15 (0.78, 1.68)	0.90 (0.59, 1.37)	1.67 (1.03, 2.70)	0.75/0.39
Race/Ethnicity Stratified Analysis					
US White (n = 276)					
n (%)	32 (11.6)	162 (58.7)	71 (25.7)	11 (4.0)	-
Mean AoCaS	62.4	105.7	90.7	381.0	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p-trend*[a]**
Model II	1.00	1.28 (0.60, 2.74)	1.13 (0.49, 2.62)	1.31 (0.33, 5.23)	0.66/0.85
Model III	1.00	1.23 (0.56, 2.73)	1.15 (0.47, 2.80)	1.69 (0.39, 7.26)	0.86/0.58
Japanese in Japan (n = 305)					
n (%)	48 (15.7)	82 (26.9)	81 (26.6)	94 (30.8)	-
Mean AoCaS	129.1	76.5	67.7	251.3	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p-trend*[a]**
Model II	1.00	1.10 (0.48, 2.49)	1.04 (0.46, 2.35)	1.79 (0.82, 3.88)	0.84/0.33
Model III	1.00	0.94 (0.41, 2.19)	1.06 (0.46, 2.45)	1.47 (0.64, 3.37)	0.70/0.39
Japanese American (n = 250)					
n (%)	71 (28.4)	75 (30.0)	59 (23.6)	45 (18.0)	-
Mean AoCaS	60.3	157.8	224.9	232.5	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p-trend*[a]**
Model II	1.00	1.41 (0.74, 2.67)	0.97 (0.49, 1.92)	1.81 (0.86, 3.80)	0.70/0.67
Model III	1.00	1.49 (0.78, 2.86)	1.02 (0.51, 2.07)	1.82 (0.80, 4.13)	0.56/0.69

OR, odds ratio; CI, confidence interval; AoCaS, aortic calcification score;

Model I: Alcohol consumption, age, race, years of education;

Model II: Model I + pack-years of smoking, BMI, diabetes, anti-lipid medication, job physical activity, meat intake, LDL-C, and CRP;

Model III: Model II + HDL-C, triglycerides, hypertension, fibrinogen;

[a]*p-trend* shows *p*-values for linear and quadratic trends across the alcohol consumption categories calculated using contrast.

Table 3-9. Tobit regression describing the association between alcohol consumption and coronary artery calcification for the ERA-JUMP Study [Reference category= nondrinkers (never + former drinkers)]

Alcohol Categories	Non-drinkers	Light Drinkers	Moderate Drinkers	Heavy Drinkers	
All Participants (n = 1006)					
n (%)	258 (25.7)	355 (35.3)	236 (23.5)	157 (15.6)	
Mean CAC score	25.8	24.9	37.3	41.0	
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**
Unadjusted	1.00	0.77 (0.42, 1.40)	0.48 (0.25, 0.95)	1.07 (0.51, 2.24)	0.82/0.03
Model I	1.00	0.99 (0.55, 1.77)	0.72 (0.38, 1.37)	2.22 (1.07, 4.58)	0.07/0.02
Model II	1.00	1.18 (0.68, 2.07)	0.98 (0.52, 1.82)	2.75 (1.36, 5.56)	0.01/0.07
Model III	1.00	1.16 (0.66, 2.05)	0.99 (0.53, 1.87)	2.37 (1.11, 5.08)	0.03/0.12
Race/Ethnicity Stratified Analysis					
US White (n = 301)					
n (%)	57 (18.9)	162 (53.8)	71 (23.6)	11 (3.7)	
Mean CAC score	35.7	23.1	17.5	26.7	
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**
Model II	1.00	1.06 (0.44, 2.57)	0.68 (0.24, 1.94)	0.79 (0.10, 6.04)	0.68/0.96
Model III	1.00	0.95 (0.39, 2.34)	0.65 (0.22, 1.89)	0.68 (0.08, 5.60)	0.63/0.95
Japanese in Japan (n = 310)					
n (%)	53 (17.10)	82 (26.45)	81 (26.13)	94 (30.32)	
Mean CAC score	10.8	5.3	1.87	25.2	
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**
Model II	1.00	0.76 (0.22, 2.56)	0.39 (0.11, 1.39)	2.05 (0.63, 6.61)	0.44/0.02
Model III	1.00	0.62 (0.17, 2.18)	0.38 (0.10, 1.39)	1.71 (0.48, 6.09)	0.57/0.02
Japanese American (n = 292)					
n (%)	113 (38.7)	75 (25.7)	59 (20.2)	45 (15.4)	
Mean CAC score	32.9	57.7	104.0	69.5	
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**
Model II	1.00	1.71 (0.55, 5.32)	2.11 (0.63, 7.06)	3.51 (0.95, 12.97)	0.06/0.98
Model III	1.00	1.79 (0.58, 5.59)	2.13 (0.63, 7.17)	2.15 (0.51, 9.09)	0.22/0.66

TR: Tobit ratio; CI: confidence interval; CAC: coronary artery calcification;
Model I: Alcohol consumption, age, race, years of education;
Model II: Model I + pack-years of smoking, BMI, diabetes, anti-lipid medication, job physical activity, meat intake, LDL-C, CRP;
Model III: Model II + HDL-C, triglycerides, hypertension, fibrinogen;
[a]*p-trend* shows *p*-value for linear and quadratic trend across the alcohol consumption categories calculated using contrast.

Table 3-10. Ordinal logistic regression describing the association between alcohol consumption and coronary artery calcification for the ERA-JUMP Study [Reference category= non-drinkers)]

Alcohol Categories	Non-drinkers	Light Drinkers	Moderate Drinkers	Heavy Drinkers	
All Participants (n = 1006)					
n (%)	258 (25.6)	355 (35.3)	236 (23.5)	157 (15.6)	-
Mean CAC score	25.8	24.8	37.3	41.0	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**
Unadjusted	1.00	0.93 (0.64, 1.36)	0.85 (0.56, 1.31)	1.28 (0.82, 2.01)	0.32/0.60
Model I	1.00	1.15 (0.76, 1.73)	1.13 (0.72, 1.77)	2.14 (1.31, 3.50)	0.74/0.03
Model II	1.00	1.31 (0.86, 2.00)	1.34 (0.84, 2.14)	2.39 (1.43, 4.00)	0.75/0.01
Model III	1.00	1.30 (0.85, 2.00)	1.36 (0.85, 2.19)	2.25 (1.29, 3.93)	0.69/0.02
Race/Ethnicity Stratified Analysis					
US White (n = 301)					
n (%)	57 (18.9)	162 (53.8)	71 (23.6)	11 (3.6)	-
Mean CAC score	35.7	23.1	17.5	26.7	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**
Model II	1.00	1.10 (0.53, 2.28)	1.09 (0.46, 2.57)	0.80 (0.15, 4.41)	0.67/0.80
Model III	1.00	1.08 (0.52, 2.25)	1.16 (0.48, 2.81)	0.95 (0.17, 5.42)	0.76/0.98
Japanese in Japan (n = 310)					
n (%)	53 (17.1)	82 (26.5)	81 (26.1)	94 (30.3)	-
Mean CAC score	10.7	5.3	1.9	25.2	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**
Model II	1.00	0.75 (0.23, 2.42)	0.26 (0.06, 1.15)	1.84 (0.64, 5.30)	0.14/0.61
Model III	1.00	0.70 (0.21, 2.40)	0.22 (0.05, 1.05)	1.73 (0.54, 5.50)	0.12/0.52
Japanese American (n = 292)					
n (%)	113 (38.7)	75 (25.7)	59 (20.2)	45 (15.4)	-
Mean CAC score	32.9	57.7	104.0	69.5	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**
Model II	1.00	1.93 (0.98, 3.79)	2.31 (1.14, 4.68)	2.69 (1.26, 5.72)	0.11/0.03
Model III	1.00	1.94 (0.97, 3.87)	2.32 (1.12, 4.78)	2.21 (0.97, 5.07)	0.08/0.09

OR: odds ratio; CI: confidence interval; CAC: coronary artery calcification;

Model I: Alcohol consumption, age, race, years of education

Model II: Model I + pack-years of smoking, BMI, diabetes, anti-lipid medication, job physical activity, meat intake, LDL-C, CRP

Model III: Model II + HDL-C, triglycerides, hypertension, fibrinogen

[a]*p- trend* shows *p*-value for linear and quadratic trend across the alcohol consumption categories calculated using contrast.

Table 3-11. Tobit regression describing the association between alcohol consumption and coronary artery calcification score for the ERA-JUMP Study [Reference category= never drinkers]

Alcohol Categories	Never drinkers	Light Drinkers	Moderate Drinkers	Heavy Drinkers	
All Participants (n = 914)					
n (%)	166 (18.2)	355 (38.8)	236 (25.8)	157 (17.2)	-
Mean CAC score	24.2	24.8	37.3	41.0	-
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**
Unadjusted	1.00	0.87 (0.43, 1.74)	0.54 (0.25, 1.16)	1.21 (0.53, 2.76)	0.94/0.07
Model I	1.00	1.01 (0.51, 2.00)	0.72 (0.35, 1.49)	2.27 (1.03, 5.03)	0.09/0.03
Model II	1.00	1.25 (0.66, 2.38)	1.05 (0.52, 2.12)	3.02 (1.40, 6.52)	0.01/0.10
Model III	1.00	1.23 (0.64, 2.37)	1.06 (0.52, 2.17)	2.60 (1.14, 5.96)	0.03/0.17
Race/Ethnicity Stratified Analysis					
US White (n = 276)					
n (%)	32 (11.6)	162 (58.7)	71 (25.7)	11 (4.0)	-
Mean CAC score	56.5	23.1	17.5	26.7	-
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**
Model II	1.00	0.77 (0.26, 2.32)	0.50 (0.14, 1.73)	0.55 (0.06, 4.71)	0.47/0.79
Model III	1.00	0.65 (0.21, 2.07)	0.45 (0.12, 1.69)	0.48 (0.08, 4.54)	0.45/0.72
Japanese in Japan (n = 305)					
n (%)	48 (15.74)	82 (26.9)	81 (26.6)	94 (30.8)	-
Mean CAC score	11.0	5.3	1.9	25.2	-
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**
Model II	1.00	0.92 (0.26, 3.32)	0.47 (0.13, 1.80)	2.51 (0.73, 8.63)	0.30/0.04
Model III	1.00	0.77 (0.20, 2.91)	0.45 (0.12, 1.76)	1.99 (0.53, 7.48)	0.45/0.04
Japanese American (n = 250)					
n (%)	71 (28.4)	75 (30.0)	59 (23.6)	45 (18.0)	-
Mean CAC score	22.9	57.7	104.0	69.5	-
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**
Model II	1.00	1.96 (0.56, 6.86)	2.61 (0.69, 9.89)	4.74 (1.13, 19.97)	0.03/0.96
Model III	1.00	1.99 (0.57, 6.96)	2.48 (0.65, 9.45)	2.98 (0.61, 14.51)	0.13/0.70

TR: Tobit ratio; CI: confidence interval; CAC: coronary artery calcification;
Model I: Alcohol consumption, age, race, years of education;
Model II: Model I + pack-years of smoking, BMI, diabetes, anti-lipid medication, job physical activity, meat intake, LDL-C, CRP;
Model III: Model II + HDL-C, triglycerides, hypertension, fibrinogen;
[a]*p trend* shows *p*-value for linear and quadratic trend across the alcohol consumption categories calculated using contrast.

