SUBCLINICAL CARDIOVASCULAR DISEASE AND ITS ASSOCIATED RISK FACTORS IN DIFFERENT POPULATION GROUPS

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Examination of the prevalence of subclinical cardiovascular disease (SCD) may help us to delineate future trends of coronary heart disease (CHD) mortality. There are substantial differences in the prevalence of SCD in different population groups. Identification of explaining factors for the difference in SCD rates across different population groups can provide new preventive strategies for reducing CHD morbidity or mortality. Based on this, the **E**BCT and **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 is an ongoing population-based-cross-sectional study to examine differences in SCD rates across Japanese, Japanese American, and U.S. white men, and Korean men aged 40-49 years.

As a part of the ERA-JUMP study, the present study demonstrated that: 1) among middle-aged men, Koreans in South Korea had a significantly higher level of carotid intima-media thickness than the Japanese in Japan. Middle-aged Korean men may continue to be at more increased risk for CHD than Japanese men at least over the near future. 2) Of polyunsaturated fatty acids, the proportion of linoleic acid was significantly and inversely associated with unfavorable levels of lipoprotein subclasses in three population groups of Japanese, Japanese American, and white men; an inverse association of docoxahexaenoic acid occurred only in white men. 3) Associations between pulse wave velocity (PWV) and

cardiovascular risk factors were segmental-specific among white men. Brachial-ankle PWV had a mixed characteristic of stiffness of the central and peripheral arteries, compared to carotid-femoral PWV having a characteristic of the central arteries.

Public health importance of these findings is that: First, we can provide scientific information imperative to addressing a health-related problem (i.e., increasing risk for cardiovascular disease in South Korea). Second, the promotion of enriched intakes of linoleic acid (e.g., sunflower, corn, safflower oils) and fish-derived n-3 fatty acids (e.g., mackerel or salmon) should be addressed to public in habitual diet in the prevention of CHD by establishing practical guidelines. Finally, incorporation of both measures of brachial-ankle PWV and carotid-femoral PWV into the assessment of arterial stiffness should be considered when screening a high-risk group for cardiovascular disease.

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1.0 INTRODUCTION

1.1 SPECIFIC AIMS

Cardiovascular disease (CVD) is the leading cause of mortality worldwide, responsible for one-third of all deaths.[1] There is considerable variation in coronary heart disease (CHD) mortality rates across countries.[1, 2] CHD mortality rate in Japan is the lowest among developed countries, which has been decreasing since the 1970s.[2, 3] Even among men in post World War II (WWII) birth cohort, who adopted a westernized lifestyle from childhood and young adulthood, the CHD mortality rate is much lower in Japan than Western countries (e.g., U.S.) as well as other Asian countries (e.g., South Korea).[4]

Examination of the prevalence of subclinical cardiovascular disease (SCD) across countries may help us to identify explaining factors for the difference in the SCD rates. Especially, SCD rates in young and middle-aged population groups may reflect recent or future trends in CHD mortality. As a result, we can provide new preventive strategies for leveling off current trends of CHD morbidity or mortality.

The **E**BCT and **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 is an ongoing, population-based, cross-sectional study of four population groups of healthy men (i.e., Japanese, Japanese American, Korean, and U.S. white men) aged 40 to 49 years. The ERA-JUMP study was primarily designed to

investigate differences in the prevalence of SCD rates (i.e., carotid intima-media thickness, coronary calcification, and pulse wave velocity) across the population groups. According to main findings of the ERA-JUMP study, Japanese in Japan had lower levels of carotid intima-media thickness and lower prevalence of coronary calcification than U.S. white men.[5] Furthermore, a serum high level of marine-derived n-3 polyunsaturated fatty acids (PUFA) explained the lower level of carotid initma-media thickness and lower prevalence of coronary calcification in Japanese men in Japan than in U.S. white men.[6] As a part of the ERA-JUMP study, we need, in the present study, to investigate the difference in SCD rates between two Asian population groups who have similar socio-cultural and genetic background. Second, we need to determine if n-3 or n-6 PUFA that are recognized as a protective factor against CHD is significantly associated with mediators for atherosclerosis. Third, we need to assess potential utility of brachial-ankle pulse wave velocity (PWV) for screening a high-risk group for CHD.

This cross-sectional study as a part of the ERA-JUMP study has three specific aims:

1) To determine if there is a significant difference in the levels of carotid intima-media thickness between 102 Korean and 250 Japanese men aged 40-49 years.
2) To examine the relation between serum polyunsaturated fatty acids and atherogenic lipoprotein subclasses (e.g., large very low-density lipoprotein particles, small low-density lipoprotein particles, and large high-density lipoprotein particles) in 285 Japanese, 212 Japanese American and 261 white men.
3) To examine segmental-specific associations between pulse wave velocity (e.g., carotid-femoral PWV and brachial-ankle PWV) and cardiovascular risk factors in 248 white men.

1.2 BACKGROUND AND SIGNIFICANCE

1.2.1 Variation in coronary heart disease mortality

CVD is the leading cause of mortality worldwide, responsible for about one-third of all deaths [1]. Among CVD, CHD is decreasing in many developed countries, but is increasing in developing and transitional countries, partly as a result of increasing longevity, urbanization, and lifestyle changes.[1] However, even among developed countries, there is considerable variation in CHD mortality rates. A landmark international cardiovascular epidemiological study, called the Seven Countries Study, initiated to identify the variation in CHD mortality across seven countries including 16 cohorts (12,467 men aged 40-59 years). Age-standardized 25-year mortality from CHD in the population from Northern Europe was the highest (100/10,000 person-years), followed by the population from the United States (73/10,000 person-years), whereas that from Japan was the lowest (17/10,000 person-years).[2]

Modern epidemiological studies have made attempts to explain reasons for the variation in CHD rates. Potential reasons for such variation include differing stages of epidemiologic transition in various countries, varying environmental effects due to dissimilar burden of CVD risk factors, inherent genetic differences, and distinct early childhood programming influences. As an environmental reason, the Seven Countries Study supported a diet-heart hypothesis through the ecological correlations of cholesterol intake to rates of CHD mortality.[7] A 20 mg/dl increase in total cholesterol corresponded to an increase in CHD mortality risk of 12%.[7] Across the countries, a level of cholesterol was linearly correlated to CHD mortality; a relative increase in CHD mortality rates with a given cholesterol increase was the same across those countries.

CHD mortality rates in Japan have been decreasing since the 1970s.[3] Even among men in post World War II (WWII) birth cohort, who adopted a westernized lifestyle from childhood and young adulthood, CHD mortality rate is much lower in Japan than in other developed countries including the United States (U.S.).[8] Therefore, we need to explore the reasons for the low CHD rates in Japan, beyond cholesterol intake, to provide a new strategy for reducing CHD mortality rates worldwide.

The Westernization of Japanese diet has resulted in a gradual increase in the intakes of total fat and animal fat since the 1960s.[3, 9] In Japan, cancer mortality rates associated with high fat intake (e.g., colon, prostate, and breast cancer) are likely to parallel the increased tends of fat intake from National Nutritional Survey in Japan.[3] However, the low CHD mortality in Japan has not paralleled the gradually-increased level of total cholesterol with the westernized diet. It may not be due to genetic susceptibility of the Japanese people, because CHD mortality in Japanese migrants to the U.S. increased.[10] A careful investigation demonstrated that the low CHD mortality in Japan did not result from the misclassification of cause of death.[11] Moreover, it is unlikely due to some lifestyle specific to Asian populations because CHD mortality in China increased as many other Asian countries. The World Health Organization MONICA study reported that CHD mortality in Beijing, China increased during 1984-1999, which was explained by the increased total cholesterol.[12] Furthermore, there is no evidence that the strength and nature of associations between CHD risk factors and CHD mortality are different between Asians and Caucasians. The Asia Pacific Cohort Studies Collaboration (APCSC) group demonstrated that hazard ratios for CHD mortality associated with risk factors were similar between Asians and Caucasians, including systolic blood pressure, total cholesterol, smoking, and diabetes (Figure 1).[13] In this regard, we assume that relative risks for CHD mortality associated with the risk

factors may be similar between the Japanese and Caucasians, because 42% of deaths caused by CHD were from the Japanese cohort of entire Asian cohorts in the APCSC study.

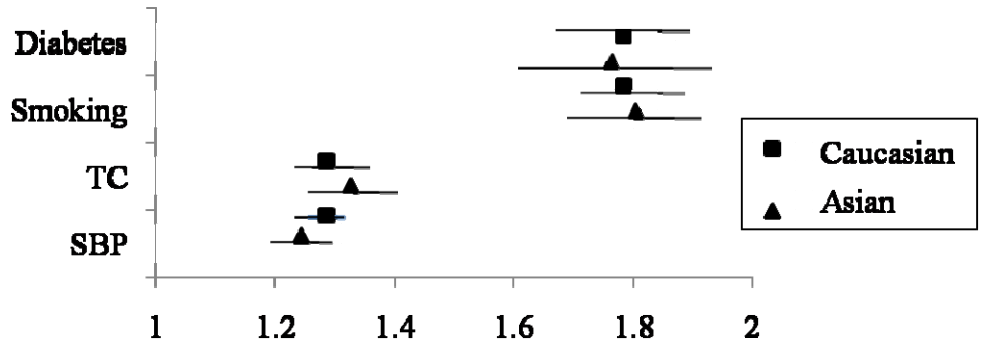

Figure 1 Relative risks of coronary heart disease mortality associated with risk factors (Asia Pacific Cohort Studies Collaboration group, 2005)

TC=total cholesterol

Most of the basis on prevention and treatment derives from studies conducted in white populations. The prevention and treatment strategies such as use of lipid-lowering medications (e.g., statins), hypertensive medications (e.g., angiotensin-converting enzyme inhibitors), or aspirin, can be applied to other ethnic populations.[14] However, there is still a need to identify the reasons for the lower CHD mortality rates in Japan so that we can find out some other risk factors for CHD than determined in the white populations. Based on this effort, we may provide a new preventive strategy for controlling newer risk factors for CHD in cardiovascular prevention.

1.2.2 ERA-JUMP study: Low rates in subclinical cardiovascular diseases in the Japanese than in Whites and Japanese Americans

The ERA-JUMP study, a population-based-cross-sectional study, investigated variation in rates of subclinical cardiovascular disease (SCD) across Japanese, Japanese American, and U.S. men aged 40 to 49 years of the post World-War-II birth cohort. The ERA-JUMP study examined three markers of SCD: 1) levels of carotid intima-media thickness (IMT), 2) prevalence of coronary calcification, and 3) levels of arterial stiffness measured by pulse wave velocity. The study participants of the ERA-JUMP study were randomly recruited: 313 Japanese from Kusatsu in Japan, 303 Japanese Americans from Honolulu, Hawaii, U.S., and 310 whites from Allegheny County, Pennsylvania, U.S. Recently 300 Koreans in South Korea were added to the study population. All participants were free of clinical cardiovascular disease, type I diabetes, or other severe disease.[5]

The ERA-JUMP study reported main findings: 1) Lower levels of carotid IMT and lower prevalence of coronary calcification in Japanese men than U.S. white men.[5] 2) Lower prevalence of coronary calcification in Japanese men than Japanese American men.[15] The study findings indicate that there are protective factors against SCD in the Japanese in Japan. The protective factors may not include a genetic factor, but environmental factors. In this regard, most recently, the ERA-JUMP study reported that high levels of marine-derived n-3 fatty acids in serum in Japanese men in Japan explained the lower levels of carotid IMT and lower prevalence of coronary calcification in Japanese in Japan than in white men.[6]

1.2.3 Different trends in CHD mortality between South Korea and Japan

CHD mortality rates rose with a concomitant rise in serum total cholesterol in most Asian countries including China, India, Indonesia, South Korea, Malaysia, Philippines, Singapore, and Thailand.[16] CHD mortality rates in South Korea and Japan were relatively low among the Asian Pacific regions.[17] Age-adjusted mortality rates from CHD in South Korea increased between 1984 and 1999.[18] However, the mortality rates in Japan have decreased since the 1970s. In particular, CHD mortality in men aged 35-44 years of the post WWII birth cohort in South Korea increased steadily from 1985 to 1992 while it did not in Japan.[4] When comparing the mortality rates between the two countries, CHD mortality in South Korea has exceeded that in Japan since the late 1980s.[4] (Figure 2)

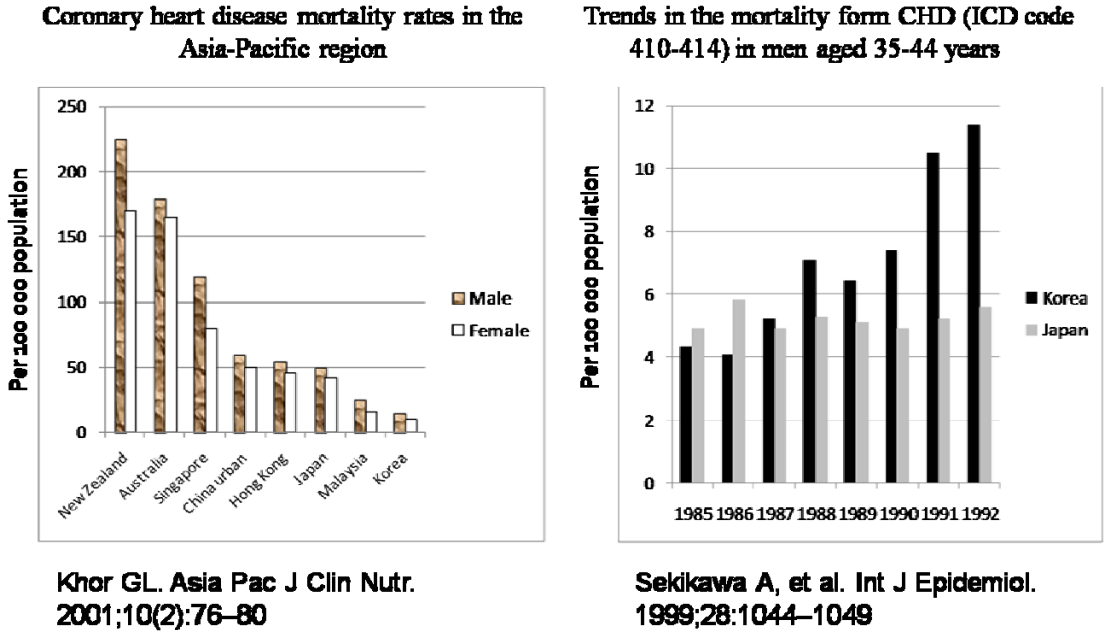

Figure 2 Coronary heart disease mortality rates in South Korea and Japan

The post WWII birth cohort may reflect recent changes in risk factors for CHD, caused by the impact of westernization of lifestyle. Hence, the risk for CHD in this birth cohort measured by the markers of SCD may better reflect the current trends in CHD mortality rather than age-adjusted mortality or the mortality in an old age group.

1.2.4 Carotid intima-media thickness as a predictor of cardiovascular disease

A level of carotid IMT is a surrogate and intermediate end point of early atherosclerosis. Increased carotid IMT is associated with cardiovascular risk factors in various population groups, even in young or middle-aged and various ethnic groups (e.g., Koreans and the Japanese) (Table 1).[19-24] Moreover, an increased level of carotid IMT is a predictor of incidences of myocardial infarction and stroke in middle-aged or elderly groups and various ethnic groups (e.g., American, Japanese, Dutch and German population groups).[25-31] (Table 2; Table 3) A meta analysis demonstrated that the risk for myocardial infarction significantly increased by 15% per an 0.10 nm increase (or 26% per a standard deviation increase) of IMT score and the risk for stroke significantly increased by 18% per an 0.10 nm increase (or 32% per a standard deviation increase) of IMT score.[25] In this regard, the identification of variation in SCD rates using carotid IMT levels may be an epidemiological tool to help delineate a recent or future variation in CHD rates across countries, especially between South Korea and Japan.

Table 1 Associations of cardiovascular risk factors with carotid IMT in young or middle-aged adults

Publ Year	Authors and origin	Outcome	Study design	Population	Results (Multivariate OR /β)
1999	Ferrieres J et al.[19] from France	Mean IMT: CCA	Cross – sectional	1,013 Caucasians Aged 35-65 y	In 536 men, β Smoking: 1.48 (p=.007) SBP: 1.44 (p<.0001), HDL-C:-28.3 (p=.02) Alcohol: -2.65 (p=.001)
2001	Davis PH et al.[20] Muscatine Study	Maximal IMT: from CCA, bif, & ICA	Prospective	725 Aged 33-42 y	In men, OR Childhood cholesterol: 1.53 (1.19-1.96) Young adulthood LDL-C: 1.39 (1.09-1.76) Young adulthood HDL-C: .70 (0.52-0.93) Current LDL-C: 2.00 (1.50-2.67)
2003	Li S et al.[21] Bogalusa Heart Study	Mean IMT: from CCA, bif, & ICA	Prospective	486 Aged 25-37 y	OR Childhood BMI: 1.25 (1.01-1.54) Childhood LDL-C: 1.42 (1.14-1.78) Adulthood SBP: 1.36 (1.08-1.72) Adulthood HDL-C: 0.67 (0.51-0.88) Adulthood LDL-C: 1.46(1.16-1.82)
2003	Raitakari OT et al.[22] The CV Risk in Young Finns Study	Left CCA IMT	Prospective	2,229 Whites Aged 24-39 y	Current, β BMI: 0.011 (p<.001) SBP: 0.010 (p<.001) Smoking: 0.011 (p=.004) Childhood, β Plus LDL-C: 0.010 (p=.001)
2004	Tatsukawa M et al.[23] from Japan	Mean IMT: CCA	Cross – sectional	3,442 Aged 20-89 y I city: 1,078 K town: 2,364	I city, OR LDL-C: 1.02 (1.01-1.02) HDL: 0.96 (0.94-0.99) Diabetes: 2.59 (1.35-4.94) K town, OR SBP:1.03 (1.01-1.04), LDL-C:1.01(1.01-1.02)
2006	Lee YH et al.[24] form Korea	Mean IMT: CCA, bif, & ICA	Cross – sectional	1,507 Aged 20-75y	In men aged <50 y (n=248), β HTN: .22 (p<.001)

Publ=publication; IMT=intima-media thickness; CCA=common carotid artery; bif=bifurcation; ICA=internal carotid artery; SBP= systolic blood pressure; HDL-C=high density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; BMI=body mass index; HTN=hypertension

Table 2 Carotid IMT as a predictor of incident CHD and Stroke in the United Studies

Publ Year	Authors	End Points	Design	Population	Results
1997	Chambless LE et al.[26] ARIC Study	MI	Prospective (4-7 y)	12,841 Aged 45-64 y	HRR: >=1mm vs. <1mm 5.07 (3.08-8.36) for women 1.85 (1.28-2.69) from men *mean IMT from bif;CCA;ICA
1999	O'Leary DH et al.[27] CHS	MI, stroke	Prospective (6.2 y)	5,858 Aged>=65 y	RR for MI per 1 SD increase 1.36 (1.23-1.52) RR for Stroke per 1 SD increase 1.33 (1.20-1.47) *CCA;ICA
2000	Chambless LE et al.[28] ARIC Study	Ischemic Stroke	Prospective (6-7 y)	15,792 Aged 45-64 y	HRR: >=1mm vs. <0.6 mm 8.5 (3.5-20.7) for women 3.6 (1.5-9.2) for men *mean IMT from bif;CCA;ICA

Publ=publication; IMT=intima-media thickness; MI=myocardial infarction; ARIC=Atherosclerosis Risk In Communities; HRR=hazard relative risk; CHS=Cardiovascular Heart Study; bif=bifurcation; CCA=common carotid artery; ICA=internal carotid artery; RR=relative risk; SD=standard deviation

Table 3 Carotid IMT as a predictor of CVD risk in Europe and Japan

Publ Year	Authors	End Points	Design	Population	Results
1997	Bots ML et al.[29] Rotterdam Study	MI , stroke	Nested case -control (2.7 y)	7,983 Aged >=55 y	OR for MI, 1.25 (0.98-1.58) OR for stroke, 1.34 (1.08-1.67) *IMT from CCA
2004	Kitamura A et al.[30] from Japan	Stroke	Prospective (4.5 y)	1,289 men Aged 60-74y	RR: 5.2 >=1.07 mm of CCA and >=1.93 of ICA vs. <1.07 mm and < 1.93 * IMT from CCA;ICA IMT
2005	Murakami S et al.[31] LILAC Study from Japan	All-cause/vascular mortality	Prospective (3.2 y)	298 Aged > 75y	All-cause mortality, left vs. right IMT RR 1.6 vs. 3.3 Cardiovascular mortality RR 2.4 vs. 2.9
2006	Lorenz MW et al.[32] CAPS (from German)	MI, stroke or death	Prospective (4.2 y)	5,056 Aged 19-90y * IMT from Bif;CCA;bulb	HRR for CCA IMT per 1SD MI: 1.16 (1.05-1.27) Stroke: 1.11 (0.97-1.28) MI, stroke, or death: 1.17 (1.08-1.26)

KIHD=Kuopio Ischemic Heart Disease; LILAC Study=Longitudinal Investigation for the Longevity and Aging in Hokkaido County; CAPS=Carotid Atherosclerosis Progression Study; OR=odds ratio

1.2.5 Polyunsaturated fatty acids (n-3 and n-6) and cardiovascular disease

Polyunsaturated fatty acids (PUFA) are essential fatty acids, which are obtained only from intake of foods. They are divided into two major compositions (Table 4). One is n-3 PUFA and the other is n-6 PUFA. The primary sources of n-3 are alpha-linolenic acids (ALA, 18:3n-3), eicosapentanoic acids (EPA, 20:5n-3) and docosahexanoic acids (DHA, 22:6n-3). ALA is called plant-derived n-3 PUFA which is found mostly in flaxeed oil or soybeans. EPA and DHA are called marine-derived n-3 PUFAs which are primarily found in fish. The primary source of n-6 is linoleic acid (LA, 18:2n-6) which is present mostly in corn, sunflower and safflower oils. Additionally, other fatty acids include monounsaturated fatty acids (MUFA), saturated fatty acids (SFA) and trans-fatty acids (TFA) (Table 4).

Table 4 Fatty acid compositions and their food sources

Fatty acids	Composition		Sources
Polyunsaturated fatty acids (PUFA)	N-3	Alpha-linolenic acid (ALA, 18:3n3)	Flaxeed oil
		Eicosapentaenoic acid (EPA, 20:5n3) Docosahexataenoic acid (DHA, 22:6n3)	Fish (e.g., salmon, herring, and anchovy)
	N-6	Linoleic acid (LA, 18:3n6)	Corn oil Sunflower oil Safflower oil
		Arachidonic acid (AA, 20:4n6)	Meat, eggs
Monounsaturated fatty acids (MUFA)	Oleic acid (18:1n9)		Olive oil
Saturated fatty acids (SFA)	Palmitic acid (16:0) Stearic acid (18:0)		Animal fat
Trans fatty acids (TFA)	16:1t 18:1t 18:2tt		Hydrogenated oil

Epidemiological or clinical studies demonstrated that n-3 PUFA protects against CHD.[33-37] Marine-derived n-3 intakes have a wide range of favorable effects on coronary risk factors, including hypotriglyceridemic, antithrombotic, anti-inflammatory and hypotensive effects. [38] There is evidence that the marine derived n-3 PUFA, especially EPA and DHA, is significantly related to the reduction of CVD risk, which is known to be mostly due to anti-arrhythmic effect. The anti-arrhythmic effects were consistently reported at least one or two servings per week of fish consumption in western countries.[39] However, it has been controversial on its antiatherogenic effect such as the reduction of non-fatal CHD (Table 5). Statistically non-significant results in the antiatherogenic effects may be possibly due to a low treatment dose of fish intake or fish oil in studies conducted in Western countries.[40, 41] However, statistically significant results have been reported in prospective or clinical trials conducted in Japanese in Japan where the population level of fish intake is relatively high.[35, 36] The Japanese are well acknowledged to be one of the populations who consume the highest intake of fish in the world. According to the International Study on Macronutrients and Blood pressure (INTERMAP) Study, Japan consumed the greatest intake of fish, estimated by marine-derived n-3 PUFA, among the studied countries (i.e., Japan, China, United Kingdom, and United States) and almost twice that of the U.S.[42] (Figure 3).

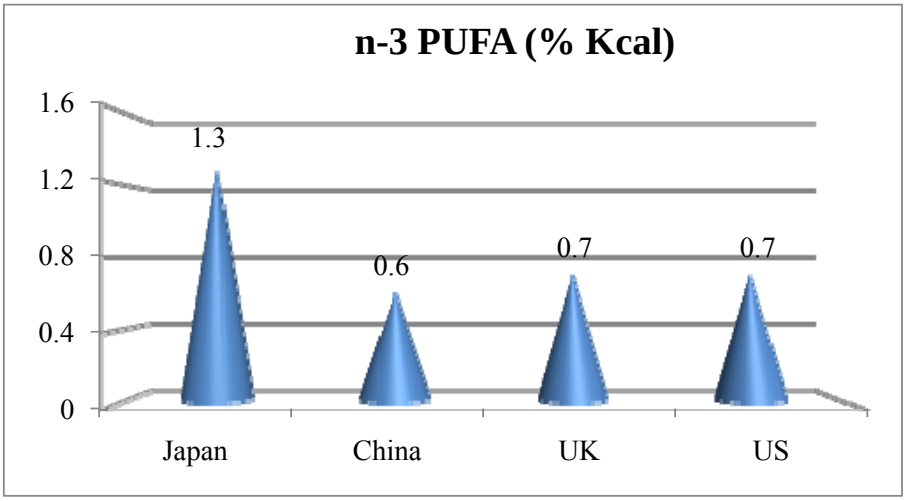

Figure 3 Marine-derived n-3 polyunsaturated fatty acids across Japan, China, United Kingdom, and United States (Zhou BF et al. [2003] from INTERMAP Study)

PUFA=polyunsaturated fatty acids

A diet-heart hypothesis may provide the foundation for an explanation of the lower level of SCD in Japan. Recently the ERA-JUMP study demonstrated that the serum level of marine-derived n-3 PUFAs attenuated the significant differences in multivariable-adjusted carotid IMT and prevalence of coronary calcification between the Japanese in Japan and U.S. whites[43]. The result indicates that the higher level of marine-derived n-3 PUFAs is a factor that may explain the low rates of SCD in the Japanese in Japan compared to U.S. Whites.

The beneficial effects of n-6 PUFA was started back to the 1960s. Linoleic acid (LA), a primary index of n-6 PUFA, has been well-recognized to decrease low-density lipoprotein cholesterol (LDL-C), when it replaces either saturated or monounsaturated fatty acids.[44] An intake of 5% - 8% energy from LA (11-20 g/day for a 2000 kcal diet) may be effective on lipid modification for healthy individuals.[38] The Nurses' Health Study, a prospective study for 14 year follow-up conducting in about 80,000 women reported that a 5 % increase of PUFA in diet

decreased the CHD risk by 38%.[45] The National Heart, Lung, and Blood Institute Family Heart Study reported that the enriched diet (4.5% - 6% energy) of LA was associated with lower coronary artery disease.[46] The cardioprotective effects of enriched LA diet have anti-thrombosis, anit-arrhythmia, and increasing insulin sensitivity.[47] Taking into consideration a robust role of circulating level of fatty acids, rather than intake, the protective effects of circulating levels of LA against cardiovascular events have been controversial in case-control studies (Table 6).[33, 48, 49] However, according to a prospective study (i.e., the Kuopio Ischemic Heart Disease Risk Factor study), an increased serum level of linoleic acid was significantly associated with the reduction in cardiovascular mortality in 1,551 men aged 42-62 years after 15 year-follow-up.[50] Therefore, an enriched diet of LA may be effective on the reduction of cardiovascular mortality.

Table 5 Marine n-3 PUFA and non-fatal CHD

Year	Authors	Effect	End Points	Design	Population	Results
1995	Simon JA et al.[33] MRFIT Study	+	CHD	Nested case-control	94/94 men Aged 35-57 y	OR of serum DPA: 0.67 (0.47-0.95) OR of serum DHA:0.66 (0.46-0.94)
1995	Guallar E et al.[40] Physicians' Health Study	-	MI	Nested case-control (5 y)	254/222 men Aged 40-84 y	RR of plasma EPA: NS RR of plasma DHA : NS
2002	Bucher HC et al.[41]	-	Nonfatal, fatal MI	Meta-analysis of RCTs	11 secondary prevention trials	RR of fish intake for nonfatal: NS RR for fish oil for nonfatal: NS
2002	Yli-Jama P et al.[34] Study from Norway	+	MI	Case-control	111/107 Aged 45-75 y	OR of serum marine n-3: 0.20 (0.06-0.63)
2002	Hu F et al.[51] Nurses' Health Study	+	CHD or CHD death	Prospective (16 y)	84,688 women Aged 34 to 59 years	RR of fish intake 0.79 for 1-3 times /month 0.64 for once per week 0.69 for 2-4 times per week 0.66 for 5 or more per week
2006	Iso H et al.[35] JPHC Study from Japan	+	CHD, MI, SCD	Prospective (5 y)	41,578 Aged 40-59 y	N-3 PUFA of the highest quintile: HRs CHD: 0.58 (0.35-0.97) MI: 0.35 (0.18-0.66) Non-fatal: 0.33 (0.17-0.63) SCD: NS; Fatal: NS
2007	Yokoyama M et al.[36] JELIS from Japan	+	CHD, SCD	Prospective RCT (5 y) EPA (1800 mg/day)	18, 645 hyper-cholesterolemic patients	HR for CHD: 0.81 (0.69-0.95) HR for SCD: 1.06 (0.55-2.07), NS
2007	Harris WS et al.[37]	+	Nonfatal CHD, fatal CHD	Meta-analysis	25 Nested Case-control or Case-control	Effect size for nonfatal EPA+DHA: NS DHA: -.26 (-.01-.50) EPA : -.09 (.03-.21)

+significant result; -non-significant result; PUFA=polyunsaturated fatty acids; CHD=coronary heart disease; CVD=cardiovascular disease; LA= linoleic acid; MI=myocardial infarction; MRFIT=Multiethnic Risk factor Intervention Trial; DPA= docosapentaenoic acid; OR=multivariate OR; RR=relative risk; JPHD=Japan Public Health Center-Based Study Cohort; SCD=sudden cardiac death; NS=non-significant; JELIS=Japan Eicosapentaenoic acid (EPA) Lipid Intervention Study

Table 6 Circulating linoleic acid and CHD

Publ Year	Authors	Effect	End Points	Design	Population	Results
1982	Miettinen TA, et al.[48]	+	MI	Nested case-control	3,400 men 40-55 y	Serum LA (cholesterol ester, phospholipid, and triglyceride): lower in cases than in controls (23.40 % vs. 26.20%)
1987	Wood DA et al.[49]	+	Angina Pectoris	Case-control		OR =1.2 (1.1-1.3) per 1% decrease in LA in adipose tissue
1995	Simon JA et al.[33] MRFIT Study	-	CHD	Nested case-control	94/94 men Aged 35-57 y	OR of serum LA(%): 0.83 (0.61-1.14), NS
1996	Ohrvall M et al.[52] Study from Sweden	-	MI	Nested case-control (19 y)	2,322 men Aged 50 y	Serum LA (cholesterol ester): lower in cases (54.1 vs. 52.9%) but NS in multivariate models
2002	Yli-Jama P et al.[34] Study from Norway	-	MI	Case-control	111/107 Aged 45-75 y	OR of serum LA (%): 1.45 (0.51-4.10), NS
2005	Laaksonen DE et al.[50] KIHD Study	+	CVD mortality	Prospective (15 y)	1,551 men Aged 42-60 y	OR of serum LA (%): 0.42 (0.21-0.80) OR of serum PUFA (%): 0.25 (0.12-0.50)

CHD=coronary heart disease; CVD=cardiovascular disease; LA=linoleic acids; KIHD=Kuopio Ischaemic Heart Disease Risk Factor; MI=myocardial infarction; MRFIT=Multiethnic Risk factor Intervention Trial

1.2.6 Potential mediators in the associations between polyunsaturated fatty acids (n-3 and n-6) and CHD: Lipoprotein classes

Lipoprotein subclasses are a feature of risk for CHD. In epidemiological studies and clinical trials, a specific lipoprotein particle concentration or size was related to CHD risk or subclinical atherosclerosis (Table 7; Table 8).

Elevated concentrations of large very-low-density lipoprotein (VLDL) particles and total LDL particles have been acknowledged to be atherogenic.[53] Particularly, small LDL particles have been paid attention to be more atherogenic than LDL-C, because its deleterious properties such as reduced affinity for the LDL receptor,[54] longer residence time in plasma,[55] and increased susceptibility to oxidation,[56] and deleterious effects on the function of vascular cells.[57] An elevated small LDL is closely associated with high triglycerides and low high-density lipoprotein cholesterol (HDL-C) and identifies metabolic syndrome with high sensitivity.[58-61] Secondary clinical trials and case-control studies demonstrated that small LDL is associated with the risk for CHD.[61-64] On the other hand, large HDL particles were reported to be a protective factor against CHD events and subclinical atherosclerosis. [65, 66] Potential capabilities for athergenesis of lipoprotein particle concentration and size are summarized in Table 9. In this regard, the lipoprotein subclasses need to be investigated as a mediator in the associations between PUFA and CHD.