Table 3-12. Ordinal logistic regression describing the association between alcohol consumption and coronary artery calcification for the ERA-JUMP Study [Reference category= never drinkers]

Alcohol categories	Never drinkers	Light Drinkers	Moderate Drinkers	Heavy Drinkers	
All Participants (n=914)					
n (%)	166 (18.2)	355 (38.8)	236 (25.8)	157 (17.2)	-
Mean CAC score	24.2	24.9	37.3	41.0	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**
Unadjusted	1.00	1.01 (0.65, 1.57)	0.92 (0.57, 1.50)	1.38 (0.84, 2.29)	0.59/0.47
Model I	1.00	1.20 (0.75, 1.93)	1.17 (0.71, 1.95)	2.20 (1.28, 3.80)	0.91/0.03
Model II	1.00	1.38 (0.84, 2.25)	1.42 (0.83, 2.41)	2.53 (1.43, 4.49)	0.07/0.01
Model III	1.00	1.40 (0.85, 2.31)	1.47 (0.85, 2.53)	2.44 (1.32, 4.51)	0.55/0.02
Race/Ethnicity Stratified Analysis					
US White (n = 276)					
n (%)	32 (11.6)	162 (58.7)	71 (25.7)	11 (4.0)	-
Mean CAC score	56.5	23.1	17.5	26.7	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**
Model II	1.00	0.91 (0.38, 2.20)	0.92 (0.34, 2.47)	0.66 (0.11, 3.88)	0.98/0.67
Model III	1.00	0.96 (0.38, 2.41)	1.05 (0.37, 3.01)	0.89 (0.14, 5.63)	0.99/0.98
Japanese in Japan (n = 305)					
n (%)	48 (15.7)	82 (26.9)	81 (26.6)	94 (30.8)	-
Mean CAC score	11.0	5.3	1.9	25.2	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**
Model II	1.00	0.77 (0.23, 2.62)	0.28 (0.06, 1.29)	1.93 (0.64, 5.83)	0.18/0.69
Model III	1.00	0.73 (0.20, 2.63)	0.23 (0.05, 1.15)	1.76 (0.53, 5.89)	0.16/0.56
Japanese American (n = 250)					
n (%)	71 (28.4)	75 (30.0)	59 (23.6)	45 (18.0)	-
Mean CAC score	22.9	57.7	104.0	69.5	-
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**
Model II	1.00	2.08 (0.99, 4.48)	2.58 (1.15, 5.76)	3.18 (1.35, 7.47)	0.11/0.02
Model III	1.00	2.16 (0.98, 4.73)	2.63 (1.16, 5.98)	2.68 (1.07, 6.78)	0.07/0.06

OR: odds ratio; CI: confidence interval; CAC: coronary artery calcification;
Model I: Alcohol consumption, age, race, years of education;
Model II: Model I + pack-years of smoking, BMI, diabetes, anti-lipid medication, job physical activity, meat intake, LDL-C, CRP;
Model III: Model II + HDL-C, triglycerides, hypertension, fibrinogen;
[a]*p- trend* shows *p*-value for linear and quadratic trend across the alcohol consumption categories calculated using contrast.

4.0 MANUSCRIPT III: A SIGNIFICANT INVERSE ASSOCIATION OF LONG-CHAIN N-3 POLYUNSATURATED FATTY ACIDS WITH AORTIC CALCIFICATION IN HEALTHY MEN AGED 40-49 YEARS FOR THE ERA-JUMP STUDY

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4.1 ABSTRACT

Background: Few studies have examined the association between long-chain n-3 polyunsaturated fatty acids (LCn-3PUFAs) and measures of atherosclerosis in the general population.

Objective: We assessed the relationship of total LCn-3PUFAs, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) to aortic calcification – a reliable marker of generalized atherosclerosis.

Methods: In a multiethnic population-based study of 1033 asymptomatic men aged 40-49 years, we examined the relationship of serum LCn-3PUFAs to aortic calcification (measured by electron-beam computed tomography and quantified using the Agatston method) using Tobit regression and ordinal regression after adjusting for cardiovascular risk factors and potential confounders.

Results: Overall, 56.5% participants had an aortic calcification score (AoCaS) >0. The means (SD) of total LCn-3PUFAs, EPA, and DHA were 5.8% (3.3%), 1.4% (1.3%), and 3.7% (2.1%) respectively. In Tobit regression a 1-SD increase in total LCn-3PUFAs, EPA, and DHA was associated with 29% (95% CI = 0.51, 1.00), 9% (95% CI = 0.68, 1.23), and 35% (95% CI = 0.46, 0.91) lower expected AoCaS respectively. Results were similar in ordinal regression analysis. There was no significant interaction between LCn-3PUFAs and race/ethnicity on AoCaS. In a race/ethnicity stratified analysis, total LCn-3PUFAs and DHA were inversely and significantly associated with AoCaS among US White but not in other races/ethnicities.

Conclusion: This study shows a significant inverse association of LCn-3PUFAs with aortic calcification independent of cardiovascular risk factors in the general population. This significant inverse association appeared to be driven by DHA but not EPA.

Keywords: Aorta, Atherosclerosis, Calcification, DHA, EPA, LCn-3PUFAs

4.2 INTRODUCTION

Major n-3 polyunsaturated fatty acids (PUFAs) playing an important role in human physiology are α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) [152]. The human body cannot produce ALA (essential fatty acid) and therefore must be acquired through diet, whereas long-chain n-3 polyunsaturated fatty acids (LCn-3PUFAs): EPA and DHA, can be synthesized in the human body (non-essential fatty acids) from ALA through several biochemical processes [152]. Less than 10% of ALA is converted to EPA and less than 5% to DHA. Therefore, the blood levels of EPA and DHA mainly reflect their dietary intake [153].

Meta-analyses of prospective observational studies and randomized controlled trials (RCTs) documented a protective effect of LCn-3PUFAs on cardiovascular health, particularly in lowering the risk of cardiac mortality [153, 165, 219, 289]. The protective effect of LCn-3PUFAs on cardiac mortality is mainly attributed to the antiarrhythmic property of LCn-3PUFAs [1, 153]. Other cardiovascular benefits of LCn-3PUFAs include lowering of triglycerides, blood pressure, resting heart rate, cytokine formation, platelet aggregation, and inflammatory markers; and improvement in endothelial dysfunction, arterial compliance, and vascular reactivity [153]. It is also speculated that LCn-3PUFAs inhibit the atherosclerosis process (the major underlying cause of coronary heart disease (CHD)) [207, 216, 290] by lowering inflammation, improving endothelial function, and increasing atherosclerotic plaque stability [218, 219, 226, 227]. Animal studies [202-204] and basic research [205] strongly support the antiatherosclerotic properties of

LCn-3PUFAs. However, a limited number of studies conducted in a healthy human population reported mixed findings with some documenting no significant association [209, 211, 212] and others reporting a significant inverse association [208]. It is important to examine the relationship between LCn-3PUFAs and atherosclerosis in the general population to gain further insight into the relationship between LCn-3PUFAs and CHD.

Aortic calcification is a reliable biomarker of generalized atherosclerosis [291] and has a graded and consistent relationship with CHD beyond traditional cardiovascular risk factors [27, 32]. Moreover, some studies have reported that aortic calcification may be a better measure of atherosclerosis than coronary artery calcification (CAC) - a well-established biomarker of coronary atherosclerosis, because it adds valuable prognostic information of cardiovascular risk beyond CAC [29, 266]; develops earlier and more extensively than in any other vascular bed [28]; and may have a stronger association with cardiovascular risk factors than CAC [29, 266, 268]. To the best of our knowledge, however, no previous study has examined the association of serum biomarkers of LCn-3PUFAs and aortic calcification in the general population.

In this study, we examined the relationship of LCn-3 PUFAs to aortic calcification in 1033 asymptomatic middle-aged men who participated in the ERA-JUMP Study [the Electron Beam Computed Tomography (**E**BCT), **R**isk-Factor **A**ssessment among **J**apanese and the **U**nited States (US) **M**en in the **P**ost-World-War-II birth cohort]. We hypothesized that serum total LCn-3PUFAs would have a significant inverse association with aortic calcification. Moreover, based on our previous finding of a significant inverse association of DHA (but not EPA) with carotid intima-media thickness (CIMT) [208] and a reported differential significant association of DHA compared to EPA with endothelial dysfunction (a precursor of atherosclerosis) [219], we also

hypothesized that DHA but not EPA would have a significant inverse association with aortic calcification.

4.3 MATERIALS AND METHODS

4.3.1 Study Population

The ERA-JUMP Study is a population-based study of 1033 men aged 40–49 years comprising US White, US Black, Japanese American, and Japanese in Japan. The details of the study protocol have been described previously [234]. Briefly, during 2002–2006, a population-based sample of 1033 men aged 40–49 years, without the clinical cardiovascular diseases (CVD) or other severe illnesses, was obtained from 3 centers: 310 White and 107 Black from Pittsburgh, Pennsylvania, US; 303 Japanese American from Honolulu, Hawaii, US; 313 Japanese from Kusatsu City, Shiga, Japan. The study protocol followed 'the 1975 Declaration of Helsinki ethical guidelines.' We obtained the study approval from the Institutional Review Boards of University of Pittsburgh, Pittsburgh, US; Kuakini Medical Center, Honolulu, US; Shiga University of Medical Science, Otsu, Japan. All participants gave written informed consent. We excluded participants with missing data for aortic calcification (n=27) and LCn-3PUFAs (n=8). Our final sample size was 998 with 300 US White, 101 US Black, 287 Japanese American, and 310 Japanese in Japan.

4.3.2 Risk Factor Assessment

Standardized data collection procedures were followed across all centers. As published elsewhere, participants underwent a physical examination, completed a set of lifestyle questionnaire, and a laboratory assessment [292, 293]. Body weight and height were measured while the participant was wearing minimal light clothing without shoes. The formula used to calculate Body mass index (BMI) was ‘weight in kilograms divided by the square of height in meters.' Participants with systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg or use of antihypertensive medications were considered as hypertensive. Participants were considered to be smokers if they reported current use of cigarettes or having stopped smoking within the past 30 days. The formula used to calculate pack-years of smoking was ‘years of smoking multiplied by the number of cigarettes per day divided by 20’. Medication use (antihypertensive, antidiabetic, and lipid-lowering) was reported as ‘yes/no’. Meat intake was defined as intake of beef, pork, or sausage ≥2 times per week. Self-reported physical activity related to the current job was categorized into sedentary, light, medium, and heavy physical activity.

Venipuncture was performed early in the clinic visit after a 12-hour fast. Blood samples were stored at -70°C and shipped on dry ice from all the centers to the University of Pittsburgh. Serum/plasma samples were assayed for glucose, lipids [including total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C)], fibrinogen, and C-reactive protein (CRP) as described previously [55]. Participants with fasting glucose ≥7.0 mmol/l or using medications for diabetes were considered as having diabetes.

4.3.3 N-3 and N-6 Polyunsaturated Fatty Acids Assessment

Serum levels of n-3 PUFAs [EPA (20:5n-3), docosapentaenoic acid (DPA (22:5n-3)), DHA (22:6n-3), and ALA (20:3n-3)], and n-6 PUFAs [linoleic (LA (18:2n-6)) and arachidonic (ARA (20:4n-6)) acids] were measured using Capillary Gas-Liquid chromatography, as previously described [234]. Serum fatty acids were measured as percentages of total fatty acids. The coefficients of variation between tests for EPA, DPA, DHA, ALA, LA, ARA, and total fatty acids were 4.5%, 4.5%, 7.2%, 1.6%, 7.9%, 2.8%, and 5.7% respectively. Total LCn-3PUFAs was defined as the sum of EPA, DPA, and DHA.

4.3.4 Aortic Calcification Assessment

To assess aortic calcification, EBCT was performed using a GE-Imatron C150 scanner (GE Medical Systems, South San Francisco, US) at the three centers [55, 57]. To evaluate aortic calcification, 6 mm images were acquired from the aortic arch to the iliac bifurcation. Readings of the scans were performed centrally at the Cardiovascular Institute, University of Pittsburgh, using a DICOM (Digital Imaging and Communications in Medicine) workstation and software by AccuImage (AccuImage Diagnostic Cooperation, San Francisco, US). The software program implements the widely accepted Agatston scoring method [16]. A trained radiology technician who was blinded to each participant's characteristics and the study centers evaluated the readings. The reproducibility of non-zero Agatston aortic calcification score (AoCaS) had an intra-class correlation of 0.98 [294].

4.3.5 Statistical Analysis

The distributions of AoCaS, triglycerides, years of education, CRP, and the pack-years of smoking were highly skewed, and therefore log transformed. Continuous variables with approximately normal distribution (i.e., total LCn-3PUFAs, EPA, DHA, ALA, LA, ARA, age, BMI, LDL-C, and HDL-C) were standardized. We created race-specific quartiles of total LCn-3PUFAs, EPA, and DHA. Across different quartiles of total LCn-3PUFAs as well as categories of AoCaS (i) Age and race/ethnicity adjusted BMI, LDL-C, and HDL-C were expressed as means±standard error (SE); (ii) Age and race/ethnicity adjusted triglycerides, years of education, and the pack-years of smoking were expressed as medians and interquartile range (IQR); (iii) Age and race/ethnicity adjusted categorical variables were expressed in percentages. A *p*-value for trend across the different quartiles of LCn-3PUFAs as well as the categories of AoCaS was determined using linear regression when a response variable was continuous, using quartile regression when it was a non-normal continuous variable, and logistic regression when it was a categorical variable.

We used Tobit conditional regression to determine the independent association of total LCn-3PUFAs, EPA, or DHA with aortic calcification [natural log of (AoCaS + 1)] adjusting for potential confounders. We considered Tobit regression because it is suited to the uncommon distribution of AoCaS (right-sided skewness and many participants with zero AoCaS) [276, 277]. Secondarily, we also performed ordinal logistic regression to assess the likelihood of study participants being in a higher category of AoCaS. For ordinal regression, four AoCaS categories were used: 0, 1-99, 100-299, and ≥300. For both Tobit regression and ordinal regression: Model I was adjusted for socio-demographic variables (age, race/ethnicity, and years of education); Model II was further adjusted for potential confounders (pack-years of smoking, alcohol

consumption, BMI, diabetes, lipid-lowering medications, LDL-C, physical activity at job, and meat intake); Model III was additionally adjusted for intermediary variables (hypertension, HDL-C, triglycerides, CRP, and fibrinogen) in the relationship between LCn-3PUFAs and atherosclerosis/CHD. In model III, we tested for an interaction between race/ethnicity and total LCn-3PUFAs (or EPA or DHA) on aortic calcification. In regression models, we treated total LCn-3PUFAs, EPA, and DHA as categorical variables (race-specific quartiles) as well as continuous variables separately. We also assessed the independent association of ALA, LA, and ARA using similar regression techniques and models mentioned above. The inclusion of variables in the regression models was mainly based on previously published literature on the relationship of LCn-3PUFAs to atherosclerosis/CHD to minimize the possibility of residual confounding. In Tobit regression as well as in ordinal regression a *p*-value for linear trend across the quartiles of LCn-3PUFAs was calculated using contrast.

Sensitivity analyses were further conducted (i) Excluding Japanese study participants, as serum median levels of LCn-3PUFAs among Japanese in Japan were ≥2 times compared to other study participants [234]; and (ii) Stratifying the analysis by race/ethnicity. All *p*-values were two-tailed and *p*-value <0.05 was considered as significant. SAS version 9.4 (SAS Institute, Cary, NC, US) and STATA version 14.0 (StataCorp LP, College Station, TX, US) were used for all statistical analyses.

4.4 RESULTS

Overall mean (SD) age of study participants was 45.3 (2.8) years. Study participants had on average BMI of 26.8 kg/m^2, 25.6% had hypertension, 7.4% had diabetes, and 56.5% had AoCaS

>0 with a median (IQR) AoCaS of 4.0 (0.0, 50.0) (Table 4-1). Among all study participants, means (SD) for LDL-C, HDL-C, total LCn-3PUFAs, EPA, and DHA were 129.6 (35.4), 50.9 (13.4), 5.8 (3.3), 1.4 (1.3), and 3.7 (2.1) respectively. Japanese in Japan had much higher serum levels of total LCn-3PUFAs, EPA, and DHA compared to participants of other race/ethnicity groups.

Table 4-2 describes the age and race/ethnicity adjusted demographic and clinical characteristics of study participants by quartiles of total LCn-3PUFAs. There was a significant decreasing trend in BMI, triglycerides, the proportion with diabetes, CRP, and AoCaS with increasing in quartile of total LCn-3PUFAs. On the contrary, serum levels of LDL-C and HDL-C increased with increasing in quartiles of total LCn-3PUFAs.

Except for HDL-C, total LCn-3PUFAs, EPA, and DHA, adjusting for age and race/ethnicity, participants with AoCaS >0 had higher: BMI, pack-years of smoking, LDL-C, triglycerides, CRP, and fibrinogen, had a greater proportion with diabetes and hypertension, and were more likely to be on lipid-lowering medications compared to zero AoCaS category (Table 4-3).

In Tobit regression, participants in the fourth quartile compared to the first quartile of total LCn-3PUFAs had 49% lower expected AoCaS after adjustment for socio-demographic and potential confounders [Model II: Tobit ratio (TR) (95% CI) = 0.51 (0.26, 0.97)] (Table 4-4). With further adjustment for intermediary variables, this significant inverse association was attenuated and became nonsignificant [Model III: TR (95% CI) = 0.55 (0.28, 1.08)]. In model II, a 1-SD (3.3%) increase in total LCn-3PUFAs was associated with 29% lower expected AoCaS [TR (95% CI) = 0.71 (0.51, 1.00)]. EPA (whether assessed as a categorical or a continuous variable) was not significantly associated with aortic calcification. In model II, a 1-SD increase

in EPA (1.3%) was associated with 9% lower expected AoCaS [TR (95% CI) = 0.91 (0.68, 1.23)]. Participants in the fourth quartile of DHA compared to the first quartile had significantly lower expected AoCaS in all models – from unadjusted to fully adjusted model III. There was a significant dose-response relationship between DHA and aortic calcification (*p* for linear trend <0.05). In model II, a 1-SD (2.1%) increase in DHA was associated with 35% lower expected AoCaS [TR (95% CI) = 0.65 (0.46, 0.91)]. This significant inverse association remained with further adjustment for intermediary variables [Model III: TR (95% CI) = 0.69 (0.49, 0.98)] (Table 4-4) and ALA, LA, and ARA [TR (95% CI) = 0.68 (0.47, 0.98)] (Table 4-7). In model II, ARA but not ALA or LA had a significant inverse association with aortic calcification [TR (95% CI) = 0.64 (0.48, 0.86)] (Table 4-6).

Using ordinal regression, participants in the fourth quartile compared to the first quartile of total LCn-3PUFAs had a significantly lower likelihood of having higher AoCaS after adjustment for socio-demographic variables and potential confounders [Model II: OR (95% CI) = 0.69 (0.48, 0.99)] (Table 4-5). With further adjustment for intermediary variables, the significant association was attenuated and became nonsignificant [Model III: OR (95% CI) = 0.74 (0.52, 1.07)]. In model II, a 1-SD (3.3%) increase in total LCn-3PUFAs was associated with 16% lower likelihood of having higher AoCaS [OR (95% CI) = 0.84 (0.70, 1.01)]. EPA, whether used as a categorical or a continuous variable, was not significantly associated with aortic calcification. Participants in the fourth compared to the first quartile of DHA had 35% lower likelihood of having higher AoCaS after adjustment for socio-demographic and potential confounders [OR (95% CI) = 0.65 (0.45, 0.92)]. In model II, a 1-SD (2.1%) increase in DHA was associated with 20% lower likelihood of having higher AoCaS [OR (95% CI) = 0.80 (0.67, 0.97)]. In model II, a 1-SD (2.4%) increase in ARA was associated with 19% lower likelihood of

having higher AoCaS [OR (95% CI) = 0.81 (0.70, 0.95)] (Table 4-6). In Tobit regression as well as in ordinal regression, there was no significant interaction between race/ethnicity and total LCn–3PUFAs, EPA, DHA, ARA, ALA, or LA on aortic calcification.

When the analysis was repeated excluding Japanese in Japan, results were similar to the analysis combining all study participants (Tables 4-8 and 4-9). A 1-SD increase in total LCn-3PUFAs and DHA but not EPA was significantly and inversely associated with aortic calcification independent of cardiovascular risk factors and potential confounders. In a stratified analysis by race/ethnicity, among US White, a 1-SD increase in total LCn-3PUFAs and DHA was associated with approximately 40% to 50% lower expected AoCaS (model II and model III). Both when using Tobit regression (Table 4-10) and ordinal regression (Table 4-11). Among Japanese in Japan as well as among Japanese American, total LCn-3PUFAs and DHA were inversely and non-significantly associated with aortic calcification (Tables 4-12, 4-13, 4-14, 4-15).

4.5 DISCUSSION

In this community-based sample of healthy middle-aged men (US White, US Black, Japanese American, and Japanese in Japan) blood levels of total LCn-3PUFAs were significantly and inversely associated with aortic calcification independent of cardiovascular risk factors. This significant inverse association appeared to be driven by DHA. DHA was significantly and inversely associated with aortic calcification after adjustment for cardiovascular risk factors and other fatty acids including ALA, LA, and ARA. Consistent results were seen in sensitivity analysis excluding Japanese in Japan from the main analysis. To the best of our knowledge, this

is the first community-based study examining the relationship between blood biomarkers of LCn-3PUFAs and aortic calcification in asymptomatic middle-aged men across different races/ethnicities from two countries in a standardized manner.

In contrast to our findings, He et al. in the Multi-Ethnic Study of Atherosclerosis [212] of 5488 healthy US adults from four different race/ethnicity, aged 45–84 years without clinical CVD and Heine-Broring et al. in the Rotterdam study [211] among 1570 asymptomatic participants aged >55 years reported no significant association of dietary intake of LCn-3PUFAs and CAC measured by EBCT. Similarly, Shang et al. in the Melbourne Collaborative Cohort Study among 312 asymptomatic participants aged 45–64 years reported no significant association between dietary intake of LCn-3PUFAs and aortic calcification measured by lateral thoraco-lumbar radiography and dual-energy X-ray absorptiometry [209]. Several plausible explanations for the contrasting findings between these studies [209, 211, 212] and ours may include differences in the age distribution, subclinical atherosclerosis assessment techniques, the examined vascular bed, and use of blood biomarkers of LCn-3PUFAs in our study as opposed to self-reported dietary assessment of fatty acids which may lead to LCn-3PUFAs misclassification.