Table 7 Lipoprotein particles as a predictor of CHD risk (incidence, mortality and progression)

Publ. Year	Authors	End Points	Design	Population	Results
1997	Lamarche B et al.[62] Quebec Cardiovascular Study	IHD	Nested case-control (5 y)	114 /103 Aged 35-64 y	RR for small LDL size (<=25.6) vs. (26.1): 3.6 NS, after adjusting for LDL-C, TG, HDL-C and apo-B *Using gradient gel electrophoresis
2002	Kuller L et al.[67] CHS	MI, angina	Nested case-cohort (2.7 y)	434/249 Aged >=65 y	Total LDL-p, higher for the case in multivariate models in women (but not in men)
2002	Blake GJ et al.[68] WHS	MI, stroke, death	Nested case-control (3 y)	130/130 Aged>=65 y	RR for highest quartile of total LDL-p: 4.17 (1.96-7.87)
2002	Rosenson RS et al.[63] PLAC-I Trial	Progression of CAD	RCT (3 y)	130/111 Mean age 57.5 y	OR for small LDL-p of >= 30 mg/dl: 9.1 (2.1-39) in the placebo
2003	Soedamah-Muthu S et al.[65] EDIC Study	CHD	Nested case-control	59/59 Patients with type I diabetes Mean age 35 y	OR:Medium HDL-p 3.79 (1.17-12.25) Large HDL-p 0.43 (0.20-0.92) Total VLDL 2.33 (1.07-5.08)
2006	Otvos JD et al.[64] VA-HIT	CHD	RCT (5.1 y)	364/697 men	OR for large LDL-p per 1 SD increase: 1.34 (1.11-1.62) OR for small LDL-p per 1 SD increase: 1.41 (1.14-1.73) OR for small HDL-p per 1 SD increase: 0.67 (0.57-0.79)

CHS=Cardiovascular Heart Study; WHS=Women Heart Study; PLAC-I=Pravastatin Limitation of Atherosclerosis in the Coronary Arteries; EDC=Epidemiology of Diabetes Complication; VA-HIT=Veterans Affairs High-Density Lipoprotein Intervention Trial; NS=non-significant; RR=relative risk; OR=odds ratio; LDL-C=low-density lipoprotein cholesterol; LDL-p=low-density lipoprotein particles; HDL-C=high-density lipoprotein cholesterol; HDL-p=high-density lipoprotein particles; RCT=randomized clinical trial; CHD=coronary heart diseae; CAD=coronary artery disease; MI=myocardial infarction; IHD=ischemic heart disease; SD=standard deviation

Table 8 Lipoprotein particles as a predictor of risk for subclinical atherosclerosis

Publ. Year	Authors and study name	End Points	Design	Population	Results
2002	Mackey RH et al.[53] Healthy Women Study	Coronary calcification	Cross -sectional	286 Postmenopausal women Aged 42-50 y	OR per 1 SD increase Small LDL: 1.36 (1.04-1.77) LDL size: 0.55 (0.31-0.99) Total LDL-p: 1.44 (1.04-1.99) Large VLDL: 1.59 (1.11-2.28)
2006	Lyon TJ et al.[69] DCCT/EDIC	Carotid IMT	Retrospective	968 with Type I diabetes Aged 13-40 y	Relation (β) to ICA Large LDL-p: 0.03 (0.01) for women vs. 0.02 (0.01) for men Small LDL-p: 0.05 (0.02) for women vs. 0.03 (0.01) for men Relation (β) to CCA Large LDL-p: 0.01 (0.01) for women vs. 0.01 (0.01) for men
2007	Mora S et al.[66] MESA	Carotid IMT	Cross -sectional	5,538 Aged 45-84 y	Δ IMT in μm per 1 SD Large LDL-p 42.9 (6.7) Small LDL-p 39.1 (7.1) Large HDL-p -25.1 (7.0) Small HDL-p -13.2 (4.5)

DCCT=Diabetes Control and Complication Trial; EDIC=Epidemiology of Diabetes Interventions and Complications; HDL-p=high-density lipoprotein particles; IMT=intima-media thickness; ICA=internal carotid artery; LDL-p=low-density lipoprotein particles; MESA=Multiethnic Study of Atherosclerosis

Table 9 Lipoprotein subclasses: Potential capability for atherogenesis

VLDL	
Total VLDL-p	Atherogenic
Large VLDL-p	Atherogenic
Small VLDL-p	Unknown
LDL	
Total LDL-p	Atherogenic
Large LDL-p	Atherogenic (controversial)
Small LDL-p	Atherogenic
HDL	
Total HDL-p	Unknown
Large HDL-p	Anti-atherogenic
Small HDL-p	Anit-atherogenic
VLDL size	Unknown
LDL size	Anti-atherogenic/null (controversial)
HDL size	Unknown

VLDL-p=very low-density lipoprotein particles; LDL-p=low-density lipoprotein particles; HDL-p=high-density lipoprotein particles

Some, but not all, clinical trials reported beneficial effects of fish intake or fish oil supplementation on the modification of lipoproteinns including large VLDL, [70, 71] small LDL, [72, 73] large HDL [70, 74, 75] and LDL size (Table 10; Table 11). [74, 76] However, few studies reported the beneficial effect of n-6 PUFA on the modification of lipoproteins (Table 12). [77-79] Furthermore, there is little evidence on the relationships of n-3/n-6 PUFA with lipoprotein subclasses across different population groups who have considerable variability in habitual diet (e.g., fish intake).

Table 10 Marine n-3 PUFA and lipoprotein subclasses in clinical trials

Publ. Year	Authors	PUFA	Tx	Subjects & design	Results
1991	Fumeron F et al.[70]	Fish oil supplement	3 wks	36 healthy, young males (23.5 y) Cross-over 1) Usual diet (70g butter) 2) + fish oil (6g/d MaxEPA) 3) + margarine instead of butter	In the fish oil group ↓ TG ↓ VLDL ↑large HDL
1993	Contacos C et al.[76]	Fish oil supplement	6 wks	32 pts with mixed hyperlipidemia RCT 1)Pravastatin (40mg/d, n=10) 2)Fish oil (6g/d, 3g/d of n-3) 3)Placebo	In pravastatin group ↓TC;↓LDL;↓Apo B (–) LDL Stokes' diameter In the fish oil group ↓ TG; – LDL ↑ LDL Stokes' diameter
1995	Suzukawa M et al.[80]	Fish oil supplement	6 wks	20 hypertensive subjects Cross-over 1)Fish oil (4g/d), 2) Corn oil	In the fish oil group ↓ TG (–) LDL ↑ LDL size
2000	Mori TA et al.[74]	Fish oil supplement	6 wks	59 men aged 20-65, overweight, mildly hyperlipidemic RCT 4g EPA oil, 4g DHA oil, 4g Olive oil	*Differential effect ↓ TG in both EPA and DHA ↑ LDL; LDL size in DHA (–) HDL;↑ large HDL in DHA ↓ small HDL in EPA
2000	Minihane AM et al.[72]	Fish oil supplement	6 wks	55 atherogenic lipoprotein phenotype males aged 34-69 y Cross-over 1) Fish oil (3g, EPA+DHA) 2) Olive oil	↓ TG in fasting and postprandial ↓ % small LDL (–) HDL-C
2002	Leigh-Firbank EC et al.[73]	Fish oil supplement	6 wks	55 mildly hyperTG men Cross-over 1) Fish oil (6g/d, 3g/d of EPA+DHA) 2) Olive oil (6g/d)	↓ TG in fasting and postprandial ↓ % small LDL ↑ LDL-C *Differential effect DHA→↑LDL-C, EPA→↓ TG

(–) denotes no changes; TG=triglycerides; LDL-p=low lipoprotein particles; NCE= national cholesterol education program; Tx=treatment; RCT=randomized clinical trial; PUFA=polyunsaturated fatty acids; DHA=docoxahexanoic acids; EPA=eicosapetaenoic acids

Table 11 Marine n-3 PUFA and lipoprotein subclasses in clinical trials/population-based studies

Publ. Year	Authors	PUFA	Tx	Subjects & design	Results
2002	Petersen M et al.[75]	Fish oil supplement	8 wks	42 moderately hyperTG/type diebeteic RCT Fish oil (2.6g of EPA+DHA) Corn oil	↓TG ↑large HDL (–) LDL-p
2004	Li Z et al.[78]	Dietary fish	24 wks	22 men and women aged >40 y RCT: 6 wk average American diet→ NCEP step 2 diet with high-fishdiet (4.5% saturated fat, 15mg chol) NCEP step 2 diet with low-fish diet (4.0% saturated fat, and 11mg chol)	↓ medium and small VLDL in high-fish diet ↓ LDL in both diets ↓ HDL in both diets (–) LDL-p and size in both diet
2007	Mostad IL et al.[71]	Fish oil supplement	9 wks	27 type 2 diabetic patients aged 40-75 y with normoTG RCT Fish oil (5.9 g /d , 1.8g EPA;3.0g DHA) Corn oil	(–) T chol; –LDL-C; –HDL-C ↓large VLDL-p ;↓VLDL size (–) All LDL-p ↓Small HDL
2007	Bos G et al.[81]	Dietary total PUFA		Cross-sectional 758 men and women with normal, impaired glucose metabolism and type II diabetes As a function of PUFA by stratification of glucose metabolic status (normal, impaired, diabetes)	No association between LDL size and total PUFA in subjects with normal glucose metabolism.

(–) denotes no changes; TG, triglycerides; LDL-p, low lipoprotein particles; NCEP, national cholesterol education program; Tx, treatment; RCT, randomized clinical trial; PUFA, polyunsaturated fatty acids; DHA, docoxahexanoic acids; EPA, eicosapetaenoic acids

Table 12 Linoleic acid and lipoprotein subclasses in clinical trials

Publ Year	Authors	PUFA	Tx	Subjects & design	Results
2005	Thijssen MA et al.[77]	Dietary LA	5 wks	45 healthy subjects (27 women and 18men) Cross-over 38% of energy from fat; 7% of energy of each fatty acid Stearic, Oleic, LA	Compared to other diet, ↑ large VLDL (NS) ↑ large HDL (NS) Compared to stearic diet, ↓ small LDL
2005	Wilkinson P et al.[82]	Dietary ALA, LA, and marine n-3	12 wks	57 healthy men aged 35-60 y, With a moderately raised TG and low HDL-C, with the predominance of small LDL (>30%) RCT, three diets FXO (rich in flaxeed oil) SO (rich in sunflower oil) SOF (SO plus fish-oil capsules; 3g/d of EPA+DHA) 0, 6, & 12 week follow-up	↓Small LDL in SOF (at both 6 and 12 weeks) ↓Small LDL in FXO & SO (NS) ↑Large HDL in SOF (at 12 weeks) ↑Large HDL in FXO & SO (at 12 weeks, NS)

LA= linoleic acid; PUFA=polyunsaturated fatty acids; Tx=treatment; NS=not significant; RCT=randomized clinical trial; FXO=flaxeed oil ; SO=sunflower oil; SOF=sunflower oil plus fish oil; EPA=eicopentaenoic acids; DH=docosahexanoic aicds

1.2.7 Arterial stiffness measured by pulse wave velocity: a measure of subclinical cardiovascular disease

Arterial stiffness can be noninvasively evaluated by measuring pulse wave velocity (PWV). Stiffer arteries have higher pulse wave velocity. PWV is defined as the speed of travel of the pressure pulse along an arterial segment. Theoretically, it can be obtained by using any two arterial locations accessible to palpation including heart-carotid, heart-femoral, heart-brachial, carotid-femoral, brachial-ankle, and femoral-ankle locations.

PWV may be a measure of subclinical cardiovascular disease. A large amount of evidence showed that aortic stiffness measured by carotid-femoral PWV is a predictor of all-cause and cardiovascular mortality, fatal and nonfatal coronary events, and fatal strokes in various diseases and healthy population groups, including patients with hypertension, type 2 diabetes, and end-stage renal disease and elderly/general population.[83-89] (Table 13). Moreover, aortic stiffness is related to early artherosclerosis.[90-92] There are several potential mechanisms by which increased PWV is associated with cardiovascular mortality. Arterial stiffness is associated with left ventricular hypertrophy in normotensive and hypertensive patients,[93, 94] which increases the risk for congestive heart failure and cardiovascular events.[95] Arterial stiffness increases systolic blood pressure and decreases diastolic blood pressure, which raises left ventricular afterload and myocardial work and reduces coronary perfusion, respectively.[96] Arterial stiffness is also associated with atherosclerosis,[97] probably through the effects of cyclic stress on arterial wall thickening.[98, 99]

1.2.8 Indices of arterial stiffness: carotid-femoral PWV versus brachial-ankle PWV

Carotid-femoral PWV measured by Doppler ultrasound is an established index of aortic stiffness. For this reason, the carotid-femoral PWV is a gold standard measure because the aorta is recognized as the important vessel to evaluate arterial stiffness. Alternatively, brachial-ankle PWV has been recently received attention, because it has potential as a new marker of cardiovascular risk and because it is a simple and practical methodology for clinical practice and large-scale population studies.[100, 101] Criticisms on the brachial-ankle PWV have been recognized, because brachial and post-tibial arteries are considered to be peripheral muscular arteries. Compared to central elastic arteries, peripheral arteries do not appear to change significantly with aging and disease state. However, recent evidence showed that brachial-ankle PWV was a better correlate of early atherosclerosis in postmenopausal women.[91] In small cohorts of either elderly community-dwelling people or coronary heart disease patients, brachial-ankle PWV was an independent predictor for cardiovascular deaths and events.[101]

An increase in PWV may be a result of structural and functional changes of the vascular tree, [102] which are mainly related to aging process and high mean arterial pressure[103, 104] and additionally related to other cardiovascular risk factors. [92, 104] The changes of stiffness in the vascular tree may differ by properties of arterial wall (i.e., the central artery vs. the peripheral artery). Studies reported that age-related increases in PWV differed by the arterial properties. The central arteries become stiffer with aging,[105-107]while the peripheral arteries may not be similarly influenced by aging.[103] The central artery wall has numerous elastic membranes and fibers, while the peripheral artery wall has slight elastic tissue and numerous smooth muscle cells.[108] In this regard, the characteristics of PWV associated with cardiovascular risk factors may differ by arterial segments, especially carotid-femoral PWV vs. brachial-ankle PWV.

Table 13 Prospective studies reporting predictive value of aortic pulse wave velocity

Year	Authors	Study design	Study population	End points	Results (multivariate)
2001	Laurent (France)[83]	Prospective cohort (≈9 y)	1980 essential hypertensive patients (mean age, 50 y)	CVD mortality	OR of 5 m/s increase in PWV Cardiovascular mortality: 1.51 (1.08 – 2.11)
2002	Boutouyrie (France)[84]	Prospective cohort (5.7 y)	1045 hypertensive patients (mean age, 51 y)	CHD mortality CVD mortality	RR of 10.0-12.3 m/s vs. <10.0 m/s CHD 1.63 (1.13 – 2.36) CVD 1.22 (0.91 – 1.65)\ RR of >12.3 m/s vs. <10.0 m/s CHD 2.66 (1.27 – 5.56) CVD 1.49 (0.82 – 2.71)
2003	Laurent (France)[85]	Prospective cohort (7.9 y)	1715 essential hypertensive patients (mean age, 51 y)	Stroke death	RR of 4 m/s increase in PWV Stroke death 1.39 (1.08 – 1.72)
2005	Sutton-Tyrrell K et al.[86] The Health, Aging and Body Composition (ABC) Study	Prospective cohort (4.6 y)	2488 men and women Aged 70-79 y (mean age, 74 y)	Total mortality CVD mortality CHD Stroke	RR compared to the lowest quartile of PWV Total mortality, 1.60 (1.10-2.32) CVD mortality, 1.98 (1.03-3.81) CHD, 1.53 (1.09-2.13) Stroke, 3.21 (1.56-6.63)
2005	Shokawa T et al.[87] Hawaii-Los Angeles - Hiroshima long-term epidemiological study	Prospective cohort (10 y)	492 men and women Japanese-Americans living in Hawaii (mean age, 64 y)	All-cause mortality CVD mortality	Using the cut-off value of PWV (9.9 m/s) RR All cause mortality, 1.42 (0.96-2.11) CVD mortality, 4.24 (1.36-12.96)
2006	Mattace-Raso FUS et al. The Rotterdam Study[88]	Prospective cohort (4.1 y)	2835 healthy men and women Aged >=55 y (mean age, 72 y; 39% of men)	CVD/CHD Stroke All-cause mortality	HR compared to the lowest tertile of PWV index CVD, 1.93 (1.16-3.21) CHD, 20.7 (1.08-3.98) Stroke, 1.94 (0.94-4.29) All-cause mortality, 1.39 (0.99-1.96)
2006	Willum-Hansen T et al.[89]	Prospective (9.4 y)	1678 Danes Aged 40-70 y	CVD CVD mortality CHD	HR CVD, 1.14 (1.00-1.29) CVD mortality, 1.14 (0.95-1.36) CHD, 1.12 (0.95-1.31)

CVD=cardiovascular disease; CHD=coronary heart disease; PWV=pulse wave velocity; HR=hazard ratio; CI=confidence interval; RR=relative risk; OR=odds ratio

2.0 ARTICLE ONE: DIFFERENCE IN CAROTID INTIMA-MEDIA THICKNESS BETWEEN KOREAN AND JAPANESE MEN

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2.1 ABSTRACT

Purpose: Koreans are recently at higher risk for coronary heart disease than are the Japanese. We aimed to evaluate levels of carotid intima-media thickness (IMT) and coronary risk factors in Korean and Japanese men in the post World War II birth cohort. **METHODS:** We conducted a population-based study of 352 randomly-selected healthy men aged 40-49 years: 102 Koreans in Ansan City, Gyeonggi-do, Korea and 250 Japanese in Kusatsu City, Shiga, Japan. Imaging carotid IMT by ultrasound and other procedures were standardized. Analyzing blood samples and reading carotid IMT were performed at the University of Pittsburgh. **RESULTS:** Despite more favorable or similar features in coronary risk factors as compared to the Japanese men, the Korean men had a higher crude IMT level than the Japanese men (mean ± s.e., .655 ± .008 mm versus .616 ± .005 mm, respectively, $p<.0001$). The difference in the levels of carotid IMT significantly remained after adjusting for metabolic and conventional risk factors (.654± .008 mm versus .616 ± .005 mm, respectively, $p<.0001$). **CONCLUSIONS:** Among men aged 40-49, Koreans had significantly higher levels of carotid IMT than the Japanese. Factors that underlie the different susceptibility to subclinical atherosclerosis need to be explored.

Keywords: atherosclerosis, carotid artery, cohort studies, coronary disease, Japanese, Koreans

2.2 INTRODUCTION

Among the Asian-Pacific region, Korea and Japan had relatively low coronary heart disease (CHD) mortality rates.[1] However, CHD mortality rate in Korea has been increasing since the 1980s as in many other Asian countries, whereas that in Japan has been decreasing since the 1970s.[1, 2] Among men in the post World War II (WWII) cohort, the CHD mortality rate in Korea has, in fact, exceeded that in Japan since the late 1980s.[3]

Compared to other Asian countries, Korea and Japan, closely located in East Asia, have relative similarity in socio-cultural and genetic background.[1, 4, 5] Furthermore, the post WWII birth cohorts of the two countries are more likely to have adopted a westernized lifestyle than the old cohorts. [3] Thus, a study on the comparison of CHD risk between Korean and Japanese populations in the post WWII birth cohorts may provide a powerful epidemiological tool for understanding the different trends in CHD mortality.

Carotid intima-media thickness (IMT) is a surrogate marker of general atherosclerosis. [6] Increased carotid IMT is positively associated with coronary risk factors [7, 8] and future cardiovascular events.[9] We have previously reported the difference in carotid IMT among men in the post WWII birth cohort between Japan and the U.S.[10] However, few studies have evaluated levels of carotid IMT in different ethnic cohorts of Asian populations. The purpose of the study is to determine whether there is a significant difference in levels of carotid IMT between Korean and Japanese men aged 40-49 years from population-based samples.

2.3 METHODS

2.3.1 Study population

Participants were population-based samples of 352 randomly-selected men aged 40-49: 102 Koreans in Ansan City, Gyeonggi-do, Korea and 250 Japanese in Kusatsu City, Shiga, Japan. Exclusion criteria were 1) clinical cardiovascular disease, 2) type I diabetes, 3) cancer except skin cancer in the past two years, 4) renal failure, and 5) genetic familial hyperlipidemias. All exclusion criteria were self-reported.

The 102 Koreans were randomly selected from the Korean Health and Genome Study (KHGS), an ongoing population-based prospective cohort study. [11] The target population was all non-institutionalized residents aged 40-69 of Ansan City, Gyeonggi-Do, Korea. By using the telephone directory of Ansan City, the KHGS randomly selected 5,020 participants, who had three consecutive cohorts (at the end of year 2002, 2004 and 2006). For this study, using the Ansan cohort in year 2004, we adopted a stratified random sampling by 2-year interval age groups (i.e. 40-41, 42-43, 44-45, 46-47, and 48-49), each including about 20 individuals. After extracting a list of 1,375 men aged 40-49, we contacted them at each age group by phone in randomly generated order. After 192 calls, 92 subjects took part in this study. The response rate was approximately 50%. Since the KHGS had an insufficient number of subjects aged 40-41 in 2004, we additionally enrolled 10 volunteers in the community.

The 250 Japanese men who resided in Kusatsu City, Shiga, Japan, were randomly selected from the Basic Residents' Register during 2003 – 2004.[10] Each selected man was mailed

an invitation to the study to determine his willingness to participation. The response rate was approximately 50%.

Informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of Korea University (in Seoul, Korea), Shiga University of Medical Science (in Otsu, Japan), and the University of Pittsburgh (in Pittsburgh, Pennsylvania, U.S.).

2.3.2 Carotid IMT

A Titan (Sonosite) high-resolution ultrasound system with a 10.5 MHz linear array was used at the Korean site. A Toshiba 140A scanner equipped with a 7.5 MHz-linear-array imaging probe was used at the Japanese site. For the common carotid arteries, both near and far walls were examined approximately 1 cm proximal to the bulb; for the bulb and internal carotid arteries, only far walls were examined. The digitally stored scans were then analyzed by trained readers, at the Ultrasound Research Laboratory of the University of Pittsburgh, who were blind to the details of the study subjects. The IMT scores from each location in right and left sides were averaged to produce an overall score. The IMT scores were highly reproducible, demonstrating the estimate of correlations between scans performed by different readers (inter-reader reliability) was 0.99.[12]

In order to reduce an observer bias and to assure the scanning quality,[12] sonographers of both sites were given 3-day training sessions for carotid scanning by a sonographer at the Ultrasound Research Laboratory of the University of Pittsburgh and the quality of the scanning was continuously monitored.

2.3.3 Coronary risk factors

Physical examinations and questionnaires were standardized between the two research sites. Body mass index (BMI) was calculated using body weight and height (kg/m^2). Waist circumference was measured at the level of the umbilicus while a participant was standing erect. Blood pressure was measured on the right arm in the seating position after a 5-minute rest with the bladder emptied, by using an automated sphygmomanometer (BP-8800, Colin Medical Technology, Komaki, Japan). An average of two measurements was used.

Venipuncture was performed early in a clinic visit after a 12-hour fast. All blood samples obtained at each research site were shipped on dry ice to the University of Pittsburgh after stored at -80 ◦C. Biochemical measurements of the blood samples were centralized at the Heinz Laboratory of the University of Pittsburgh. Serum lipids were determined with the standardized methods according to the Centers for Disease Control and Prevention, including total cholesterol (TC), low-density-lipoprotein cholesterol (LDL-C), high-density-lipoprotein cholesterol (HDL-C), and triglycerides.[13] Serum fasting glucose was determined by a hexokinase-glucose-6-phosphate-dehydrogenase-enzymatic assay; serum fasting insulin by a radio-immuno assay (Linco Research Inc., St. Charles, Missouri).

Data for smoking were collected with categories of non-, past- and current smoker. Current smokers were defined as cigarette smokers over the last 30 days. Pack-years of smoking were calculated as years of smoking multiplied by number of cigarettes smoked per day divided by 20. Alcohol drinkers were defined as those who drank alcohol two days per week or more. Ethanol consumption (g/day) was estimated, assuming that concentrations of alcohol were 5% for beer, 12% for wine, 40% for liquor, 16% for soju (Korean rice wine), and 16% for sake (Japanese rice wine). Hypertension was defined as systolic blood pressure ≥ 140 mmHg,

diastolic blood pressure ≥ 90 mmHg, or use of antihypertensive medications. Diabetes mellitus (type II) was defined as a serum fasting glucose level ≥ 126 mg/dL or use of diabetic medications. Education years were also collected. Prevalence of metabolic syndrome was assessed as having three or more components among the criteria proposed by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III).[14]

2.3.4 Statistical analyses

To compare coronary risk factors between the two populations, a t-test or Wilcoxon rank sum test for continuous variables and the chi-square test or Fisher's exact test for dichotomous variables were used. To examine associations of carotid IMT with coronary risk factors within each population, Pearson (r) or Spearman (ρ) correlation coefficients for continuous variables and regression coefficients (β) of the linear regression for dichotomous or ordinal variables were obtained. Two dummy variables for smoking (past and current) were designed and a p-value for smoking was obtained by p-for-trend test.

To examine an independent association between ethnicity (Korean or Japanese) and carotid IMT, the linear regression model was conducted by adjustment for potential confounders in four steps of modeling (crude, model I, II, and III,) where adjusted-means of carotid IMT for the two populations were calculated. Ethnicity was entered to calculate the crude means of IMT in both populations. Traditional risk factors (age, BMI, DBP, LDL-C, TC/HDL-C ratio, log-transformed TG, glucose, insulin, and pack-years of smoking) were entered in Model I, followed by metabolic syndrome and ethanol consumption in model II, and by other risk factors (education years, hypertension, diabetes, and use of lipid lowering medications) in model III.

All p-values were two-tailed. A P-value of <.05 was considered as significant. STATA 9.0 (StataCorp LP, College Station, Texas, USA) was used for all statistical analyses.

2.4 RESULTS

The Korean men had more favorable features than the Japanese men in TC, LDL-C, fasting glucose, smoking, and pack years of smoking; similar features in age, SBP, triglycerides, TC/HDL-C ratio, ethanol consumption, hypertension, diabetes, and use of lipid-lowering medications (Table 14). However, the Korean men had less favorable features in BMI, DBP, HDL-C, and fasting insulin.

The Korean men had significantly positive associations of carotid IMT with age, TC, systolic and diastolic BP, and prevalence of metabolic syndrome. The Japanese men had significantly positive associations of the carotid IMT with age, BMI, systolic and diastolic BP, LDL-C, TC /HDL-C ratio, fasting glucose, prevalence of metabolic syndrome, hypertension and diabetes (Table 15).

The distribution of carotid IMT was near-normal both in the Korean and Japanese men (Figure 4). The crude IMT was significantly greater in the Korean men than in the Japanese men (Table 16); the difference in the crude IMT was .039 mm. After adjusting for age, BMI, DBP, TC/HDL-C ratio, LDL-C, log-transformed TG, glucose, insulin, and pack-years of smoking (model I), the significant difference remained in the IMT. Further adjusting for metabolic syndrome and ethanol consumption (model II) and further adjusting for other factors (model III) did not alter the result.

To examine if light-to-moderate ethanol consumption explained the difference in IMT between the populations, we categorized the continuous variable of ethanol consumption into 0, >0-15, >15-30, and >30 g/day. We found no significant difference in IMT across the categories of ethanol consumption in either population; moreover, no change in the difference in IMT between populations when we placed the categorical ethanol consumption in the crude model.

2.5 DISCUSSION

This report is the first to examine the difference in levels of carotid IMT between Koreans and the Japanese in a population-based study. We found that, among men aged 40-49 years, the levels of carotid IMT were significantly greater in the Korean men than in the Japanese men. This difference was independent of metabolic and conventional risk factors.

Since increased carotid IMT is associated with CHD incidence and recurrence,[9, 15] our results suggest that middle-aged Korean men may continue to be at more increased risk for CHD than the Japanese men at least over the near future. Increased carotid IMT is also associated with stroke incidence and mortality.[9] Our observation of significantly greater IMT levels in the Korean men than in the Japanese men may be in accordance with the fact from the vital statistics that Korean men have higher stroke mortality than the Japanese men.[16]

The Korean men had lower levels of TC, LDL-C, and HDL-C than the Japanese men. These observations were consistent to those in previous studies.[8, 17] Lower TC and LDL-C levels in Koreans may reflect relatively lower fat intake of total energy intake than those in the Japanese. Recent rapid economic growth has allowed Koreans to have an accelerating concurrent shift to westernized diet pattern, which has led to an increase in average total fat intake, from

14% of energy intake in 1986 to ≈20% in 2002[18]. The total fat intake, however, is still lower in Korea than that in Japan (23.7%).[19]

Although many coronary risk factors were associated with carotid IMT as expected in each population, some risk factors showed weaker associations with IMT in the Korean men than in the Japanese men, including SBP, BMI, LDL, fasting glucose, hypertension and diabetes. The weaker associations are likely to be due to the small sample size of the Koreans. In addition, the lack of the significant association of LDL-C with IMT in the Korean men may be due to a small variability of LDL-C level.

The significant difference in carotid IMT scores between the two populations remained after adjusting for metabolic and conventional risk factors. This finding indicates that metabolic and conventional risk factors hardly explained the higher IMT in the Korean men than in the Japanese men. Genetic factors may be unlikely to explain the difference in the carotid IMT since it has been well known that Koreans and the Japanese are genetically similar[4, 5]. It was also reported that Japanese Americans have higher IMT levels than the native Japanese or Caucasians.[20, 21] In this context, we may assume that higher carotid IMT in the Korean men than in the Japanese men may be explained by environmental factors other than factors examined in the present study.

Some epidemiological studies reported a J-shaped association between ethanol consumption and carotid atherosclerosis,[22] although conflicting results have been reported.[22-25] We did not observe a J-shaped association in either population. Furthermore, the significant difference in IMT remained after adjusting for ethanol consumption. Thus, we have no evidence that ethanol consumption is likely to explain the difference in IMT between the two populations.

Differences in fat intake quality (e.g. polyunsaturated fatty acids, omega-3 fatty acids) between two populations may contribute to the difference in carotid IMT. Koreans consume relatively less fish of 60g per day than the Japanese population who has been well known to uniquely have high fish consumption, 120g per day.[26, 27] Hino et al. reported that omega-3 fatty acids intakes (e.g. eicosapentaenoic acid [EPA] or docosahexaenoic acid [DHA]) in fish had an inverse association with carotid IMT.[28] Thus, high fish consumption, rich in EPA and DHA, may be protective against carotid atherosclerosis, which might in part explain the higher carotid IMT in Koreans than the Japanese.

The study has several limitations. First, the fact that the study population is confined to men aged 40 to 49 years may limit generalization to older individuals and women. Second, we had a relatively small sample size of Koreans (n=102) compared to the Japanese (n=250). However, the levels of coronary risk factors and carotid IMT level in Koreans were not different from those reported in other studies.[29] Third, we had limited data for socio-environmental and behavioral factors (i.e. income, lifestyle, physical activity) that may be potential confounders. The two urban cities, chosen in this study, locate within a closed geographic zone determined by longitude and latitude. However, they are fairly different by population and industry. Ansan (in Korea) located near in Seoul, is a planned urban city of over 700,000 populations with a large industrial complex; Kusatsu (in Japan), located near in Kyoto City, is a city of over 110,000 populations, developed with service industry (hotels, etc.). Finally, with regard to IMT measurement by different machines, the difference was no greater than the variation between sonographers when evaluating between-machine differences. In addition, the two machines were comparable in the image quality. Thus, we believe that the variation in measurement due to the machine is relatively small.

Our finding suggests that higher level of carotid IMT in Koreans in the post World War II birth cohort may reflect a more increase risk for CHD than the Japanese. Further research is required to explore factors explaining the variation in carotid IMT between the two populations.

Table 14 Coronary risk factors between Korean and Japanese men

	Korean (n=102)	Japanese (n=250)	P-value
Age (years)	44.8±2.8	45.2±2.8	.283
BMI (kg/m^2)	24.6±2.5	23.8±3.1	.001
Waist circumference (cm)	84.2±7.2	85.3±8.3	.421
SBP (mmHg)	123.8±15.0	125.1±16.4	.601
DBP (mmHg)	80.0±11.5	76.5±11.9	.006
TC (mg/dL)	182.0±27.6	218.5±36.3	<.001
LDL-C (mg/dL)	111.3±27.2	134.1±35.5	<.001
HDL-C (mg/dL)	41.9±9.3	53.6±12.9	<.001
TG (mg/dL)	122.5 (92-173)	137.5 (104-181)	.124
TC/HDL-C ratio	4.5±1.1	4.3±1.3	.117
Fasting glucose (mg/dL)	100.0±12.2	105.9±16.0	<.001
Fasting insulin (μIU/mL)	11.5±4.8	10.4±4.5	.026
Education (years)	13.3±.3	14.3±.1	.001
Metabolic syndrome, yes (%)	14.7	12.8	.633
Smoking			.009
Current, yes (%)	33.3	49.2	
Past, yes (%)	34.0	38.2	
Pack-years of smoking	10.3 (0-23)	18.9 (3-30)	.003
Alcohol drinking, yes (%)	38.0	66.8	>.999
Ethanol consumption (g/day)	10.0 (1.8-71.4)	14.3 (2.0-42.5)	.184
Hypertension, yes (%)	21.6	26.4	.416
Diabetes mellitus, yes (%)	3.9	4.8	>.999
Lipid lowering medication, yes (%)	2.0	3.2	.730

Abbreviation: BMI, body-mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol; TG, triglycerides

Values are expressed as mean and standard deviation (SD) or median and inter-quartile range for continuous variables.

Metabolic syndrome denotes > three risk factors defined by NCEP-ATP III

Alcohol drinking denotes > two times of alcohol drinking per week

For continuous variables, a t-test or Wilconxon rank sum test was performed; for dichotomous variables, the chi-square test or Fisher's exact test

Table 15 Associations between carotid IMT and coronary risk factors within each population

	Korean (n=102)	Japanese (n=250)
Age (years)	.34***	.25***
BMI (kg/m^2)	.11	.32***
Waist circumference (cm)	.11	.26***
SBP (mmHg)	.24*	.19**
DBP (mmHg)	.29**	.17**
TC (mg/dL)	.24*	.12
LDL-C (mg/dL)	.15	.16*
HDL-C (mmol/L)	.03	-.12
TG (mmol/L)	.03	.01
TC/HDL-C ratio	.12	.19**
Fasting glucose (mg/dL)	.14	.19**
Fasting insulin (μIU/mL)	-.01	.04
Pack-years of smoking (years)	<-.001	.05
Ethanol consumption (g/day)	.05	-.01
Education (years)	-.05	-.04
Metabolic syndrome, yes	.07*	.03**
Smoking		
Current, yes	-.02	.01
Past, yes	.02	.01
Alcohol drinking, yes	.01	-.01
Hypertension, yes	.04	.02*
Diabetes mellitus, yes	-.01	.05*
Lipid lowering medication, yes	.06	<.01

***p <.001, **p<.01, *p<.05

Pearson (r) or Spearman (rho) correlation coefficients for continuous variables; regression coefficients (β) for dichotomous or ordinal variables (two dummy variables for smoking were designed [current and past] and a p-value was obtained by using p-for-trend test)

Abbreviation as Table 1.