In our study, the association of total LCn-3PUFAs, EPA, and DHA with aortic calcification was partly attenuated after adjusting for intermediary variables suggesting that the relationship was partly mediated through the effects of LCn-3PUFAs on blood pressure, lipids, and inflammation. Atherosclerosis is a systemic chronic inflammatory disease of the vessel walls. Inflammation resulting from the interaction of modified atherogenic lipoproteins, inflammatory cells, and smooth muscle cells of vessel wall plays a major role in the initiation and progression of the atherosclerotic plaque [11]. Available evidence from epidemiological and experimental studies suggest that LCn-3PUFAs exerts antiatherosclerotic effects through several

anti-inflammatory pathways including lowering the expression of nuclear factor κ-B, regulators of inflammation, and oxidative stress, improving endothelial function, and increasing atherosclerotic plaque stability [218, 219, 226, 227].

Our study shows a significant inverse association of DHA but not EPA with aortic calcification. This finding concords with our previous finding of a significant inverse association of DHA but not EPA with CIMT [208] as well as results of a prospective cohort study among postmenopausal women with CHD where DHA but not EPA was significantly associated with less progression of coronary atherosclerosis [295]. Additionally, indirect evidence from short-term clinical trials in humans reported that DHA compared to EPA is more potent in lowering blood pressure [296, 297], resting heart rate [298], the expression of pro-inflammatory cytokines, cell adhesion molecules, and monocyte adhesion to endothelial cells [299].

To date, no RCT has assessed the effect of pure DHA or compared the effect of pure DHA with EPA on cardiovascular outcomes or atherosclerosis. However several RCTs of pure EPA conducted among diabetics [207], patients with stable angina [216], and CHD [290] reported an inverse association of EPA with measures of atherosclerosis. Moreover, the RCTs in diabetics [207] and CHD patients [290] reported an increase in serum EPA but not DHA (although EPA theoretically can be metabolized to DHA) among intervention groups supporting the antiatherogenic effect of EPA. The significant inverse association of DHA but not EPA with aortic calcification in our study may imply that DHA may be more antiatherogenic than EPA. RCTs are warranted to disentangle the differential association of EPA and DHA with cardiovascular outcomes and atherosclerosis.

In our study, the robust inverse association of total LCn-3PUFAs and DHA with aortic calcification was seen among US White but not in Japanese in Japan or Japanese American.

There was no significant interaction between LCn-3PUFAs and race/ethnicity on aortic calcification. Plausible reasons for the nonsignificant inverse association of LCn-3PUFAs with aortic calcification among Japanese in Japan and Japanese American include suboptimal sample size and fewer number of participants with an AoCaS >0.

Our study also shows a significant inverse association of ARA with aortic calcification. This finding concords with findings reported in a meta-analysis of prospective observational studies and RCTs [300]. Although ARA-derived eicosanoids leukotriene-B4 and thromboxane-A2 are thought to be pro-inflammatory, other ARA-derived eicosanoids, epoxyeicosatrienoic acid, and lipoxins are considered to lower inflammation [301]. This anti-inflammatory mechanism was further supported by animal studies and observational studies in humans showing an inverse association of ARA metabolites and cardiovascular risk [153].

Our study has several limitations. First, blood levels of LCn-3PUFAs reflect short-term intake and may not reflect long-term dietary intake. However, blood levels of LCn-3PUFAs vary randomly, therefore the actual association between blood levels of LCn-3PUFAs and aortic calcification could be stronger than reported in the current study. Second, we examined healthy men aged 40-49 years in Japan and the US; therefore, the results of the study cannot be generalized to females, other populations, or age groups. Future studies should be performed on women and participants of other age groups, to assess whether the association differs by sex or different age groups. Third, although we have controlled for a variety of sociodemographic and clinical characteristics, the possibility of residual confounding cannot be excluded. However, any remaining potential confounder would need to be strongly associated with both blood levels of LCn-3PUFAs and atherosclerosis and not related to other covariates included in the regression

models. Fourth, we cannot establish a causal association between blood levels of LCn-3PUFAs and aortic calcification based on our cross-sectional analyses.

Strengths of the current study include (i) the community-based nature of the study design with randomly selected study participants which increases the external validity of the study; (ii) Standardized measurement techniques were used across all centers; (iii) The use of EBCT to detect aortic calcification, allowing the accurate visualization of small calcific deposits without image blurring; (iv) and the use of blood biomarkers of LCn-3PUFAs as opposed to self-reported dietary assessment of fatty acids, which reduces the possibility of recall bias.

4.6 CONCLUSION

Our study demonstrated for the first time that in a general male population, LCn-3PUFAs are significantly inversely associated with subclinical atherosclerosis, defined by aortic calcification, independent of cardiovascular risk factors. This significant inverse association was mainly attributed to DHA but not EPA. Follow-up population-based studies are needed to further clarify the effect of LCn-3PUFAs on the incidence and progression of atherosclerosis as well as to disentangle the differential effect of DHA and EPA, and the underlying biological mechanisms.

4.7 TABLES

Table 4-1. Descriptive characteristics of study participants for the ERA JUMP Study, 2002-2006

Race/ethnicity	Overall	US White	US Black	Japanese in Japan	Japanese Americans
Total number (%)	998 (100)	300 (30.1)	101 (10.1)	310 (31.1)	287 (28.8)
Age[a], years	45.33 (2.8)	45.0 (2.8)	44.9 (2.8)	45.1 (2.8)	46.1 (2.8)
BMI[a], kg/m^2	26.8 (4.6)	27.8 (4.2)	29.7 (5.8)	23.7 (3.1)	27.9 (4.3)
Pack-years of smoking[b]	0.0 (0.0, 15.0)	0.0 (0.0, 1.4)	0.0 (0.0, 8.8)	18.9 (3.3, 29.0)	0.0 (0.0, 3.4)
LDL-C[a], mg/dL	129.6 (35.4)	134.9 (33.7)	129.0 (41.2)	132.3 (36.0)	121.1(33.0)
HDL-C[a], mg/dL	50.9 (13.4)	47.8 (12.8)	50.9 (15.4)	54.1 (13.7)	50.8 (12.3)
Triglycerides[b], mg/dL	133.0 (95.0, 188.0)	128.0 (92.5, 185.5)	108.0 (80.0, 166.0)	137.0 (104.0, 182.0)	142.0 (93.0, 227.0)
Hypertension[c]	255 (25.6)	43 (14.3)	33 (32.7)	83 (26.8)	96 (33.5)
Diabetes[c]	74 (7.4)	9 (3.0)	9 (6.1)	19 (6.1)	37 (12.9)
Anti-lipid med[c]	123 (12.2)	36 (12.0)	9 (8.9)	11 (3.6)	67 (23.3)
Meat intake[c]	756 (75.8)	231 (77.0)	74 (73.3)	207 (66.8)	244 (85.0)
Years of education[b]	16.0 (14.0, 16.0)	16.5 (16.0, 18.0)	14.0 (12.0, 16.0)	16.0 (12.0, 16.0)	16.0 (14.0, 16.0)
CRP[b], mg/dL	0.7 (0.3, 1.4)	0.9 (0.5, 1.8)	1.5 (0.9, 3.1)	0.3 (0.2, 0.7)	0.7 (0.3, 1.3)
Fibrinogen[a], mg/dL	289.4 (74.5)	290.8 (70.3)	314.2 (73.7)	255.8 (65.8)	315.6 (73.9)
Alcohol[b], gm/day	7.4 (0.0, 25.9)	4.5 (1.0, 16.3)	3.1 (0.0, 24.7)	16.5 (2.5, 42.4)	1.0 (0.0, 25.9)
AoCaS[b]	4.0 (0.0, 50.0)	9.0 (0.0, 45.0)	14.0 (0.0, 46.0)	0.0 (0.0, 41.0)	5.0 (0.0, 76.0)
Total LCn-3PUFAs[a], %	5.8 (3.3)	3.8 (1.7)	3.8 (1.5)	9.3 (3.0)	4.8 (2.2)
EPA[a], %	1.4 (1.3)	0.8 (0.5)	0.7 (0.5)	2.5 (1.4)	1.0 (0.9)
DHA[a], %	3.7 (2.1)	2.4 (1.2)	2.5 (1.1)	5.9 (1.7)	3.2 (1.4)
ALA[a], %	0.3 (0.3)	0.3 (0.3)	0.4 (0.4)	0.2 (0.2)	0.5 (0.4)
LA[a], %	28.8 (4.4)	29.9 (4.1)	29.0 (4.2)	26.5 (4.1)	30.0 (4.3)
ARA[a], %	8.5 (2.4)	9.0 (1.9)	11.3 (1.9)	6.6 (1.3)	8.9 (2.4)

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein; AoCaS, aortic calcification score; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; ALA, α-linolenic acid, LA, linoleic acid; ARA, Arachidonic acids; Total LCn-3PUFAs, long chain n-3 polyunsaturated fatty acids defined as sum of EPA, DPA, and DHA; n(%): number(%);

[a]Continuous normally distributed variables expressed as mean (standard deviation);

[b]Continuous non-normally distributed variables expressed as median (inter-quartile range);

[c]Categorical variables expressed as numbers (%);

SI conversion factors: To convert LDL-C and HDL-C to mmol/L, multiply values by 0.0259. To convert triglycerides to mmol/L, multiply values by 0.01129. To convert fibrinogen to µmol/L, multiply values by 0.0294. To convert CRP to nmol/L, multiply values by 9.524.

Table 4-2. Demographic and clinical characteristics by race/ethnicity specific quartiles of total LC n-3 PUFAs for the ERA JUMP Study, 2002-2006 (n=998)

Total LCn-3PUFAs quartiles	Q1	Q2	Q3	Q4	*p*-trend[d]
Total number (%)	251 (25.3)	249 (24.9)	249 (24.9)	249 (24.9)	-
LCn-3PUFAs[b], %	2.7 (2.3, 5.0)	3.8 (3.1, 7.7)	4.9 (4.0, 9.0)	7.3 (5.9, 11.8)	-
Age[a], years	45.1 (0.2)	45.5 (0.2)	45.0 (0.2)	45.8 (0.2)	0.01
BMI[a], kg/m^2	28.3 (0.3)	28.3 (0.3)	27.7 (0.3)	27.1 (0.3)	0.01
Pack-years of smoking[b]	0.0 (0.0, 6.2)	0.0 (0.0, 2.7)	0.0 (0.0, 2.7)	0.0 (0.0, 2.6)	0.10
Alcohol[b], gm/day	2.8 (1.0, 10.8)	5.1 (1.0, 16.8)	6.1 (1.0, 16.1)	5.6 (1.0, 16.3)	0.20
LDL-C[a], mg/dL	131.3 (2.8)	132.5 (2.8)	135.9 (2.8)	140.6 (2.8)	0.01
HDL-C[a], mg/dL	44.7 (1.1)	48.8 (1.1)	48.5 (1.1)	49.5 (1.1)	0.01
Triglycerides[b], mg/dL	154.1 (104.2, 214.3)	126.1 (93.2, 181.4)	122.1 (90.2, 158.1)	120.8 (82.2, 163.0)	0.01
Hypertension[c]	40 (15.6)	37 (14.8)	32 (12.9)	35 (13.9)	0.39
Diabetes[c]	12 (4.8)	9 (3.4)	6 (2.3)	4 (1.7)	0.01
Anti-lipid med[c]	30 (11.8)	32 (12.6)	35 (13.8)	26 (10.2)	0.68
Meat intake[c]	210 (83.4)	201 (80.5)	194 (77.9)	166 (66.7)	0.01
Years of education[b]	17.0 (16.0, 18.0)	16.0 (16.0, 18.0)	17.0 (16.0, 18.0)	17.0 (16.0, 18.0)	0.32
CRP[b], mg/dL	1.1 (0.6, 1.8)	1.0 (0.5, 1.9)	1.0 (0.5, 1.6)	0.9 (0.5, 1.7)	0.05
Fibrinogen[a], mg/dL	290.5 (5.6)	292.7 (5.6)	288.2 (5.7)	294.8 (5.6)	0.66
AoCaS[b]	9.2 (0.0, 91.7)	8.7 (0.0, 67.0)	8.2 (0.0, 67.0)	6.7 (0.0, 57.0)	0.35

Q1, Q2, Q3, Q4: quartiles of total LCn-3PUFAs; BMI: body mass index; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; CRP: C-reactive protein; AoCaS: aortic calcification score; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; Total LCn-3PUFAs: total long-chain n-3 polyunsaturated fatty acids defined as sum of EPA, DPA, and DHA; n(%): number(%);

Values for all variables except age were adjusted for 'age' and 'race/ethnicity': value of age was fixed at 45.3 years and race was fixed as 'US White'.