Table 16 Multivariate-adjusted mean of carotid IMT between Korean and Japanese men

	Korean (n=102)		Japanese (n=250)		
	Mean (SE)	95% CI	Mean (SE)	95% CI	P-value
Crude	.655(.008)	.640-.670	.616 (.005)	.606-.625	<.0001
Model I	.654 (.008)	.638-.670	.616 (.005)	.607-.626	<.0001
Model II	.654 (.008)	.638-.670	.616 (.005)	.607-.626	<.0001
Model III	.654 (.008)	.638-.671	.616 (.005)	.606-.626	<.0001

Mean (SE), mean and standard error; IMT, intima-media thickness; CI, confidence interval
Model I: adjusted for age, BMI, DBP, TC/HDL ratio, LDL-C, logTG, glucose, insulin, and pack-years of smoking
Model II: further adjusted for metabolic syndrome and ethanol consumption
Model III: further adjusted for education years, hypertension, diabetes, and use of lipid lowering medications

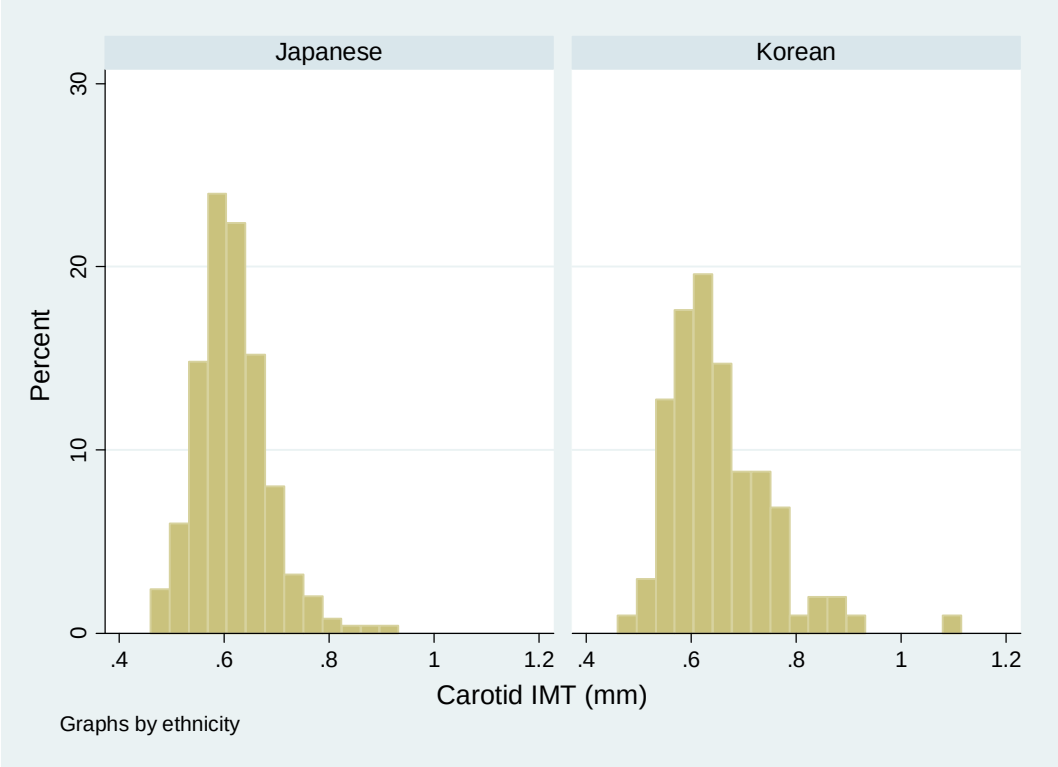

Figure 4 Distribution of carotid IMT scores by ethnicity

IMT=intima-media thickness

2.6 LITERATURE CITED

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3.0 ARTICLE TWO: RELATION BETWEEN SERUM POLYUNSATURATED FATTY ACIDS AND LIPOPROTEIN SUBCLASSES IN U.S. WHITE, JAPANESE AND JAPANESE AMERICAN MEN

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3.1 ABSTRACT

Objective: Different population groups have variability in habitual diet, especially in dietary polyunsaturated fatty acids (PUFA). Evaluating serum PUFA than dietary PUFA may be more robust for eliciting relations with lipoproteins. We aimed to identify relations between serum n-3/n-6 PUFA and lipoproteins in white, Japanese, and Japanese American men aged 40-49. **Methods and Results:** We conducted a cross-sectional study in healthy 758 men by using a robust regression analysis. We analyzed serum PUFAs (% of total fat) by capillary-gas-liquid chromatography. After adjusting confounders, linoleic acid (LA) was significantly and inversely related to large very low-density lipoprotein particles (VLDL-p) and total LDL-p, and positively related to large high-density lipoprotein particles (HDL-p) across three population groups. LA had a consistent pattern of significant and inverse relationships with small LDL-p across three population groups (β = -40.6, -37.6, and -34.2 in white, Japanese, and Japanese American men, respectively). However, docosahexaenoic acid (DHA) had a population-specific pattern, showing significant and inverse relationships with small LDL-p only for whites (β = -108.2). **Conclusions:** LA was related to a favorable shifting of VLDL-p, LDL-p and HDL-p. DHA may influence the reduction of small LDL-p within a relatively moderate range of marine-derived n-3 PUFA.

Keywords: Lipoproteins; fatty acids/blood; fatty acids, Omega-3; fatty acids, Omega-6; population groups

3.2 INTRODUCTION

Atherogenic capabilities of lipoproteins may be determined by their particle concentration. Epidemiological studies demonstrated that concentrations of total low-density lipoprotein particles (LDL-p) and small LDL-p were predictors of clinical coronary heart disease (CHD)[1-4] and subclinical atherosclerosis,[5] independent of LDL cholesterol (LDL-C). Large very low-density lipoprotein particle concentration (VLDL-p) was also reported to be positively related to prevalence of subclinical atherosclerosis.[6] On the other hand, large high-density lipoprotein particle concentration (HDL-p) was reported to be a protective factor against CHD events or progression.[1, 7]

Blood (plasma or serum) proportions of polyunsaturated fatty acid (PUFA) may be markers for CHD risk. Epidemiological studies reported that circulating marine-derived n-3 PUFAs, especially docosahexaenoic acid (DHA; 22:6n-3) with or without eicosapentaenoic acid (EPA; 20:5n-3), was inversely associated with fatal or non-fatal CHD events.[8-11] Moreover, studies reported an inverse association of circulating linoleic acid (LA, 18:2n6) with non-fatal CHD events[8] or cardiovascular mortality.[12]

Some, but not all, clinical trials reported that enriched intakes of either dietary fish or fish oil supplement favorably influenced lipoproteins (e.g., large VLDL-p, small LDL-p [or LDL size] and large HDL-p).[13-20] On the other hand, enriched LA diet has been well recognized to have a prominent effect of decreasing LDL-C.[21] However, few clinical trials reported beneficial effects of LA on particle concentration or mean size of lipoproteins.[18, 22] It is not clear that, in the

clinical trials, the outcomes of lipoprotein modification were the result from an enriched treatment diet or from exchanged proportions of other fatty acids (i.e. saturated fatty acids).[18, 22] Circulating PUFA (i.e. n-3 or n-6) is a biomarker of dietary PUFA intake.[12, 23] Evaluating serum PUFA, rather than dietary PUFA assessed in most previous studies, may be more robust for eliciting relations with lipoprotein subclasses.

Some evidence supports a different pattern of the relation between PUFA and coronary risk factors by population.[24] Fish intake in the Japanese in Japan is one of the highest in the world, much higher than that in Americans.[25, 26] No previous studies identified patterns of the relation between serum PUFA and lipoproteins in different population groups with considerable variability in marine-derived n-3 PUFA in habitual diet. Therefore, the purpose of the present study was to identify the relation between serum n-3 and n-6 PUFA and lipoprotein subclasses of VLDL, LDL and HDL, in the **E**BCT and **R**isk Factor **A**ssessment among **J**apanese and **U**.S. **M**en in the **P**ost World War II Birth Cohort (ERA-JUMP).[27, 28] a population-based-cross-sectional study of white, Japanese, and Japanese American men aged 40-49.

3.3 METHODS

3.3.1 Study population

During 2002 to 2006, 926 men aged 40-49 were randomly selected: 310 Whites from Allegheny County, Pennsylvania, U.S., 313 Japanese from Shiga, Japan, and 303 Japanese Americans, Honolulu, Hawaii, U.S.[28] All participants were without clinical cardiovascular disease, type 1

diabetes, or other severe diseases.[27] Of the original sample, we excluded those (n=168) taking lipid-lowering medications and having type 2 diabetes and missing data. The final sample was 758 men (white=261, Japanese=285 and Japanese American=212).

Written informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of the University of Pittsburgh, PA, U.S.; Shiga University of Medical Science in Otsu, Japan; and the Kuakini Medical Center, Honolulu, U.S.

3.3.2 Nuclear Magnetic resonance (NMR) lipoprotein measurements

Venipuncture was performed early in the clinic visit after a 12-hour fast. The samples were stored at -80°C and shipped on dry ice to the University of Pittsburgh.

Using NMR spectroscopy at LipoScience, Inc (Raleigh, U.S.), lipoproteins of different size were quantified including VLDL, LDL, and HDL on serum.[29, 30] Particle concentrations of the following lipoproteins were determined: 3 VLDL subclasses (large, >60nm; medium, 35-60nm; and small, 27-35nm), 3 LDL subclasses (intermediate-density lipoprotein, 23-27nm; large, 21.3-23nm; small, 18.3-21.2nm), and 3 HDL subclasses (large, 8.8-13nm; medium, 8.2-8.8nm; and small, 7.3-8.2nm).[31] Weighted average particle sizes of VLDL, LDL, and HDL were calculated.

3.3.3 Determination of serum fatty acids

The fatty acid composition of serum lipids (i.e., phospholipids, triglycerides and cholesteryl esters) was determined by capillary-gas-liquid chromatography.[32, 33] Coefficients of variation between runs for major PUFAs, EPA (20:5n-3), DHA (22:6n-3) and LA (18:2n-6), were 2.5%,

7.0% and 1.6%, respectively. Coefficients of variation for other major FAs: palmitic (16:0), stearic (18:0), oleic (18:1n-9), alpha-linolenic (ALA, 18:3n-3), arachidonic (AA, 20:4n-6), docosapentaenoic (DPA, 22:5n-3) acids, and total fatty acid amount were 1.2%, 4.0%, 2.3%, 7.9%, 2.8%, 4.5%, and 5.7%, respectively.

3.3.4 Physical and biochemical measurements

The study protocol is described in detail elsewhere.[27] Data collection was were standardized across three research sites. A self-administered questionnaire was used to obtain information on smoking, ethanol (g/day), and exercise.[27] Exerciser was defined as those who do exercise more than three times per week.

3.3.5 Statistical Analysis

All fatty acids were analyzed as proportions (%) of total fatty acid amount (mg/dL). Marine-derived n-3 PUFA was defined as a sum of EPA, DPA, and DHA; total n-3 PUFA as a sum of ALA and marine-derived n-3 PUFA; total n-6 PUFA as a sum of LA, 18:3n6, 20:2n6, 20:3n6, 20:4n6 (AA), and 22:5n6. Saturated fatty acids were defined as a sum of 14:0, 16:0 and 18:0; monounsaturated fatty acids as a sum of 16:1n7, 18:1n7, and 18:1n9.

To examine differences in lipoproteins and fatty acids by population, we performed an ANOVA test and Bonferroni test for multiple comparisons. We performed a robust regression analysis for each lipoprotein as a function of each fatty acid, after adjusting for covariates, including age, BMI, current smoking, ethanol consumption, glucose, insulin, trans fatty acids, and further adjusting for total n-6 or total n-3 PUFA according to each model. In the models

including marine-derived n-3, DHA or EPA as a primary predictor of interest, we placed total n-6 PUFA; in the models including LA as a primary predictor of interest, we placed n-3 PUFA. To illustrate a population-specific association of each of DHA and LA with small LDL-p, we calculated a multivariable-adjusted mean of small LDL-p by a tertile group of either DHA or LA within each population group.

Statistical significance was considered to be $P<0.05$. All statistical analyses were performed with STATA 9.0 for Windows (StataCorp LP, College Station, TX).

3.4 RESULTS

Our cohort had an average age of 45±3 years. The distribution of coronary risk factors across three population groups was reported in our previous studies.[28]

3.4.1 Standard lipids and lipoprotein subclasses

The white men had the highest level of LDL-C and the lowest level of HDL-C (Table 17). The Japanese men had the highest level of HDL-C. Total cholesterol and triglycerides did not differ significantly across three population groups.

Overall the Japanese men had the most favorable features of VLDL-p and LDL-p (Table 17). The Japanese men had the lowest levels of large VLDL-p and small VLDL-p, and the smallest size of VLDL-p. The white men showed the highest total LDL-p; Japanese American men showed the highest small LDL-p. The Japanese men had the most favorable features of

HDL subclasses: the highest large HDL-p and the largest HDL size. In contrast, white men had the lowest large HDL-p and the smallest size of HDL-p.

The Japanese men had the highest levels of marine-derived n-3 (i.e., DHA+DPA+EPA) and its compositions (i.e., DHA and EPA) (Table 17). The Japanese men had more than 2 times higher marine-derived n-3 PUFA and DHA than white men. There was no significant difference in ALA across three population groups (0.3%, 0.4%, and 0.2% for white, Japanese and Japanese American men, respectively). The Japanese men had the lowest levels of total n-6 PUFA, LA and AA.

3.4.2 Multivariable-adjusted models: Relations between fatty acids and lipoprotein subclasses

Each of marine-derived n-3 (i.e., DHA+DPA+EPA; DHA; EPA) showed a consistent pattern of significant and inverse relationships with large VLDL-p and VLDL size across three population groups (Table 18). LA also held the same pattern.

DHA and LA showed consistent patterns of significant and positive relationships with large HDL-p (Table 18) across three population groups. ALA showed a significant and positive relationship with large HDL-p only in the Japanese men; AA also held the same pattern as ALA.

DHA showed significant and inverse relationships with total LDL-p and small LDL-p only in white men (Table 19). DHA, but not EPA, showed a significant relationship with total LDL-p. All the marine-derived n-3 and LA showed consistent patterns of significant and positive relationships with large LDL-p across three population groups. LA showed a consistent pattern of significant and inverse relationships with total LDL-p and small LDL-p across three

population groups. ALA had no significant relationships with any LDL-p. AA showed significant and inverse relationships with small LDL-p in the white and Japanese men.

Figure 5 illustrated population-specific associations of DHA and LA with small LDL-p after adjusting for all the covariates using tertile groups of DHA and LA. DHA showed a negative and linear trend of the association with small LDL (p for trend=<0.001) among the white men, but not Japanese and Japanese American men. LA showed consistent and negative trends of relationships with small LDL-p across three population groups (p for trend=<0.001, 0.013, and 0.005 in white, Japanese, and Japanese American men, respectively).

3.5 DISCUSSION

We found that the serum levels of both LA and DHA had significant and inverse relationships with large VLDL-p (or size) and significant and positive relationships with large HDL-p (or size) in our cohort of white, Japanese, and Japanese American men aged 40 to 49. LA showed a consistent pattern of significant and inverse relationships with total LDL-p and small LDL-p across three population groups. DHA showed a population-specific pattern of significant and inverse relationships with total/small LDL-p (only in white men).

Our findings on serum marine-derived n-3 PUFAs and large VLDL-p are in accordance with previous findings on enriched intakes of marine-derived n-3 PUFAs.[16, 17] Some clinical trials reported that either dietary intake or fish oil supplementation decreased large VLDL-p.[16, 17] Large VLDL is positively correlated with plasma TG levels.[34] Evidence shows a hypotriglyceridemic effect of marine derived n-3 PUFA,[35] which may be related to our findings on marine-derived n-3 PUFA and large VLDL-p. On the other hand, very few studies reported

the effect of dietary LA on large VLDL-p.[36] Our study supports a beneficial effect of dietary LA intake (e.g., safflower and sunflower oils) on large VLDL-p.

We found that LA had inverse relationships with total and small LDL-p across three population groups. LA decreases LDL-C when it replaces either saturated fatty acids[37, 38] or monounsaturated fatty acids.[39]. An enriched LA diet has been reported in animal studies to increase hepatic LDL-receptor number.[40] Hence, the inverse relationships between LA and LDL-p in our findings may support this mechanism.