[a]Continuous normally distributed variables were expressed as mean (standard error);

[b]Continuous non-normally distributed variables were expressed as median (inter-quartile range);

[c]Categorical variables were expressed as numbers (%);

[d]*p*-trend shows a *p*-value for linear trend across the quartiles of total LCn-3PUFAs;

SI conversion factors: To convert LDL-C and HDL-C to mmol/L, multiply values by 0.0259. To convert triglycerides to mmol/L, multiply values by 0.01129. To convert fibrinogen to µmol/L, multiply values by 0.0294. To convert CRP to nmol/L, multiply values by 9.524.

Table 4-3. Demographic and clinical characteristics by aortic calcification score categories for the ERA-JUMP Study, 2002-2006 (n=998)

AoCaS categories	AoCaS= 0	AoCaS 0 - 99	AoCaS 100-299	AoCaS ≥300	*p*-trend[d]
Total number (%)	434 (43.5)	367 (36.8)	89 (8.9)	108 (10.8)	-
Age[a], years	44.8 (0.1)	45.4 (0.2)	46.2 (0.3)	46.3 (0.3)	0.01
BMI[a], kg/m^2	26.2 (0.3)	28.7 (0.3)	29.0 (0.5)	27.3 (0.5)	0.01
Pack-years of smoking[b]	0.0 (0.0, 0.9)	0.0 (0.0, 1.0)	0.0 (0.0, 12.8)	11.0 (0.8, 19.7)	0.01
Alcohol[b], gm/day	5.0 (1.0, 15.9)	3.6 (1.0, 13.9)	3.5 (1.0, 11.5)	24.5 (2.1, 35.0)	0.01
LDL-C[a], mg/dL	130.6 (2.6)	137.9 (2.3)	131.8 (4.1)	138.2 (3.8)	0.16
HDL-C[a], mg/dL	50.4 (1.0)	46.3 (0.9)	46.2 (1.5)	49.8 (1.5)	0.71
Triglycerides[b], mg/dL	114.7 (80.7, 154.4)	130.7 (95.7, 184.9)	148.7 (105.7, 207.9)	138.7 (95.7, 240.5)	0.01
Hypertension[c]	42 (9.5)	59 (16.1)	18 (19.9)	17 (15.2)	0.02
Diabetes[c]	9 (2.1)	10 (2.7)	6 (6.5)	5 (4.6)	0.01
Anti-lipid med[c]	39 (9.0)	41 (11.0)	23 (25.8)	16 (14.5)	0.01
Meat intake[c]	324 (74.7)	287 (78.0)	72 (80.0)	83 (76.4)	0.61
Years of education[b]	17.0 (16.0, 18.0)	17.0 (16.0, 18.0)	16.0 (16.0, 18.0)	16.0 (16.0, 18.0)	0.01
CRP[b], mg/dL	0.8 (0.4, 1.6)	1.0 (0.6, 1.9)	1.0 (0.5, 2.0)	1.0 (0.5, 1.9)	0.05
Fibrinogen[a], mg/dL	284.6 (5.3)	293.0 (4.8)	300.0 (8.2)	298.6 (7.7)	0.05
Total LCn-3PUFAs[a], %	4.2 (0.2)	3.7 (0.2)	3.6 (0.3)	3.7 (0.3)	0.08
EPA[a], %	0.9 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.74
DHA[a], %	2.6 (0.1)	2.3 (0.1)	2.2 (0.2)	2.3 (0.2)	0.01
ALA[a], %	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)	0.63
LA[a], %	30.3 (0.3)	30.0 (0.3)	29.2 (0.5)	28.9 (0.5)	0.01
ARA[a], %	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)	0.17

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein; AoCaS, aortic calcification score; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; ALA, α-linolenic acid; LA: linoleic acid; ARA, Arachidonic acids; Total LCn-3PUFAs, total long chain omega 3 polyunsaturated fatty acids defined as the sum of EPA, DPA, and DHA; n(%), number(%);
Values for all variables except age were adjusted for 'age' and 'race/ethnicity': value of age was fixed at 45.3 years and race was fixed as 'US White'.
[a]Continuous normally distributed variables were expressed as mean (standard error);
[b]Continuous non-normally distributed variables were expressed as median (inter-quartile range);
[c]Categorical variables were expressed as numbers (%);
[d]*p*-trend shows a *p*-value for linear trend across the AoCaS categories;
SI conversion factors: To convert LDL-C and HDL-C to mmol/L, multiply values by 0.0259. To convert triglycerides to mmol/L, multiply values by 0.01129. To convert fibrinogen to µmol/L, multiply values by 0.0294. To convert CRP to nmol/L, multiply values by 9.524.

Table 4-4. Tobit conditional regression describing the association between fatty acids and aortic calcification for the ERA-JUMP Study, 2002-2006 (n=998)

LCn-3PUFAs quartiles	Q1	Q2	Q3	Q4		LCn-3PUFAs as a continuous variable
Total LCn-3PUFAs						
Median (IQR)	2.7 (2.3, 5.0)	3.8 (3.1, 7.7)	4.9 (4.0, 9.0)	7.3 (5.9, 11.8)	-	Total LCn-3PUFAs
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	**TR (95% CI)**
Unadjusted	1	0.81 (0.40, 1.63)	0.64 (0.31, 1.29)	0.43 (0.21, 0.87)	0.02	0.44 (0.34, 0.57)
Model I	1	0.74 (0.38, 1.46)	0.73 (0.37, 1.44)	0.38 (0.19, 0.75)	0.01	0.62 (0.43, 0.88)
Model II	1	0.85 (0.45, 1.60)	0.91 (0.48, 1.72)	0.51 (0.26, 0.97)	0.06	0.71 (0.51, 1.00)
Model III	1	0.87 (0.46, 1.65)	1.03 (0.54, 1.98)	0.55 (0.28, 1.08)	0.14	0.76 (0.54, 1.08)
EPA						
Median (IQR)	0.4 (0.4, 0.9)	0.6 (0.6, 1.8)	0.9 (0.7, 2.4)	1.9 (1.2, 3.6)	-	EPA
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	**TR (95% CI)**
Unadjusted	1	0.58 (0.29, 1.18)	0.56 (0.28, 1.13)	0.54 (0.26, 1.08)	0.09	0.56 (0.43, 0.72)
Model I	1	0.59 (0.30, 1.17)	0.64 (0.32, 1.28)	0.57 (0.29, 1.12)	0.14	0.92 (0.67, 1.26)
Model II	1	0.60 (0.32, 1.13)	0.59 (0.31, 1.11)	0.58 (0.30, 1.11)	0.10	0.91 (0.68, 1.23)
Model III	1	0.61 (0.32, 1.15)	0.65 (0.34, 1.24)	0.64 (0.33, 1.24)	0.22	0.96 (0.71, 1.29)
DHA						
Median (IQR)	1.6 (1.3, 3.4)	2.5 (1.8, 4.9)	3.2 (2.6, 5.9)	4.9 (4.0, 7.3)	-	DHA
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	**TR (95% CI)**
Unadjusted	1	0.90 (0.45, 1.82)	0.56 (0.28, 1.12)	0.36 (0.18, 0.73)	0.01	0.42 (0.33, 0.54)
Model I	1	0.80 (0.41, 1.58)	0.59 (0.30, 1.17)	0.30 (0.15, 0.60)	0.01	0.52 (0.36,0.74)
Model II	1	0.85 (0.46, 1.60)	0.74 (0.39, 1.40)	0.43 (0.22, 0.83)	0.01	0.65 (0.46, 0.91)
Model III	1	0.86 (0.45, 1.62)	0.83 (0.43, 1.57)	0.47 (0.24, 0.93)	0.03	0.69 (0.49, 0.98)

Q1, Q2, Q3, Q4, quartiles of total LCn-3PUFAs; IQR, interquartile range; TR, Tobit ratio; CI: confidence interval; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; Total LCn-3PUFAs, total long chain omega 3 polyunsaturated fatty acids defined as the sum of EPA, DPA, and DHA; One standard deviation of total LCn-3PUFAs, EPA, and DHA equals to 3.3%, 1.3%, and 2.1% respectively.

Model I: Fatty acids, age, race, years of education;

Model II: Model I + pack-years of smoking, alcohol consumption, BMI, diabetes, lipid-lowering medications, LDL-C, physical activity at the job, and meat intake;

Model III: Model II + hypertension, HDL-C, triglycerides, CRP, and fibrinogen;

[a]*p*-trend shows a *p*-value for linear trend across the quartiles of LCn-3PUFAs calculated using contrast.

Table 4-5. Ordinal logistic regression describing the association between fatty acids and aortic calcification for the ERA-JUMP Study, 2002-2006 (n=998)

LCn-3PUFAs Quartiles	Q1	Q2	Q3	Q4		LCn-3PUFAs as a continuous variable
Total LCn-3PUFAs						
Median (IQR)	2.7 (2.3, 5.0)	3.8 (3.1, 7.7)	4.9 (4.0, 9.0)	7.3 (5.9, 11.8)		**Total LCn-3PUFAs**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Unadjusted	1	0.90 (0.65, 1.25)	0.81 (0.59, 1.13)	0.67 (0.48, 0.93)	0.82	0.65 (0.58, 0.74)
Model I	1	0.86 (0.62, 1.20)	0.85 (0.61, 1.19)	0.61 (0.43, 0.85)	0.83	0.77 (0.65, 0.92)
Model II	1	0.91 (0.65, 1.29)	0.95 (0.67, 1.35)	0.69 (0.48, 0.99)	0.93	0.84 (0.70, 1.01)
Model III	1	0.94 (0.66, 1.33)	1.04 (0.73, 1.48)	0.74 (0.52, 1.07)	0.81	0.88 (0.73, 1.06)
EPA						
Median (IQR)	0.4 (0.4, 0.9)	0.6 (0.6, 1.8)	0.9 (0.7, 2.4)	1.9 (1.2, 3.6)		**EPA**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Unadjusted	1	0.78 (0.56, 1.07)	0.78 (0.56, 1.08)	0.73 (0.53, 1.02)	0.18	0.73 (0.64, 0.82)
Model I	1	0.78 (0.56, 1.09)	0.82 (0.59, 1.14)	0.72 (0.52, 1.01)	0.25	0.94 (0.81, 1.09)
Model II	1	0.78 (0.55, 1.10)	0.76 (0.54, 1.07)	0.73 (0.51, 1.04)	0.19	0.95 (0.81, 1.12)
Model III	1	0.78 (0.55, 1.11)	0.80 (0.56, 1.14)	0.78 (0.55, 1.12)	0.21	0.98 (0.84, 1.16)
DHA						
Median (IQR)	1.6 (1.3, 3.4)	2.5 (1.8, 4.9)	3.2 (2.6, 5.9)	4.9 (4.0, 7.3)		**DHA**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Unadjusted	1	0.96 (0.69, 1.33)	0.77 (0.55, 1.06)	0.63 (0.45, 0.87)	0.89	0.65 (0.57, 0.73)
Model I	1	0.91 (0.65, 1.26)	0.78 (0.56, 1.08)	0.55 (0.40, 0.78)	0.93	0.72 (0.60, 0.86)
Model II	1	0.94 (0.67, 1.33)	0.87 (0.62, 1.23)	0.65 (0.45, 0.92)	0.83	0.80 (0.67, 0.97)
Model III	1	0.95 (0.67, 1.35)	0.93 (0.65, 1.32)	0.70 (0.49, 1.01)	0.82	0.84 (0.70, 1.02)

Q1, Q2, Q3, Q4, quartiles of total LCn-3PUFAs; IQR, interquartile range; OR, Odds ratio; CI, confidence interval; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; Total LCn-3PUFAs, total long chain omega 3 polyunsaturated fatty acids defined as sum of EPA, DPA, and DHA; One standard deviation of total LCn-3PUFAs, EPA, and DHA equals to 3.3%, 1.3%, and 2.1% respectively.