The significant and inverse relationships between all the marine-derived n-3 and small LDL-p occurred only in white men. Consistently, intervention studies reported a decreasing effect of fish oil supplementation (DHA+EPA) on small LDL-p in Caucasians.[13, 14, 19] One possible explanation of the population-specific pattern may be different levels of the marine-derived n-3 PUFA across population groups. It was noted that the Japanese men had > 2 times higher levels of marine-derived n-3 PUFA than white men; Japanese American men also had higher than white men. It appeared that an intake of two fish meals per week was associated with reduction of small LDL-p in white men, and this corresponds to a serum level of marine n-3 of 5 %. This intake has been recommended by several countries.[35, 41] Higher intakes observed among either Japanese American or Japanese men may not provide additional benefit. At the same time, the population-specific pattern may be resulted from different levels in multivariable-adjusted small LDL-p across population groups. In figure 5, the Japanese and Japanese American men showed lower levels of multivariable-adjusted small LDL-p at the lowest tertiles of DHA (853.6 nmol/L and 935.0 nmol/L, respectively) than white men (1,009.4 nmol/L). This may indicate that a long-term effect in habitual diet with relatively high intake of fish among the Japanese American and Japanese men might have produced a saturation of decreasing small LDL-p.

DHA, but not EPA, was inversely related to total LDL-p in white men, and positively related to large HDL-p across three population groups. DHA may have a stronger effect on lipoprotein modification. An meta-analysis reported that serum DHA is a stronger predictor of cardiovascular risk including both fatal and nonfatal events than serum EPA.[8] Particularly, Mori et al. (2000) reported that a fish oil supplementation of DHA, compared to EPA, was effective on raising LDL size and increasing large HDL-p.[15] However, we argue that the lack of association of EPA with total LDL-p may be in part attributed to a low proportion of EPA compared to DHA in circulating levels. Nevertheless, we assume that DHA may be dominant in decreasing total LDL-p and large HDL-p, compared to EPA.

The serum levels of both DHA and LA were significantly and positively related to the level of large HDL-p. Otvos et al. (2002) demonstrated that large HDL-p contributed, to a large extent, to an increase in HDL-C, showing a fairly linear relationship.[42, 43] Furthermore, there is evidence that fish oil supplementations increase large HDL-p, despite little effect on HDL-C.[15, 19] However, there have been no studies reporting on the beneficial effect of LA on large HDL-p, along with little evidence on its effect on HDL-C. Thus, our finding suggests that large HDL-p may have a favorable shifting by the effect of each of DHA and LA, regardless of its effects on HDL-C.

The study has limitations. The study population of men aged 40-49 years limits the generalizability of the results to elderly and women groups.

We found that higher serum levels of DHA and LA were related to beneficial modification of VLDL-p and HDL-p in a cohort of healthy white, Japanese, and Japanese American men aged 40-49. The present study supports an anti-atherogenic effect of LA through the modification of atherogenic lipoprotein profile. In addition, we suggest that marine-derived

n-3 PUFA may influence a reduction of total and small LDL-p within a relatively moderate range of serum levels.

Table 17 Standard lipids, lipoprotein subclasses, and fatty acids across population groups in 2002-2006 (N=758)

	Mean (SD)				
	U.S. white (n=261)	Japanese (n=285)		Japanese American (n=212)	
Total cholesterol (mg/dl)	215.56 (2.3)	216.02 (2.1)		211.0 (36.0)	
LDL-C (mg/dl)	137.72 (2.1)	131.47 (2.1)		127.8 (33.0)	b
HDL-C (mg/dl)	48.43 (0.80)	54.21 (0.82)	a	51.1 (12.3)	c
Triglycerides (mg/dl) [a]	125 (91-186)	134 (101-180)		136 (89-211)	
VLDL					
Total VLDL-p (nmol/L)	93.0 (44.6)	90.3 (46.7)		107.7 (53.4)	b c
Large VLDL-p (nmol/L) [*]	1.6 (0.6-5.8)	0.4 (0.1-2.8)	a	1.8 (0.6-6.9)	c
Small VLDL-p (nmol/L)	47.6 (21.4)	42.8 (24.2)		50.9 (24.1)	c
VLDL size (nm)	49.8 (7.8)	43.9 (7.5)	a	48.2 (6.7)	c
LDL					
Total LDL-p (nmol/L)	1,478.0 (400.9)	1,381.8 (443.0)	a	1,385.3 (514.2)	
Large LDL-p (nmol/L)	533.2 (279.4)	511.5 (229.0)		343.1 (250.0)	b c
Small LDL-p (nmol/L)[*]	813.0 (483.2-1,275.6)	806 (471-1,179)		992.1 (592.3-1,296.2)	c
LDL size (nm)	21.0 (0.9)	21.1 (0.9)		20.6 (0.9)	b c
HDL					
Total HDL-p (µmol/L)	31.3 (5.6)	35.2 (60.5)	a	35.9 (5.9)	b
Large HDL-p (µmol/L)	5.1 (3.2)	8.7 (4.1)	a	6.2 (3.3)	b c
Small HDL-p (µmol/L)	25.1 (4.5)	23.8 (5.6)	a	26.8 (4.8)	b c
HDL size (nm)	8.6 (0.5)	9.1 (0.5)	a	8.8 (0.4)	b c
PUFA (%)	46.2 (4.5)	44.7 (3.8)	a	47.7 (4.2)	b c
DHA+DPA+EPA (%)	3.9 (1.8)	9.4 (3.0)	a	5.0 (2.3)	b c
DHA (%)	2.4 (1.3)	6.0 (1.7)	a	3.3 (1.4)	b c
EPA (%)	0.8 (0.6)	2.6 (1.5)	a	1.1 (1.1)	b c
Total n-6 (%)	41.9 (4.2)	35.0 (4.3)	a	42.2 (4.0)	c
LA (%)	30.1 (4.1)	26.4 (4.1)	a	30.7 (4.2)	c
AA (%)	8.9 (1.9)	6.6 (1.3)	a	8.9 (2.2)	c
SFA (%)	31.0 (2.5)	31.7 (2.2)	a	30.7 (2.1)	c
MUFA (%)	20.1 (3.2)	21.2 (3.1)	a	18.9 (3.2)	b c
Trans fatty acids (%)	1.0 (0.5)	0.6 (0.2)	a	0.9 (0.4)	b c

[*] Values are expressed as median and inter-quartile range. SD=standard deviation; LDL-C=low density lipoprotein cholesterol; HDL-C=high density lipoprotein cholesterol; VLDL-p=very low-density lipoprotein particles; LDL-p=low-density lipoprotein particles; HDL-p=high-density lipoprotein particles; PUFA=polyunsaturated fatty acids; DHA=docosahexaenoic acid; DPA= docosapentaenoic acid; EPA=eicosapentaenoic acid; ALA=alpha-linolenic acid; LA=linoleic acid; AA=arachidonic acid; SFA=saturated fatty acids; MUFA=monounsaturated fatty acids

a : $p<0.05$ White versus Japanese, **b**: $p<0.05$ White versus Japanese American, **c**: $p<0.05$ Japanese versus Japanese American

Table 18 Relation of % PUFAs to VLDL and HDL subclasses in U.S. white (n=261), Japanese (n=285) and Japanese American men (n=212)

	β coefficient											
	Large VLDL (nmol/L)			VLDL size (nm)			Large HDL (μmol/L)			Mean HDL size (nm)		
	W	J	JA	W	J	JA	W	J	JA	W	J	JA
Model: age + covariates[a] +(total n-3 or total n-6)[b] + trans fatty acids												
Marine-derived n-3												
DHA+DPA+ EPA	-0.56*	-0.16*	-0.33*	-1.46*	-1.80*	-1.03*	0.37*	0.43*	0.17	0.45*	0.03*	0.00
DHA	-0.67*	-0.28*	-0.46*	-1.87*	-2.83*	-1.45*	0.50*	0.54*	0.33†	0.06*	0.05*	0.01
EPA	-1.51*	-0.19*	-0.70*	-4.00*	-3.19*	-2.10*	1.11*	0.89*	0.18	0.11†	0.04†	-0.05
Plant-derived n-3												
ALA	0.92	-0.42	-0.53*	0.66	-5.60†	-1.43	-0.81	4.16*	-0.81	-0.10	0.21	-0.00
n-6												
LA	-0.24*	-0.09*	-0.32*	-0.21†	-0.94*	-0.31*	0.18*	0.37*	0.12†	0.02*	0.03*	0.01†
AA	-0.32*	-0.05	-0.17†	-0.32	-1.20*	-0.41†	0.15	0.70*	0.05	0.02	0.04†	0.00

*p<0.01, †p<0.05

Abbreviation as Table 1. total n-3= DHA+DPA+EPA+ALA; total n-6 = sum of all n-6 compositions

[a] Covariates indicate body mass index, current smoking, ethanol consumption, glucose, and insulin.

[b] Marine n-3, DHA, EPA and ALA were adjusted for total n-6; LA and AA were adjusted for total n-3.

W, J and JA denote white men, Japanese men, and Japanese American men, respectively.

Table 19 Relation of % PUFAs to LDL lipoprotein subclasses in U.S. white (n=261), Japanese (n=285) and Japanese American men (n=212)

	β coefficient											
	Total LDL (nmol/L)			Large LDL (nmol/L)			Small LDL (nmol/L)			Mean LDL size (nm)		
	W	J	JA	W	J	JA	W	J	JA	W	J	JA
Model: age + covariates [a] + (total n-3 or total n-6) [b] + trans fatty acids												
Marine-derived n-3												
DHA+DPA+EPA	-20.43	11.13	5.03	38.61*	26.78*	15.91†	-65.83*	-10.11	-5.83	0.14*	0.05*	0.02
DHA	-46.46†	20.81	-1.85	56.81*	38.27*	26.38†	-108.15*	-11.82	-18.86	0.22*	0.07†	0.06
EPA	-15.48	18.11	17.47	83.59*	9.07*	29.68†	-123.16†	-18.64	-4.13	0.30*	0.09*	0.04
Plant-derived n-3												
ALA	75.03	-142.42	-15.62	8.15	108.34	-65.29	82.67	258.85	-48.87	-0.15	0.53	-0.02
n-6												
LA	-29.25*	-17.25†	-25.62*	15.20*	22.74*	13.63*	-40.64*	-37.57*	-34.16*	0.07*	0.08*	0.06*
AA	10.79	-21.57	39.26*	43.00*	40.79*	7.12	-37.13*	-62.16*	25.41	0.09*	0.13*	-0.01

*p<0.01, †p<0.05 Abbreviation as Table 1 and 2.

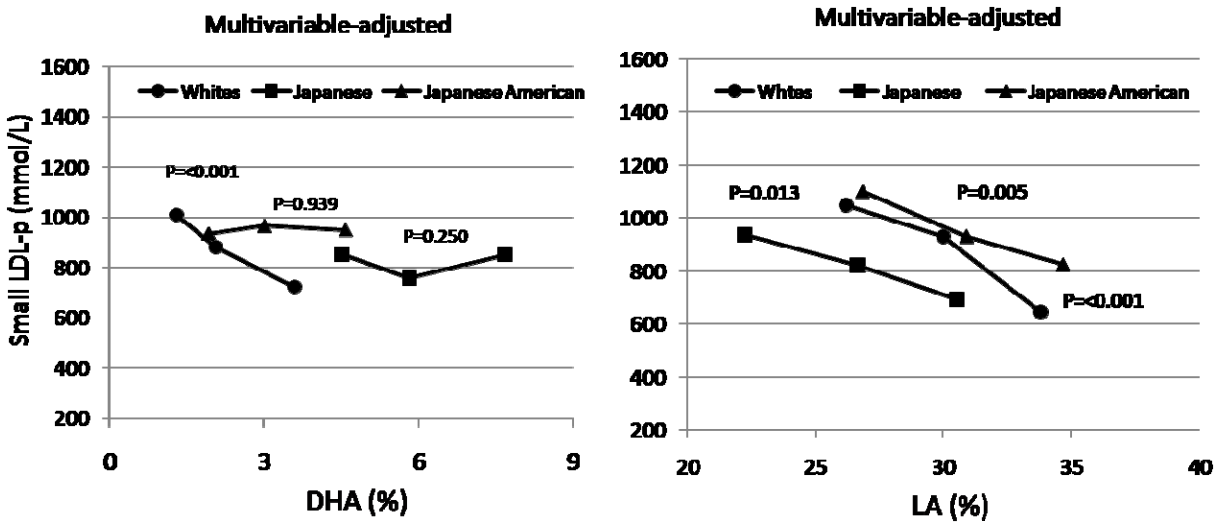

Figure 5 Population-specific associations of DHA and LA with small LDL particles in multivariable-adjusted models in white, Japanese and Japanese American men.

Abbreviation as Table 1; Tertile groups of DHA and LA were placed into the models

3.6 SUPPLEMENT MATERIALS: ASSOCIATION BETWEEN SERUM PUFA AND FISH INTAKE

3.6.1 Methods

Frequency of fish intake was collected as more than once daily; every day; 4 to 5 times a week; 2 to 3 times a week; once a week; 1 to 3 times a month; less than once a month; rare or never. To examine associations between weekly fish intake and serum fatty acids, we performed a regression analysis for a specific fatty acid as a function of weekly fish intake as a continuous variable. For this analysis, we collapsed the data of frequency of fish intake using different categories by population because of incomparable distributions between U.S. white and Japanese men. For white and Japanese American men, we collapsed into < once, once, and ≥ 2 times per week; for the Japanese men, ≤ once, ≥ 2 times, and ≥ 4 times.

3.6.2 Summary of Results and Discussion

The proportions of marine-derived n-3 and its composition (i.e., DHA and EPA) were significantly associated with fish intake frequency (per week) across three population groups (Table 1). Among the Japanese men, total n-6 PUFA and LA were inversely associated with the frequency of fish intake (per week).

In our finding, the serum proportions of marine-derived n-3 PUFA reflected the dietary intakes of fish across three population groups. The Pearson correlation coefficients for DHA (r=0.37) and EPA (r=0.35) in white men were similar to those reported in the Atherosclerosis Risk in Community (ARIC) study (r=0.42 and r=0.23 for DHA and EPA,

respectively, measured by phospholipid in plasma).[23] Because of lack of data, we were unable to observe correlations between serum LA and dietary LA. However, epidemiological studies demonstrated that dietary LA significantly correlated with serum LA proportion (r=0.50 in the Kuopio Ischemic Heart Disease Risk Factor study and r=.22 in the ARIC study).[12, 23]

Table 20 Associations between serum PUFA and dietary intake of fish within each population (N=758)

Fish intake (per week)	% by weight of total fatty acids, Mean (SD)				
	< Once	Once	≥ 2 times	≥ 4 times	P-value by fish intake
U.S. white					
Number	117	85	29		
DHA+DPA+EPA	3.3 (1.3)	4.0 (1.6)	5.0 (2.1)		<0.001
DHA	2.0 (1.0)	2.5 (1.2)	3.1 (1.4)		<0.001
EPA	0.6 (0.3)	0.8 (0.6)	1.1 (0.7)		<0.001
Total n-6	42.4 (4.3)	41.3 (4.1)	41.9 (4.3)		0.279
LA	30.3 (4.2)	29.2 (3.8)	30.9 (4.2)		0.753
Japanese					
Number		45	121	119	
DHA+DPA+EPA		7.8 (2.6)	8.7 (2.4)	10.8 (3.1)	<0.001
DHA		5.1 (1.5)	5.7 (1.6)	6.6 (1.6)	<0.001
EPA		1.9 (1.2)	2.2 (.9)	3.2 (1.7)	<0.001
Total n-6		36.8 (3.8)	35.8 (4.0)	33.4 (4.3)	<0.001
LA		27.9 (3.9)	27.2 (3.8)	25.0 (4.1)	<0.001
Japanese American					
Number	83	63	66		
DHA+DPA+EPA	4.5 (2.0)	4.7 (1.9)	6.0 (2.8)		<0.001
DHA	2.9 (1.3)	3.1 (1.1)	3.9 (1.5)		<0.001
EPA	0.9 (0.8)	1.0 (0.9)	1.4 (1.4)		0.008
Total n-6	42.8 (3.7)	42.0 (3.9)	41.6 (4.4)		0.072
LA	31.2 (3.8)	30.5 (4.0)	30.2 (4.7)		0.123

PUFA=polyunsaturated fatty acids; DHA=docosahexaenoic acid; DPA= docosapentaenoic acid; EPA=eicosapentaenoic acid; ALA=alpha-linolenic acid; LA=linoleic acid; AA=arachidonic acid; SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; total n-3= DHA+DPA+EPA+ALA; total n-6 = sum of all n-6 compositions

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4.0 ARTICLE THREE: SEGMENTAL PULSE WAVE VELOCITY AND ITS RISK FACTORS IN APPARENTLY HEALTHY MEN: A CROSS-SECTIONAL STUDY

Manuscript submitted for publication

SHORT TITLE: Segmental Pulse Wave Velocity

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4.1 ABSTRACT

An assessment of segmental pulse wave velocity (PWV) allows for perspective on stiffening characteristics by type of the artery, i.e., central elastic artery vs. peripheral muscular artery. We aimed to examine correlations between segmental PWVs and to identify correlates of each segmental PWV with cardiovascular risk factors in a population-based sample of healthy white men aged 40 -49. We conducted a cross-sectional study in 248 men without diabetes, hypertension and cardiovascular disease. PWVs were simultaneously measured by an automated tonometry/plethysmogrphy system, heart-carotid (*hc*), heart-femoral (*hf*), carotid-femoral (*cf*), heart-brachial (*hb*), femoral-ankle (*fa*), and brachial-ankle (*ba*) PWVs. *cf*PWV correlated strongly with *hf*PWV (r=0.87, p<0.001). *ba*PWV correlated strongly with *fa*PWV (r=0.68, p<0.001), followed by *hf*PWV (r=0.18, p=0.005). *ba*PWV correlated partially with *cf*PWV (r=0.16, p=0.028) . In stepwise regression analyses, all the PWVs had a strong correlate of mean arterial pressure. The PWV of the central elastic arteries, e.g., *hf*PWV or *cf*PWV, had the correlates of age, body mass index, low-density lipoprotein cholesterol, and alcohol drinking. The PWV of the peripheral muscular arteries, i.e, *fa*PWV, had the correlates of fasting insulin and smoking. *ba*PWV had the correlates of fasting insulin and alcohol drinking. Among healthy middle-aged white men, *ba*PWV correlated partially with *cf*PWV. *ba*PWV showed a mixed characteristic of stiffness of the central and peripheral arteries. A primary independent correlate of PWV was mean arterial pressure across all the arterial segments. Other correlates of PWV differed by arterial segments.