Model I: Fatty acids, age, race, years of education;

Model II: Model I + pack-years of smoking, alcohol consumption, BMI, diabetes, lipid-lowering medications, LDL-C, physical activity at the job, and meat intake;

Model III: Model II + hypertension, HDL-C, triglycerides, CRP, and fibrinogen;

[a]*p*-trend shows a *p*-value for linear trend across the quartiles of LCn-3PUFAs calculated using contrast.

4.8 SUPPLEMENTARY TABLES

Table 4-6. Tobit conditional regression and ordinal logistic regression describing the association between ALA, LA, and ARA with aortic calcification for the ERA-JUMP Study, 2002-2006 (n=998)

	Tobit Regression	**Ordinal Regression**
	TR (95% CI)	**OR (95% CI)**
Model II		
ALA	0.99 (0.78, 1.25)	1.01 (0.89, 1.15)
LA	0.94 (0.73, 1.22)	0.94 (0.82, 1.08)
ARA	0.64 (0.48, 0.86)	0.81 (0.70, 0.95)
Model III		
ALA	0.98 (0.77, 1.23)	1.00 (0.88, 1.14)
LA	1.14 (0.86, 1.51)	1.05 (0.90, 1.23)
ARA	0.74 (0.54, 1.02)	0.91 (0.76, 1.08)

TR, Tobit ratio; OR, Odds ratio; CI, confidence interval; ALA, α-linolenic acid; LA, linoleic acid; ARA, Arachidonic acids; One standard deviation of ALA, LA, and ARA equals to 0.3%, 4.4%, and 2.4% respectively.
Model I: Fatty acids, age, race, years of education;
Model II: Model I + pack-years of smoking, alcohol consumption, BMI, diabetes, lipid-lowering medications, LDL-C, physical activity at the job, and meat intake;
Model III: Model II + hypertension, HDL-C, triglycerides, CRP, and fibrinogen;

Table 4-7. Tobit conditional regression and ordinal logistic regression describing the association between LCn-3PUFAs, EPA, and DHA with aortic calcification for the ERA-JUMP Study, 2002-2006 (n=998)

	Tobit regression	**Ordinal Regression**
Model II	**TR (95% CI)**	**OR (95% CI)**
Total LC-n3PUFAs	0.71 (0.50, 1.01)	0.82 (0.68, 1.00)
EPA	0.92 (0.67, 1.25)	0.94 (0.79, 1.11)
DHA	0.65 (0.46, 0.91)	0.80 (0.66, 0.96)
Model III		
Total LC-n3PUFAs	0.75 (0.52, 1.10)	0.88 (0.71, 1.07)
EPA	0.99 (0.71, 1.37)	0.99 (0.83, 1.19)
DHA	0.68 (0.47, 0.98)	0.84 (0.69, 1.02)

TR, Tobit ratio; OR, Odds ratio; CI, confidence interval; ALA, α-linolenic acid; LA, linoleic acid; ARA, Arachidonic acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; Total LCn-3PUFAs, total long chain omega 3 polyunsaturated fatty acids defined as sum of EPA, DPA, and DHA; One standard deviation of total LCn-3PUFAs, DHA, and EPA equals to 3.3%, 2.1%, and 1.3% respectively.
Model II: Total LC n-3 PUFAs/DHA/EPA + age, race, years of education + pack-years of smoking, alcohol consumption, BMI, diabetes, lipid-lowering medications, LDL-C, physical activity at the job, and meat intake, ALA, LA, ARA;
Model III: Model II + hypertension, HDL-C, triglycerides, CRP, and fibrinogen;

Table 4-8. Tobit conditional regression describing the association between fatty acids and aortic calcification for the ERA-JUMP Study, 2002-2006 (n=688) (*excluding Japanese in Japan*)

LC-n3 PUFAs Quartiles	**Q1**	**Q2**	**Q3**	**Q4**		**LCn-3PUFAs as a continuous variable**
			Total LCn-3PUFAs			
Median (IQR)	2.5 (2.2, 2.7)	3.3 (3.0, 3.9)	4.4 (3.9, 5.0)	6.3 (5.4, 7.4)		**Total LCn-3PUFAs**
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	**TR (95% CI)**
Unadjusted	1	1.20 (0.60, 2.40)	0.81 (0.40, 1.62)	0.32 (0.16, 0.65)	0.01	0.39 (0.25, 0.60)
Model I	1	0.82 (0.41, 1.63)	0.87 (0.44, 1.74)	0.51 (0.25, 1.01)	0.01	0.40 (0.26, 0.63)
Model II	1	1.30 (0.68, 2.48)	1.14 (0.59, 2.19)	0.52 (0.26, 1.01)	0.04	0.56 (0.36, 0.85)
Model III	1	1.28 (0.66, 2.46)	1.26 (0.64, 2.45)	0.53 (0.27, 1.07)	0.09	0.58 (0.37, 0.89)
			EPA			
Median (IQR)	0.4 (0.3, 0.4)	0.6 (0.5, 0.6)	0.8 (0.7, 0.9)	1.4 (1.1, 2.0)		**EPA**
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	**TR (95% CI)**
Unadjusted	1	0.78 (0.39, 1.57)	0.69 (0.34, 1.39)	0.43 (0.21, 0.86)	0.02	0.53 (0.34, 0.85)
Model I	1	0.82 (0.41, 1.63)	0.87 (0.44, 1.74)	0.51 (0.25, 1.01)	0.08	0.61 (0.38, 0.97)
Model II	1	0.82 (0.43, 1.57)	0.81 (0.42, 1.56)	0.60 (0.30, 1.16)	0.16	0.75 (0.48, 1.17)
Model III	1	0.79 (0.41, 1.51)	0.88 (0.45, 1.70)	0.63 (0.32, 1.24)	0.27	0.77 (0.50, 1.20)
			DHA			
Median (IQR)	1.4 (1.1, 1.6)	2.0 (1.7, 2.6)	2.9 (2.5, 3.3)	4.2 (3.7, 5.1)		**DHA**
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	**TR (95% CI)**
Unadjusted	1	1.20 (0.60, 2.40)	0.76 (0.38, 1.52)	0.30 (0.15, 0.62)	0.01	0.42 (0.28, 0.62)
Model I	1	1.09 (0.56, 2.15)	0.85 (0.43, 1.68)	0.28 (0.14, 0.56)	0.01	0.41 (0.27,0.61)
Model II	1	1.17 (0.61, 2.23)	1.06 (0.55, 2.03)	0.43 (0.22, 0.85)	0.01	0.55 (0.37, 0.81)
Model III	1	1.12 (0.58, 2.14)	1.12 (0.58, 2.16)	0.43 (0.21, 0.87)	0.03	0.57 (0.38, 0.85)

Q1, Q2, Q3, Q4, quartiles of LC n-3 PUFAs; IQR, interquartile range; TR, Tobit ratio; CI, confidence interval; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; Total LCn-3PUFAs, total long chain omega 3 polyunsaturated fatty acids defined as sum of EPA, DPA, and DHA; One standard deviation of total LCn-3PUFAs, EPA, and DHA equals to 1.9%, 0.7%, and 1.3% respectively.

Model I: Fatty acids, age, race, years of education;

Model II: Model I + pack-years of smoking, alcohol consumption, BMI, diabetes, lipid-lowering medications, LDL-C, physical activity at the job, and meat intake;

Model III: Model II + hypertension, HDL-C, triglycerides, CRP, and fibrinogen;

[a]*p*-trend shows a *p*-value for linear trend across the quartiles of LCn-3PUFAs calculated using contrast.

Table 4-9. Ordinal regression describing the association between fatty acids and aortic calcification for the ERA-JUMP Study, 2002-2006 (n=688) (*excluding Japanese in Japan*)

LCn-3 PUFAs Quartiles	**Q1**	**Q2**	**Q3**	**Q4**		**LCn-3PUFAs as a continuous variable**
			Total LC-n3 PUFAs			
Median (IQR)	2.5 (2.2, 2.7)	3.3 (3.0, 3.9)	4.4 (3.9, 5.0)	6.3 (5.4, 7.4)		**Total LCn-3 PUFAs**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Unadjusted	1	1.09 (0.74, 1.62)	0.90 (0.60, 1.33)	0.52 (0.35, 0.77)	0.20	0.58 (0.46, 0.74)
Model I	1	1.06 (0.71, 1.57)	0.96 (0.65, 1.45)	0.50 (0.33, 0.75)	0.20	0.58 (0.45, 0.75)
Model II	1	1.14 (0.76, 1.72)	1.11 (0.74, 1.67)	0.63 (0.42, 0.97)	0.14	0.68 (0.52, 0.89)
Model III	1	1.16 (0.77, 1.75)	1.21 (0.80, 1.85)	0.68 (0.44, 1.05)	0.13	0.72 (0.55, 0.95)
			EPA			
Median (IQR)	0.4 (0.3, 0.4)	0.6 (0.5, 0.6)	0.8 (0.7, 0.9)	1.4 (1.1, 2.0)		**EPA**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Unadjusted	1	0.87 (0.59, 1.29)	0.82 (0.56, 1.22)	0.60 (0.40, 0.89)	0.88	0.70 (0.54, 0.90)
Model I	1	0.90 (0.61, 1.34)	0.93 (0.63, 1.39)	0.64 (0.43, 0.96)	0.93	0.74 (0.57, 0.97)
Model II	1	0.88 (0.59, 1.33)	0.86 (0.57, 1.29)	0.69 (0.45, 1.04)	0.82	0.81 (0.62, 1.07)
Model III	1	0.87 (0.58, 1.32)	0.91 (0.60, 1.38)	0.73 (0.47, 1.11)	0.78	0.84 (0.64, 1.11)
			DHA			
Median (IQR)	1.4 (1.1, 1.6)	2.0 (1.7, 2.6)	2.9 (2.5, 3.3)	4.2 (3.7, 5.1)		**DHA**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Unadjusted	1	1.07 (0.72, 1.59)	0.84 (0.57, 1.25)	0.49 (0.33, 0.73)	0.25	0.61 (0.49, 0.76)
Model I	1	1.02 (0.68, 1.51)	0.89 (0.60, 1.33)	0.46 (0.31, 0.69)	0.27	0.59 (0.47, 0.74)
Model II	1	1.09 (0.72, 1.64)	1.05 (0.70, 1.59)	0.57 (0.37, 0.87)	0.19	0.69 (0.54, 0.88)
Model III	1	1.07 (0.71, 1.62)	1.10 (0.73, 1.68)	0.60 (0.39, 0.94)	0.22	0.72 (0.56, 0.93)

Q1, Q2, Q3, Q4: quartiles of total LCn-3 PUFAs; IQR: interquartile range; OR: Odds ratio; CI: confidence interval; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; Total LCn-3PUFAs: total long chain omega 3 polyunsaturated fatty acids defined as sum of EPA, DPA, and DHA; One standard deviation of total LCn-3PUFAs, EPA, and DHA equals to 1.9%, 0.7%, and 1.3% respectively.