Key words: arterial stiffness; pulse wave velocity; aorta; arteries, carotid, brachial and femoral

4.2 INTRODUCTION

Arterial stiffness is a predictor of the risk for cardiovascular disease in patients with hypertension[1] as well as in the general population.[2] Increased arterial stiffness is mainly related to aging process and high blood pressure and can be accelerated by cardiovascular risk factors.[3-5] Moreover, arterial stiffness is related to early atherosclerosis.[6-8]

Arterial stiffness can be noninvasively evaluated by measuring pulse-wave velocity (PWV) through distance and time of traveling wave estimated by a specific segment of the arterial tree. Carotid-femoral PWV (*cf*PWV) measured by Doppler ultrasound is the most widely accepted index of aortic stiffness that has been considered a proxy for stiffness in other vessels. For this reason, *cf*PWV is recognized as a gold standard measure for evaluating arterial stiffness. Alternatively, brachial-ankle PWV (*ba*PWV) measured by oscillometric/plethysmographic system has been recently paid attention, because of its high validity of aortic stiffness and practically simple methodology for large-scale population studies.[9, 10] However, very few studies reported a correlation between *cf*PWV and *ba*PWV using the same device system in apparently healthy adults.[11]

An assessment of segmental PWVs allows for perspective on stiffening characteristics of arterial type determined by proximity from the heart (i.e. the central vs. the peripheral arteries), and the content of elastin and the number of smooth muscle cells (i.e., elastic vs. muscular arteries). The PWVs of arterial segments from the heart include heart-carotid PWV (*hc*PWV), heart-brachial PWV (*hb*PWV) and heart-femoral PWV (*hf*PWV). Technically, *cf*PWV may reflect the stiffness of the aorta, proximal aortic arteries and large arteries, while *ba*PWV may reflect the stiffness of both central and peripheral arteries. Femoral-ankle PWV (*fa*PWV) may entirely reflect the stiffness of the peripheral arteries, while *hf*PWV may entirely reflect the

stiffness of the aorta. An age-related increase in arterial stiffness may differ by the arterial type. The elastin-rich central arteries become stiffer with aging because of a decreased ratio of elastin to collagen contents with aging, which is probably related to elevated *cf*PWV.[12-14] The elastin-less peripheral arteries containing numerous smooth muscle cells may not be similarly influenced by aging.[5] However, there is lack of evidence on segmental-specific relationships of arterial stiffness measured by PWV to other atherosclerotic or cardiovascular risk factors.

We hypothesized that characteristics of arterial stiffness in relation to cardiovascular risk factors would differ by arterial segments. The objectives of the present study were twofold. First, we examined correlations among *hc*PWV, *hf*PWV, *cf*PWV, *hb*PWV, *fa*PWV and *ba*PWV (especially between *cf*PWV and *ba*PWV) obtained simultaneously by using an automated oscillometric/tonometry system. Second, we identified correlates of each segmental PWV with cardiovascular risk factors and examined if the correlates differed by segmental PWVs. This study was conducted in a cohort of healthy white men aged 40 – 49 years of the **E**BCT and **R**isk Factor **A**ssessment among **J**apanese and **U**.S. **M**en in the **P**ost World War II Birth Cohort (ERA-JUMP), [15-17] a population-based-cross-sectional study.

4.3 METHODS

4.3.1 Study population

During 2002 to 2006, 310 white men aged 40-49 were randomly selected from Allegheny County, Pennsylvania, U.S.[18] All participants were without clinical cardiovascular disease, type 1 diabetes, or other severe diseases.[15] Of the original samples, we excluded 49 participants who had hypertension and type 2 diabetes and 9 participants who had having neither covariates nor parameters of PWV. Then 8 participants were further excluded having > 2,500 cm/sec of *cf*PWV because of measurement errors. [19] Our final sample was 248.

Written informed consent was obtained from all participants. The study was approved by the Institutional Review Board of the University of Pittsburgh, Pittsburgh, PA, U.S.

4.3.2 PWV measurement

The PWV was automatically generated using a noninvasive and automated waveform analyzer (Colin-VP2000/1000, Omron, Japan/WaveNexus,TX). This device records the electrocardiography (ECG), phonocardiogram, and pressure waveforms; additionally measures arterial BP at the left and right brachial and ankles. ECG electrodes were placed on both wrists. A phonocardiogram was placed on the left edge of the 4th-rib-case; heart sounds S1 and S2 were detected. Pressure waveforms of the brachial and tibial arteries were recorded by a plethysmographic sensor and an oscillometric pressure sensor using the occlusion/sensing cuffs adapted to both arms (i.e., brachial arteries) and both ankles (i.e., tibial arteries). Pressure

waveforms of the carotid and femoral arteries were recorded using multiarray tonometry sensors placed at the left carotid and the left femoral arteries. Following 10 minutes of rest in a supine position, pressure waveforms were stored for a sampling time of 10 seconds with automatic gain analysis and quality adjustment.

PWV (cm/second) was calculated as (path length between arterial sites) / (time interval). The path length for *cf*PWV was obtained by using the actual distance of participants' body (in cm): ([cm from suprasternal notch to bottom of umbilicus] + [cm from botton of umbilicus to femoral sensor]) – [cm from carotid to suprasternal notch]. Also, the path length for heart-carotid (D*hc*), the heart-brachial (D*hb*), the heart-femoral (D*hf*), the femoral-ankle (D*fa*), and the brachial-ankle (D*fa* – D*hb*) segments on the basis of height (in cm) were obtained by using the following formulas: D*hc* = 0.2437*HT-18.999; D*hb*= 0.2195*HT -2.0734; D*hf* =0.5643*HT-18.381; and D*fa*= 0.2486 *HT-30.709. Excellent reproducibility of the automated PWV measurement has been reported, with coefficients of variation of 6.0, 3.3, 4.9, and 3.3% for *hc*PWV, *hb*PWV, *hf* PWV, and *fa*PWV, respectively.[20]

4.3.3 Blood pressure measurement

Blood pressure (BP) was simultaneously measured at the supine position by the oscillometric method of Colin-VP2000/1000 using the above cuffs at brachial arteries when PWV was measured. The standard brachial cuffs (No. 20 and 21) were used for participants whose arm circumference is 23 to 33 cm. For the study, we took systolic and diastolic BP derived from the average of the left and right brachial arteries. Mean arterial pressures were obtained from the

formula: (2*diastolic BP + systolic BP)/3. Pulse pressure was systolic BP subtracted by diastolic BP.

4.3.4 Other measurement

Venipuncture was performed early in the clinic visit after a 12-hour fast. The samples were stored at -80°C before their analysis. Biochemical markers (lipids, fasting insulin, and high sensitivity C-reactive protein [hs-CRP]) were described in detail elsewhere.[15] A self-administered questionnaire was used to obtain information on demography and smoking habits. Current smokers were defined as cigarette smokers over the last 30 days. Pack years of smoking were calculated as years of smoking multiplied by number of cigarettes per day smoked divided by 20. Alcohol drinking was defined as drinking more than twice per week. Exercise was defined as the regular exercise more than three times per week. Heart rate was measured after a 5-min rest by using an automated sphygmomanometer (BP-8800, Colin Medical Technology, Komaki, Japan).

4.3.5 Statistical analysis

Mean (standard deviation) and median (interquartile) are presented for basic characteristics of the study participants. To obtain correlation coefficients with adjustment for age and mean arterial pressure, we performed the partial correlation analysis between segmental PWVs. To examine univariate associations between each segmental PWV and cardiovascular risk factors, we used Spearman correlation analysis. To examine the determinants of each segmental PWV

(i.e., age, body mass index [BMI], MAP, heart rate, LDL-C, log-transformed triglycerides, high-density lipoprotein cholesterol [HDL-C], glucose, log-transformed insulin, log-transformed hs-CRP, pack-years of smoking, and exercise), we performed the stepwise regression analysis with backward removal ($p < 0.2$) for variable selection, simultaneously using the robust regression for the satisfaction of underlying assumptions (i.e., normality and heteroscedasticity). Statistical significance was considered to be $P<0.05$. All statistical analyses were performed with STATA 9.0 for Windows (StataCorp LP, College Station, TX).

4.4 RESULTS

Our cohort of white men had an average age of 45 years (Table 21). They were overweight at average. The average of blood pressure was 127/78 mmHg. Triglycerides and HDL-C were within normal limits; LDL-C was borderline high; Fasting glucose and insulin levels were within normal limits. Seven % of participants were current cigarette smokers, 45% were alcohol drinkers, and 54% were exercisers at three times or greater (per week).

The PWV of the peripheral muscular artery, i.e., *fa*PWV was higher than the PWVs of the central elastic arteries, i.e., *hc*PWV, *hf*PWV and *cf*PWV (Figure 6). *ba*PWV showed the highest value; *hb*PWV showed the lowest value. *hc*PWV, *hf*PWV and *cf*PWV showed similar values. *hc*PWV, *hf*PWV, *cf*PWV and *hb*PWV showed right-skewed distributions, whereas *ba*PWV and *fa*PWV showed normal distributions.

4.4.1 Partial correlation between PWVs

*cf*PWV had a strong positive correlation with *hf*PWV (r=0.87, p<0.001), but had no significant correlation with *fa*PWV (Table 22). *ba*PWV had a strong positive correlation with *fa*PWV (r=0.68, p<0.001), followed by *hf*PWV (r=0.18, p=0.005) and *cf*PWV (r=0.16, p=0.028) . *hf*PWV had a negative correlation with *fa*PWV (r=-0.18, p<0.005). *hc*PWV had a positive correlation with *hf*PWV (r=0.28, p<0.001), but no significant correlation with *fa*PWV. *hb*PWV had a positive correlation with both *hf*PWV (r=0.25, p=0.015). *hc*PWV and *hb*PWV showed a modest positive correlation (r=0.47, p<0.001).

4.4.2 Association between PWV and cardiovascular risk factors

The univariate analyses showed that MAP correlated strongly with all the PWVs (Table 23). Age had significant positive correlations with the PWVs of arterial segments derived from the heart, i.e., *hc*PWV, *hf*PWV and *hb*PWV, but not with *cf*PWV, *fa*PWV or *ba*PWV. BMI and hs-CRP had significant and positive correlations with the PWVs of the central arteries (*hc*PWV, *hf*PWV and *cf*PWV). Triglycerides and fasting insulin had significant and positive correlations with the PWVs of both central and peripheral arteries, i.e., *hc*PWV, *hf*PWV and *cf*PWV; *fa*PWV and *ba*PWV. HDL-C had significant and negative correlations with the PWVs of the central arteries (i.e., *hf*PWV and *cf*PWV). Heart rate had significant and positive correlations with *cf*PWV, *fa*PWV and *ba*PWV. Pack years of smoking had significant and positive associations with *hb*PWV and *fa*PWV. Alcohol drinking had significant and negative associations with the PWVs of both central and peripheral arteries (i.e., *hf*PWV and *cf*PWV; *fa*PWV and *ba*PWV).

Multivariable-adjusted analyses showed that MAP had significant associations with PWVs across all the segments (Table 24), after adjusting age, BMI, heart rate, LDL-C, triglycerides, HDL-C, glucose, insulin, hs-CRP, smoking, alcohol drinking, and exercise. Age had significant associations with *hf*PWV, *hc*PWV and *hb*PWV. BMI was significantly and positively associated with *hf*PWV and *cf*PWV, but significantly and negatively associated with *fa*PWV and *hb*PWV. Heart rate had a significant and positive association with *ba*PWV. LDL-C had a significant and positive association with *hf*PWV. Fasting insulin levels and pack years of smoking had significant and positive associations with *fa*PWV. hs-CRP levels had a significant and positive association with *hc*PWV. Alcohol drinking had significant and negative associations with *hf*PWV and *cf*PWV.

*cf*PWV had common predictor variables with *hf*PWV (i.e., MAP, BMI, LDL-C, insulin, and alcohol drinking). *ba*PWV had common predictor variables with *fa*PWV (i.e., MAP, insulin, and BMI). Moreover, *ba*PWV had common predictor variables with *cf*PWV and *hf*PWV (i.e., MAP and alcohol drinking).

4.5 DISCUSSION

We found that *ba*PWV and *cf*PWV correlated partially, in a cohort of middle-aged white men without diabetes, hypertension and clinical cardiovascular disease. We also found that although MAP was a primary independent correlate of PWVs across all the arterial segments, other correlates of PWV differed by arterial segments. The PWV of the central elastic arteries, e.g., *hf*PWV, *hc*PWV or *cf*PWV, had the correlates of age, BMI, LDL-C, hs-CRP, and alcohol

drinking. The PWV of the peripheral muscular arteries, i.e, *fa*PWV, had the correlates of fasting insulin and smoking. *ba*PWV had the correlates of fasting insulin and alcohol drinking.

Our finding that *ba*PWV weakly correlated with *cf*PWV (r=0.16) is inconsistent with previous findings. In a previous finding, the correlation (r=0.76) was much stronger in 406 Japanese healthy adults aged 18-76 years.[11] The fairly different magnitude of the correlations may be attributable to different ranges of age between the two studies. Our cohort was a narrow range-aged group of 40 to 49 years. Given gradual loss of the elastic content in the aorta/proximal arteries with aging, variability in aortic stiffness may be minimal within our aged-group. Therefore, our finding is of importance to select a PWV measure when conducting a study and to interpret study findings according to different distribution of age.

*ba*PWV might be explained by cardiovascular risk factors to a greater extent than *cf*PWV. It is of note that the total variance of *ba*PWV (R^2=0.30) in the regression model was greater than that of *cf*PWV (R^2=0.15). In our data, *ba*PWV reflected the stiffness of mixed arterial segments of the central and peripheral arteries. Compared to the central arteries, the peripheral arteries have been considered to be less clinically important, especially in the relation to diseases. Kimoto et al. reported that among cardiovascular factors, diabetes were significantly associated with *hf*PWV, but not with *fa*PWV in 290 healthy and diabetic patients .[20] However, no evidence demonstrated the associations of other diseases or their risk factors with the PWV of the peripheral arteries in apparently healthy individuals. Therefore, we suggest that characteristics of stiffness involving the peripheral arteries may also play a role in the progression of cardiovascular disease in apparently healthy men.

The association of age with arterial stiffness differed by arterial segments. As reported consistently in previous studies,[5, 13] age was significantly associated with stiffness selectively

apparent in the aorta and proximal large elastic arteries, i.e., *hf*PWV and *hc*PWV, rather than the peripheral and muscular arteries, i.e., *fa*PWV. Unlike previous findings,[12] age showed a lack of the significant association with *cf*PWV. It may be due to small variability in *cf*PWV within one-decade aged group.

Alcohol drinking may be a correlate of the PWV of the central artery. *cf*PWV had a significant and inverse association with alcohol drinking (≥ twice per week), whereas *ba*PWV had a similar trend of the association with alcohol drinking (p=0.088). Sierksma et al. reported that moderate alcohol consumption had an inverse association with aortic PWV measured by *cf*PWV in 370 men aged 40-80 years.[21] The Rotterdam Study reported a similar trend in men aged >55[22] The trend of *ba*PWV in our finding may be an expected finding because *ba*PWV had a mixed characteristic of stiffness of the central and peripheral arteries.

The association between BMI and PWVs may differ by arterial segments. Interestingly, our finding showed that increased BMI was inversely related to the peripheral arterial stiffness (i.e., *fa*PWV and *hb*PWV). Increased BMI was reported to be a predictor of aortic stiffness measured by *cf*PWV in men, in some, but not all, epidemiological studies.[3, 8, 23] Arterial stiffness may be related to distensibility and arterial diameter of the artery. Zebekakis PE et al. (2005) reported that BMI was related to increased diameter and decreased distensibility in peripheral arteries including both brachial and femoral arteries.[23] Experimental studies elucidated the effect of obesity of increasing regional blood flow to adipose as well as non-adipose tissues.[24] Therefore, the inverse association between peripheral PWVs and BMI might be attributable to a greater effort of increasing diameters, by a compensating mechanism to minimize decreased distensibility.