Model I: Fatty acids, age, race, years of education;

Model II: Model I + pack-years of smoking, alcohol consumption, BMI, diabetes, lipid-lowering medications, LDL-C, physical activity at the job, and meat intake;

Model III: Model II + hypertension, HDL-C, triglycerides, CRP, and fibrinogen;

[a]*p*-trend shows a *p*-value for linear trend across the quartiles of LCn-3PUFAs calculated using contrast.

Table 4-10. Tobit conditional regression describing the association between fatty acids and aortic calcification among *US Whites* for the ERA JUMP Study, 2002-2006 (n=300)

LCn-3PUFAs quartiles	**Q1**	**Q2**	**Q3**	**Q4**		**LCn-3PUFAs as a continuous variable**
			Total LC n-3 PUFAs			
Median (IQR)	2.3 (2.0, 2.5)	3.0 (2.8, 3.1)	3.9 (3.6, 4.2)	5.6 (5.1, 7.2)	-	**Total LCn-3PUFAs**
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	**TR (95% CI)**
Model II	1	1.04 (0.43, 2.49)	1.56 (0.63, 3.83)	0.48 (0.19, 1.21)	0.25	0.48 (0.25, 0.92)
Model III	1	0.93 (0.38, 2.29)	1.49 (0.58, 3.86)	0.40 (0.15, 1.06)	0.20	0.43 (0.22, 0.86)
			EPA			
Median (IQR)	0.4 (0.3,0.4)	0.6 (0.5, 0.6)	0.7 (0.7, 0.8)	1.2 (1.0, 1.7)	-	**EPA**
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	**TR (95% CI)**
Model II	1	0.98 (0.39, 2.45)	1.06 (0.44, 2.59)	0.65 (0.25, 1.67)	0.52	0.57 (0.26, 1.26)
Model III	1	1.01 (0.40, 2.56)	1.31 (0.53, 3.28)	0.71 (0.27, 1.88)	0.84	0.60 (0.27, 1.34)
			DHA			
Median (IQR)	1.2 (1.0, 1.3)	1.7 (1.6, 1.9)	2.5 (2.3, 2.7)	3.8 (3.4, 4.5)	-	**DHA**
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	**TR (95% CI)**
Model II	1	0.91 (0.38, 2.20)	1.46 (0.60, 3.60)	0.47 (0.19, 1.18)	0.25	0.54 (0.31, 0.94)
Model III	1	0.72 (0.30, 1.77)	1.28 (0.51, 3.20)	0.35 (0.13, 0.95)	0.13	0.47 (0.26, 0.85)

Q1, Q2, Q3, Q4, quartiles of LCn-3PUFAs; IQR, interquartile range; TR, Tobit ratio; CI, confidence interval; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; Total LCn-3PUFAs, total long chain omega 3 polyunsaturated fatty acids defined as sum of EPA, DPA, and DHA; One standard deviation of total LCn-3PUFAs, EPA, and DHA equals to 1.7%, 0.5%, and 1.2% respectively.

Model I: Fatty acids, age, race, years of education;

Model II: Model I + pack-years of smoking, alcohol consumption, BMI, diabetes, lipid-lowering medications, LDL-C, physical activity at the job, and meat intake;

Model III: Model II + hypertension, HDL-C, triglycerides, CRP, and fibrinogen;

[a]*p*-trend shows a *p*-value for linear trend across the quartiles of LCn-3PUFAs calculated using contrast.

Table 4-11. Ordinal logistic regression describing the association between fatty acids and aortic calcification among US White for the ERA JUMP Study, 2002-2006 (n=300)

Total LCn-3PUFAs Quartiles	**Q1**	**Q2**	**Q3**	**Q4**		**LCn-3PUFAs as a continuous variable**
			Total LCn-3PUFAs			
Median (IQR)	2.3 (2.0, 2.5)	3.0 (2.8, 3.1)	3.9 (3.6, 4.2)	5.6 (5.1, 7.2)	-	**Total LCn-3 PUFAs**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Model II	1	0.95 (0.50, 1.79)	1.35 (0.71, 2.59)	0.50 (0.26, 0.98)	0.41	0.58 (0.36, 0.92)
Model III	1	0.93 (0.48, 1.79)	1.41 (0.71, 2.79)	0.49 (0.24, 0.99)	0.42	0.60 (0.35, 0.94)
			EPA			
Median (IQR)	0.4 (0.3,0.4)	0.6 (0.5, 0.6)	0.7 (0.7, 0.8)	1.2 (1.0, 1.7)	-	**EPA**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Model II	1	0.92 (0.48, 1.75)	0.97 (0.52, 1.82)	0.65 (0.34, 1.28)	0.90	0.66 (0.37, 1.16)
Model III	1	0.93 (0.48, 1.80)	1.14 (0.59, 2.17)	0.70 (0.35, 1.38)	0.81	0.70 (0.40, 1.28)
			DHA			
Median (IQR)	1.2 (1.0, 1.3)	1.7 (1.6, 1.9)	2.5 (2.3, 2.7)	3.8 (3.4, 4.5)	-	**DHA**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	OR (95% CI)
Model II	1	0.91 (0.48, 1.72)	1.38 (0.72, 2.63)	0.53 (0.27, 1.02)	0.51	0.63 (0.43, 0.93)
Model III	1	0.82 (0.43, 1.56)	1.33 (0.69, 2.58)	0.48 (0.23, 0.98)	0.70	0.61 (0.40, 0.94)

Q1, Q2, Q3, Q4: quartiles of total LCn-3PUFAs; IQR: interquartile range; OR: Odds ratio; CI: confidence interval; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; Total LCn-3PUFAs: total long chain omega 3 polyunsaturated fatty acids defined as sum of EPA, DPA, and DHA; One standard deviation of total LCn-3PUFAs, EPA, and DHA equals to 1.7%, 0.5%, and 1.2% respectively.

Model I: Fatty acids, age, race, years of education;

Model II: Model I + pack-years of smoking, alcohol consumption, BMI, diabetes, lipid-lowering medications, LDL-C, physical activity at the job, and meat intake;

Model III: Model II + hypertension, HDL-C, triglycerides, CRP, and fibrinogen;

[a]*p*-trend shows a *p*-value for linear trend across the quartiles of LCn-3PUFAs calculated using contrast.

Table 4-12. Tobit conditional regression describing the association between fatty acids and aortic calcification among *Japanese in Japan* for the ERA JUMP Study, 2002-2006 (n=310)

Total LCn-3PUFAs Quartiles	**Q1**	**Q2**	**Q3**	**Q4**		**LCn-3PUFAs as a continuous variable**
			Total LCn-3PUFAs			
Median (IQR)	6.2 (5.2, 6.8)	8.1 (7.8, 8.5)	9.6 (9.2, 10.2)	12.8 (11.9, 14.0)	-	**Total LCn-3 PUFAs**
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	TR (95% CI)
Model II	1	0.17 (0.03, 1.03)	0.40 (0.07, 2.41)	0.35 (0.06, 2.18)	0.42	0.82 (0.40, 1.68)
Model III	1	0.21 (0.03, 1.23)	0.45 (0.07, 2.76)	0.47 (0.08, 2.96)	0.61	0.95 (0.46, 1.96)
			EPA			
Median (IQR)	1.1 (0.9, 1.3)	1.9 (1.8, 2.1)	2.6 (2.4, 2.8)	4.0 (3.5, 5.0)	-	**EPA**
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	TR (95% CI)
Model II	1	0.18 (0.03, 1.09)	0.18 (0.03, 1.17)	0.35 (0.06, 2.20)	0.26	0.94 (0.53, 1.65)
Model III	1	0.23 (0.04, 1.38)	0.21 (0.03, 1.34)	0.49 (0.08, 3.23)	0.43	1.08 (0.61, 1.92)
			DHA			
Median (IQR)	4.1 (3.4, 4.5)	5.2 (4.9, 5.5)	6.3 (6.0, 6.6)	8.0 (7.3, 8.6)	-	**DHA**
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	TR (95% CI)
Model II	1	0.31 (0.05, 1.77)	0.15 (0.02, 0.97)	0.36 (0.06, 2.16)	0.21	0.75 (0.34, 1.64)
Model III	1	0.43 (0.07, 2.46)	0.20 (0.03, 1.24)	0.52 (0.08, 3.24)	0.37	0.86 (0.38, 1.91)

Q1, Q2, Q3, Q4, quartiles of LCn-3PUFAs; IQR: interquartile range; TR: Tobit ratio; CI: confidence interval; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; Total LCn-3PUFAs: total long chain omega 3 polyunsaturated fatty acids defined as sum of EPA, DPA, and DHA; One standard deviation of total LCn-3PUFAs, DHA, and EPA equals to 3.0%, 1.4%, and 1.7% respectively.

Model I: Fatty acids, age, race, years of education;

Model II: Model I + pack-years of smoking, alcohol consumption, BMI, diabetes, lipid-lowering medications, LDL-C, physical activity at the job, and meat intake;

Model III: Model II + hypertension, HDL-C, triglycerides, CRP, and fibrinogen;

[a]*p*-trend shows a *p*-value for linear trend across the quartiles of LCn-3PUFAs calculated using contrast.

Table 4-13. Ordinal logistic regression describing the association between fatty acids and aortic calcification among Japanese in Japan for the ERA-JUMP Study, 2002-2006 (n=310)

LCn-3PUFAs Quartiles	**Q1**	**Q2**	**Q3**	**Q4**		**LCn-3PUFAs as a continuous variable**
			Total LCn-3PUFAs			
Median (IQR)	6.2 (5.2, 6.8)	8.1 (7.8, 8.5)	9.6 (9.2, 10.2)	12.8 (11.9, 14.0)	-	**Total LCn-3PUFAs**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Model II	1	0.49 (0.24, 0.99)	0.67 (0.33, 1.36)	0.69 (0.34, 1.41)	0.06	0.96 (0.72, 1.27)
Model III	1	0.52 (0.25, 1.06)	0.69 (0.33, 1.42)	0.77 (0.36, 1.57)	0.07	1.02 (0.75, 1.34)
			EPA			
Median (IQR)	1.1 (0.9, 1.3)	1.9 (1.8, 2.1)	2.6 (2.4, 2.8)	4.0 (3.5, 5.0)	-	**EPA**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Model II	1	0.51 (0.25, 1.05)	0.52 (0.25, 1.07)	0.68 (0.33, 1.40)	0.05	0.98 (0.79, 1.23)
Model III	1	0.54 (0.26, 1.13)	0.54 (0.26, 1.13)	0.79 (0.37, 1.67)	0.06	1.04 (0.83, 1.31)
			DHA			
Median (IQR)	4.1 (3.4, 4.5)	5.2 (4.9, 5.5)	6.3 (6.0, 6.6)	8.0 (7.3, 8.6)	-	**DHA**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Model II	1	0.69 (0.34, 1.37)	0.51 (0.25, 1.04)	0.76 (0.38, 1.53)	0.17	0.94 (0.69, 1.28)
Model III	1	0.81 (0.40, 1.63)	0.54 (0.26, 1.13)	0.86 (0.42, 1.77)	0.32	0.98 (0.71, 1.34)

Q1, Q2, Q3, Q4, quartiles of total LC n-3 PUFAs; IQR: interquartile range; OR: Odds ratio; CI: confidence interval; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; Total LCn-3PUFAs: total long chain omega 3 polyunsaturated fatty acids defined as sum of EPA, DPA, and DHA; One standard deviation of total LCn-3PUFAs, DHA, and EPA equals to 3.0%, 1.4%, and 1.7% respectively.

Model I: Fatty acids, age, race, years of education;

Model II: Model I + pack-years of smoking, alcohol consumption, BMI, diabetes, lipid-lowering medications, LDL-C, physical activity at the job, and meat intake;

Model III: Model II + hypertension, HDL-C, triglycerides, CRP, and fibrinogen;

[a]*p*-trend shows a *p*-value for linear trend across the quartiles of LCn-3PUFAs calculated using contrast.

Table 4-14. Tobit conditional regression describing the association between fatty acids and aortic calcification among Japanese Americans for the ERA JUMP Study, 2002-2006 (n=287)

LCn-3 PUFAs Quartiles	**Q1**	**Q2**	**Q3**	**Q4**		**LCn-3PUFAs as a continuous variable**
			Total LC n-3 PUFAs			
Median (IQR)	2.8 (2.5, 3.1)	3.9 (3.7, 4.2)	5.1 (4.7, 5.4)	7.0 (6.3, 8.4)	-	**Total LCn-3 PUFAs**
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	**TR (95% CI)**
Model II	1	1.40 (0.46, 4.24)	0.88 (0.28, 2.75)	0.81 (0.25, 2.61)	0.50	0.72 (0.38, 1.35)
Model III	1	1.52 (0.51, 4.56)	1.07 (0.35, 3.32)	0.87 (0.27, 2.78)	0.64	0.71 (0.38, 1.33)
			EPA			
Median (IQR)	0.4 (0.3, 0.5)	0.7 (0.6, 0.7)	0.9 (0.8, 1.0)	1.7 (1.3, 2.3)	-	**EPA**
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	**TR (95% CI)**
Model II	1	0.50 (0.17, 1.51)	0.83 (0.27, 2.53)	0.60 (0.19, 1.87)	0.55	0.92 (0.51, 1.64)
Model III	1	0.48 (0.16, 1.42)	0.90 (0.30, 2.66)	0.54 (0.17, 1.66)	0.50	0.85 (0.48, 1.51)
			DHA			
Median (IQR)	1.7 (1.5, 1.9)	2.6 (2.4, 2.8)	3.5 (3.2, 3.6)	4.8 (4.3, 5.5)	-	**DHA**
	TR	**TR (95% CI)**	**TR (95% CI)**	**TR (95% CI)**	***p*-trend[a]**	**TR (95% CI)**
Model II	1	1.26 (0.41, 3.85)	0.92 (0.30, 2.87)	0.60 (0.18, 1.97)	0.27	0.66 (0.35, 1.24)
Model III	1	1.36 (0.45, 4.10)	1.09 (0.36, 3.33)	0.63 (0.19, 2.04)	0.32	0.68 (0.36, 1.27)

Q1, Q2, Q3, Q4, quartiles of LCn-3PUFAs; IQR, interquartile range; TR, Tobit ratio; CI, confidence interval; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; Total LCn-3PUFAs, total long-chain omega 3 polyunsaturated fatty acids defined as sum of EPA, DPA, and DHA; One standard deviation of total LCn-3PUFAs, DHA, and EPA equals to 2.2%, 0.9%, and 1.4% respectively.

Model I: Fatty acids, age, race, years of education;

Model II: Model I + pack-years of smoking, alcohol consumption, BMI, diabetes, lipid-lowering medications, LDL-C, physical activity at the job, and meat intake;

Model III: Model II + hypertension, HDL-C, triglycerides, CRP, and fibrinogen;

[a]*p*-trend shows a *p*-value for linear trend across the quartiles of LCn-3PUFAs calculated using contrast.

Table 4-15. Ordinal logistic regression describing the association between fatty acids and aortic calcification among *Japanese Americans* for the ERA-JUMP Study, 2002-2006 (n=287)

LCn-3PUFAs Quartiles	**Q1**	**Q2**	**Q3**	**Q4**		**LCn-3PUFAs as a continuous variable**
			Total LCn-3PUFAs			
Median (IQR)	2.8 (2.5, 3.1)	3.9 (3.7, 4.2)	5.1(4.7, 5.4)	7.0(6.3, 8.4)	-	**Total LCn-3PUFAs**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Model II	1	1.20 (0.64, 2.25)	0.95 (0.50, 1.82)	0.88 (0.45, 1.70)	0.55	0.83 (0.58, 1.19)
Model III	1	1.25 (0.66, 2.37)	1.04 (0.54, 2.20)	0.92 (0.46, 1.80)	0.45	0.83 (0.58, 1.19)
			EPA			
Median (IQR)	0.4 (0.3, 0.5)	0.7 (0.6, 0.7)	0.9 (0.8, 1.0)	1.7 (1.3, 2.3)	-	**EPA**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Model II	1	0.67 (0.36, 1.25)	0.85 (0.45, 1.61)	0.71 (0.37, 1.35)	0.32	0.94 (0.67, 1.30)
Model III	1	0.63 (0.33, 1.20)	0.89 (0.47, 1.69)	0.67 (0.35, 1.30)	0.29	0.90 (0.65, 1.26)
			DHA			
Median (IQR)	1.7 (1.5, 1.9)	2.6 (2.4, 2.8)	3.5 (3.2, 3.6)	4.8 (4.3, 5.5)	-	**DHA**
	OR	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***p*-trend[a]**	**OR (95% CI)**
Model II	1	1.08 (0.57, 2.05)	0.94 (0.49, 1.79)	0.74 (0.38, 1.44)	0.64	0.81 (0.57, 1.15)
Model III	1	1.09 (0.57, 2.08)	1.00 (0.52, 1.94)	0.75 (0.38, 1.48)	0.59	0.82 (0.57, 1.18)

Q1, Q2, Q3, Q4, quartiles of total LCn-3PUFAs; IQR, interquartile range; OR, Odds ratio; CI: confidence interval; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; Total LCn-3PUFAs, total long chain omega 3 polyunsaturated fatty acids defined as sum of EPA, DPA, and DHA; One standard deviation of total LCn-3PUFAs, DHA, and EPA equals to 2.2%, 0.9%, and 1.4% respectively.

Model I: Fatty acids, age, race, years of education;

Model II: Model I + pack-years of smoking, alcohol consumption, BMI, diabetes, lipid-lowering medications, LDL-C, physical activity at the job, and meat intake;

Model III: Model II + hypertension, HDL-C, triglycerides, CRP, and fibrinogen;

[a]*p*-trend a *p*-value for linear trend across the quartiles of LCn-3PUFAs calculated using contrast.

5.0 DISCUSSION AND PUBLIC HEALTH SIGNIFICANCE

Atherosclerosis - the major underlying cause of CHD/CVD events, is a chronic condition of the arterial vessel wall characterized by the deposition of lipids in the arterial intima leading to plaque development. Many CHD events occur suddenly and 2/3rd of the patients die before getting any treatment. Therefore, primary prevention is very important in the field of CVD. Research on subclinical atherosclerosis provides an opportunity to measure atherosclerosis in its subclinical phase, to explore related important risk factors, to understand the progression of subclinical atherosclerosis into clinical atherosclerosis, to identify high risk people, and to implement early life intervention to prevent CHD attack. This work focuses on the determinants of subclinical atherosclerosis among US White, US Black, Japanese in Japan, and Japanese American.

US White compared to Japanese, have a higher prevalence of subclinical atherosclerosis measured by CAC and CIMT [55]. US White and Japanese in Japan have a differential distribution of NMR-measured lipoprotein particles [56]. NMR-measured lipoprotein particles have been documented to be better measures of CHD/CDV risk that their cholesterol counterparts. Therefore, the first manuscript examined whether NMR-measured lipoproteins account for differences in the prevalence of subclinical atherosclerosis measured by CAC between US White and Japanese. The two populations had different levels of total HDL-P and total VLDL-P and similar levels of total LDL-P. White men were 3.25 (95% CI = 1.55, 6.84)

times more likely to have CCS ≥10 compared to Japanese men after adjusting for traditional cardiovascular risk factors, alcohol, and inflammatory markers. Major attenuation (~16 to 20%) in the odds of CCS ≥10 (OR = 2.58, 95% CI = 1.16, 5.77) for white men was seen after including total LDL-P, large HDL-P, and VLDL-P/ VLDL particle size together in a model having traditional cardiovascular risk factors, alcohol, and inflammatory markers. Thus, differences in the distribution of lipoproteins may partially account for differences in the prevalence of CAC between US white men and Japanese men.

Differences in the prevalence of subclinical atherosclerosis between US white men and Japanese men opens several areas of research to gain a better understanding of the etiology of CHD. Differences in the prevalence of subclinical atherosclerosis between US white men and Japanese men independent of traditional and novel cardiovascular risk factors suggest that the higher prevalence of subclinical atherosclerosis among US white men or the lower prevalence of subclinical atherosclerosis among Japanese men may be attributed to unknown risk factors. Future adequately powered prospective studies are required to determine the role of other factors accounting for the variation in the prevalence of subclinical atherosclerosis between white men and Japanese men.

It is well-established that alcohol consumption has a J-shaped association with CHD. However, studies assessing the relationship between alcohol consumption and subclinical atherosclerosis have reported inconsistent findings. Investigating the relationship between alcohol and atherosclerosis, however, may help clarify the mechanisms underlying the association between alcohol and CHD. Therefore, the second manuscript of this dissertation assessed the independent association of alcohol consumption with aortic calcification. Heavy drinkers [TR (95% CI) = 2.15 (1.01, 4.57), OR (95% CI) =1.60 (1.07, 2.41)] had significantly

higher expected AoCaS compared to nondrinkers after adjusting for potential confounders. Findings of the second manuscript suggest that the heavy alcohol consumption may be an independent risk factor for atherosclerosis and light to moderate alcohol consumption may decrease cardiovascular risk through mechanisms other than those associated with the reduced deposition of calcium in the atherosclerotic lesions. This work is of a great public health significance. Studies examining the relationship between alcohol consumption and atherosclerosis are scarce. Heavy alcohol consumption is a risk factor for range of diseases including communicable, non-communicable, behavioural disorders etc. Findings of the current study further add to the serious health hazards of heavy alcohol consumption among asymptomatic middle-aged men. Larger longitudinal studies are needed, however, to assess the relationship between alcohol consumption and atherosclerosis incidence and progression.

The third aim of the dissertation examined the relationship of LCn-3PUFAs to aortic calcification. Total LCn-3PUFAs was significantly and inversely associated with aortic calcification, an association mainly attributed to the effects of DHA and not EPA. Adjusting for cardiovascular risk factors, a 1-SD increase in total LCn-3PUFAs, EPA, and DHA was associated with 29% (95% CI = 0.51, 1.00), 9% (95% CI = 0.68, 1.23), and 35% (95% CI = 0.46, 0.91) lower expected AoCaS respectively. Large longitudinal studies are needed to further clarify the effect of LCn–3PUFAs on the incidence and progression of atherosclerosis as well as to disentangle the differential effect of DHA and EPA, and the underlying biological mechanisms. Worldwide LCn-3PUFAs are used as a dietary supplement. If our findings are replicated in future studies in different population settings, newer areas would be opened to lower the public health burden of atherosclerosis/CHD/CVD.

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