Assuming that *hf*PWV indicates the stiffness of the aorta, we can assume *cf*PWV may indicate the stiffness of the aortic/proximal aortic large arteries. In this context, the strong correlation between *cf*PWV and *hf*PWV in our findings was expected, along with the common predictor variables (i.e., MAP, BMI and LDL-C).

LDL-C was a predictor variable of *hf*PWV. There is little evidence on the ability of LDL-C as a predictor of aortic stiffness measured by PWV in previous studies. However, a few clinical trials reported that statin therapy (i.e., simvastatin or atorvastatin) had a beneficial effect of decreasing aortic stiffness, suggesting that the improvement of aortic stiffness may be related to the reduction of LDL-C.[25, 26]

We found that hs-CRP level was significantly associated with *hc*PWV of the proximal large and elastic arterial stiffness. *hc*PWV was partially correlated with *hf*PWV of aortic stiffness (r=0.28). Consistently, Yasmin et al. (2004) reported hs-CRP as a predictor of aortic PWV in a healthy population.[27] Hence, we speculate that a higher level of hs-CRP in our cohort of healthy men may be related to stiffness in the carotid artery in which atherosclerosis may play a role frequently.

Our data showed that fasting insulin levels were significantly and positively associated with *fa*PWV. It was reported that, among patients with diabetes, arterial stiffness was predominantly increased in the femoral arteries.[28] Thus stiffness in the lower peripheral arteries might be the result of earlier deterioration from hyperinsulinemia than stiffness in the central arteries.

We also noted a positive association between *ba*PWV and heart rate. However, the significant association between *cf*PWV and heart rate attenuated after multivariable-adjustment. The Cardiovascular Heart Study reported that the strongest predictor of aortic stiffness measured

by *cf*PWV was heart rate in elderly men.[8] Elevated heart rate is a predictor of cardiovascular morbidity and mortality.[29, 30] Sympathetic overactivity induces fasting heart rates, which increases arterial wall stress and causes hyperinsulinemia and insulin resistance.[30, 31] Those mechanical and functional factors may involve across entire arterial tree. Hence, in this middle-aged group, the significant association between *ba*PWV and heart rate may be reasonable, because *ba*PWV consisted of combined properties of aortic and peripheral arteries.

The present study includes the cross-sectional nature of study design, which cannot distinguish between cause and effect. Our study also limits generalizability to female, elderly or other racial/ethnic subgroups. With regard to measurement errors of *cf*PWV derived from our device system, the errors may have occurred while capturing pressure waveforms because of a characteristic of participants (e.g, obesity). In fact, there was a significant difference in BMI between those >2,500 cm/sec and those ≤ 2500 cm/sec of cfPWV. Additionally, heart rate was not obtained by hand, but by the automated BP machine.

In conclusion, we found that, among healthy middle-aged white men, *cf*PWV correlated partially with *ba*PWV. *ba*PWV showed a mixed characteristic of stiffness of aortic and peripheral arteries. Each segmental PWV may provide information on vascular responses to cardiovascular risk factors according to the type of central elastic vs. peripheral muscular arteries.

4.6 PERSPECTIVES

We have shown for the first time that the correlates of PWV with cardiovascular risk factors differed by arterial segments. *ba*PWV was a combined stiffness of the central and peripheral

arteries, explained by cardiovascular risk factors to a great extent. These findings indicate that *ba*PWV may be another surrogate marker of arterial stiffness. A cross-sectional study reported that *ba*PWV was a better correlate of subclinical cardiovascular disease than *cf*PWV.[7] In this regard, *ba*PWV may be a useful tool in screening a high risk group of cardiovascular disease. This may have important implications for the potential utility of *ba*PWV measured by very simple methodology for clinical practice or large-scaled population-based epidemiological studies.

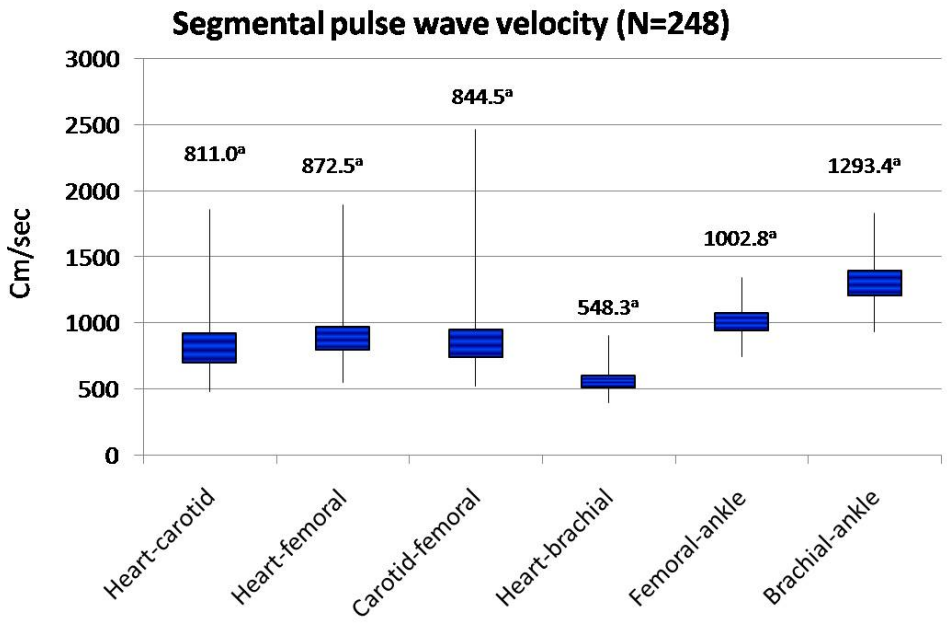

Figure 6 Distribution of segmental pulse wave velocity

[a] Median; interquartile, and range

Table 21 Basic characteristics of the study participants (N=248)

Age, years	44.9	(2.8)
BMI, kg/m^2	27.3	(3.9)
Height, cm	179.9	(6.8)
Systolic BP, mmHg	127.1	(11.0)
Diastolic BP, mmHg	77.8	(7.5)
MAP, mmHg	94.2	(8.3)
Pulse pressure, mmHg	49.3	(6.6)
Heart rate, beats/min	64.2	(9.4)
LDL-C, mg/dL	135.6	(33.4)
Triglycerides, mg/dL	122.5	(90.5 - 179.0)
HDL-C, mg/dL	48.5	(12.9)
Fasting glucose, mg/dL	99.2	(7.9)
Fasting insulin, μIU/dL	12.4	(10.2 - 17.0)
hs-CRP, mg/dL	0.9	(0.5, 1.7)
Current cigarette smoker, n (%)	18.0	(7.3)
Pack years of smoking, years	0.0	(0.0 - 1.1)
Alcohol drinking, n (%)	112	(45.2)
Exercise, n (%)	134	(54.0)

Values are means (standard deviations) or median (interquartile) for continuous variables and total number (percentages) for categorical variables

BMI = body mass index; BP = blood pressure; MAP=mean arterial pressure; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; hs-CRP = high sensitivity C-reactive protein; Exercise was defined as regular exercise ≥ three times per week.

Table 22 Age- and MAP-adjusted correlations[a] between segment-specific pulse wave velocity (n=248)

Pulse wave velocity	r					
	Heart-carotid	Heart-femoral	Carotid-femoral	Heart-brachial	Femoral-ankle	Brachial-ankle
Heart-carotid	1.00					
Heart-femoral	0.28*	1.00				
Carotid-femoral	-0.02	0.88*	1.00			
Heart-brachial	0.47*	0.16†	0.02	1.00		
Femoral-ankle	-0.09	-0.19*	-0.10	0.23	1.00	
Brachial-ankle	-0.01	0.18*	0.16†	0.15†	0.68*	1.00

[a] Partial correlation was performed.

*p<0.01, †p<0.05

MAP=mean arterial pressure

Table 23 Univariate associations[a] between pulse wave velocity and cardiovascular risk factors (N=248)

	Pulse wave velocity					
	Heart-carotid	Heart-femoral	Carotid-femoral	Heart-brachial	Femoral-ankle	Brachial-ankle
	Spearman rho					
Age, years	0.19*	0.16†	0.03	0.24*	0.02	0.09
BMI, kg/m^2	0.31*	0.27*	0.29*	0.03	0.00	0.12
Systolic BP, mmHg	0.42*	0.46*	0.40*	0.42*	0.34*	0.44*
Diastolic BP, mmHg	0.51*	0.45*	0.35*	0.53*	0.47*	0.48*
MAP, mmHg	0.50*	0.47*	0.38*	0.52*	0.44*	0.48*
Heart rate, beats/min	0.04	0.10	0.15†	-0.06	0.23*	0.27*
LDL-C, mg/dL	0.05	0.08	0.06	0.05	0.00	0.05
Triglycerides, mg/dL	0.22*	0.18*	0.16†	0.03	0.14*	0.17*
HDL-C, mg/dL	-0.11	-0.15†	-0.16†	-0.01	0.00	-0.04
Fasting glucose, mg/dL	0.11	0.08	0.15†	0.05	0.02	0.12
Fasting insulin, μIU/dL	0.23*	0.22*	0.27*	0.12	0.19§	0.22*
C-reactive protein, mg/dL	0.26*	0.16†	0.14†	0.06	0.09	0.08
Pack years of smoking, years	0.12	0.04	0.02	0.19*	0.18*	0.10
Alcohol drinker, n (%)	-0.11	-0.19*	-0.20§	-0.04	-0.13†	-0.17*
Exercise, n (%)	-0.04	-0.04	-0.14†	0.10	-0.16	-0.13†

*p<0.01, †p<0.05

Table 24 Stepwise regression analysis[a]: Associations between pulse wave velocity and cardiovascular risk factors (N=248)

	β	p-value	95% CI		R^2		β	p-value	95% CI		R^2
Heart-carotid PWV					0.25	**Heart-brachial PWV**					0.37
Age	13.58	0.001	(5.64,	21.52)		Age	4.80	0.000	(2.19,	7.41)	
MAP	8.99	0.000	(6.22,	11.75)		MAP	4.73	0.000	(3.75,	5.71)	
Heart rate	-3.75	0.005	(-6.38,	-1.12)		Heart rate	-1.36	0.000	(-2.07,	-0.65)	
Insulin	48.23	0.079	(-5.71,	102.17)		BMI	-3.72	0.000	(-5.72,	-1.71)	
hs-CRP	30.65	0.019	(5.01,	56.30)		Insulin	17.66	0.096	(-3.18,	38.50)	
						Smoking	1.10	0.020	(0.17,	2.02)	
						Exercise	18.49	0.011	(4.21,	32.76)	
Heart-femoral PWV					0.22	**Femoral-ankle PWV**					0.28
Age	8.14	0.016	(1.55,	14.73)		Age	-0.23	0.919	(-4.76,	4.30)	
MAP	6.87	0.000	(4.20,	9.54)		MAP	5.76	0.000	(4.05,	7.47)	
BMI	8.77	0.018	(1.49,	16.05)		BMI	-6.98	0.000	(- 10.63,	-3.34)	
LDL-C	0.66	0.040	(0.03,	1.29)		Insulin	40.49	0.029	(4.26,	76.72)	
Insulin	-32.53	0.197	(-82.11,	17.04)		Smoking	1.26	0.052	(-0.01,	2.54)	
Alcohol	-70.77	0.001	(-111.45,	-30.08)							
Carotid-femoral PWV					0.15	**Brachial-ankle PWV**					0.30
Age	2.93	0.638	(-9.31,	15.16)		Age	3.04	0.322	(-3.00,	9.08)	
MAP	7.37	0.002	(2.64,	12.09)		MAP	8.07	0.000	(5.89,	10.25)	
BMI	19.70	0.001	(8.07,	31.33)		Heart rate	2.08	0.017	(0.37,	3.79)	
LDL-C	0.81	0.159	(-0.32,	1.93)		BMI	-4.32	0.064	(-8.88,	0.25)	
Insulin	-62.61	0.144	(-146.85,	21.64)		Insulin	32.09	0.161	(-12.87,	77.04)	
Smoking	-2.26	0.155	(-5.37,	0.86)		Alcohol	-25.95	0.088	(-55.82,	3.92)	
Alcohol	-108.40	0.003	(-179.47,	-37.33)							

MAP = mean arterial pressure; BMI = body mass index; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; hs-CRP = high sensitivity C-reactive protein; Smoking=pack years of smoking, Alcohol=drinker ≥2 times per week [a]The stepwise regression was performed for backward removal ($p < 0.2$). In each model, we placed age, MAP, BMI, heart rate, LDL-C, triglycerides, HDL-C, glucose, insulin, hs-CRP, pack-years of smoking, alcohol drinker, and exercise into models. Triglycerides, insulin, and C-reactive protein were log-transformed

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5.0 DISCUSSION

5.1 SIGNIFICANCE OF FINDINGS AND FUTURE RESEARCH

The three articles demonstrated that: 1) Among middle-aged men, Koreans in South Korea had a significantly higher level of carotid IMT than the Japanese in Japan.[109] 2) Of polyunsaturated fatty acids, the proportion of DHA in serum was significantly and inversely associated with unfavorable levels of lipoprotein subclasses in white men; the proportion of LA in serum were significantly and inversely associated with unfavorable levels of lipoprotein subclasses in all three population groups of Japanese, Japanese American, and U.S. white men. 3) Associations between PWV and cardiovascular risk factors differed by arterial segments, i.e., the central elastic vs. the peripheral muscular arteries. Brachial-ankle PWV had a mixed characteristic of the stiffness of the central elastic and peripheral muscular arteries and shared common predictor variables with carotid-femoral PWV and heart-femoral PWV (i.e., the stiffness of the central elastic arteries) as well as with femoral-ankle PWV (i.e., the stiffness of the peripheral muscular arteries).

The significantly higher level of carotid IMT in Korean men than Japanese men may indicate that middle-aged Korean men may continue to be at more increased risk for CHD than Japanese men at least over the near future. In addition, the observation may be in accordance with the fact from the vital statistics that Korean men have higher stroke mortality than the

Japanese men.[110] Generally, the difference in risk for CHD by ethnicity can be explained by variation in the distribution of coronary risk factors.[13] However, our finding showed that the distribution of coronary risk factors in Korean men was more favorable than or similar to those in Japanese men. Moreover, the coronary risk factors hardly explained the higher IMT in the Korean men than in the Japanese men. In this context, other factors than the factors examined in the study may be explaining factors for the difference in carotid IMT between the two population groups. Therefore, a study on the exploration of the explaining factors is needed, which can provide a preventive strategy for reducing the risk for CHD in South Korea.

Differences in fat intake quality (e.g., marine-derived n-3 fatty acids) between two populations may differentiate the difference in carotid IMT. Koreans consume relatively less fish of 60g per day than the Japanese population who has been well known to uniquely have high fish consumption, 120g per day.[111, 112] Hino et al. reported that n-3 fatty acids intakes (e.g. eicosapentaenoic acid or docosahexaenoic acid) in fish had an inverse association with carotid IMT.[113] The ERA-JUMP study reported that the serum proportion of marine-derived n-3 fatty acids had an inverse association with carotid IMT.[6] Thus, high fish consumption, rich in EPA and DHA, may be protective against carotid atherosclerosis, which might in part explain the higher carotid IMT in Koreans than the Japanese. Therefore, we need to examine if marine-derived n-3 fatty acids are an explaining factor for the difference in carotid IMT between Korean and Japanese men in future research.

Of polyunsaturated fatty acids, a higher proportion of LA, a primary index of n-6 PUFA was significantly and inversely associated with unfavorable levels of lipoprotein subclasses in all three population groups of Japanese, Japanese American, and U.S. white men. The significant associations were independent of all potential covariates and n-3 PUFA. Our finding indicates

that a higher proportion of serum linoleic acid have anti-atherogenic effect through the action of the modification of atherogenic lipoproteins.

Very few clinical trials reported the effects of enriched LA diet on atherogenic lipoproteins. One study reported a significant effect of enriched LA diet on small LDL particle concentration compared to enriched stearic diet; the other study reported non-significant effects on small LDL and large HDL particle concentrations. However, it is unclear about whether the effects of enriched LA in those clinical trials were the result of the substitution of saturated fatty acids or other fatty acids (i.e., monounsaturated fatty acids, or n-3 fatty acids). Furthermore, it needs to examine in clinical trials that the compliance of enriched LA diet was achieved by using circulating levels because the circulating levels reflect the LA intake. Therefore, a well-organized randomized clinical trial needs to clarify the effects of enriched LA diet on atherogenic lipoproteins in future research.

Correlates of pulse wave velocity with cardiovascular risk factors differed according to arterial segments, i.e., central elastic vs. peripheral muscular arteries. The mean arterial pressure was a primary independent correlate of all the segmental PWVs. Age, body mass index, LDL-C, and alcohol drinking were associated with the PWVs of the segments of the central elastic arteries; fasting insulin was associated with the PWV of the segments of the peripheral muscular arteries. Fasting insulin and alcohol drinking were associated with the PWV of mixed segments of the central and peripheral arteries. Thus, this study highlights that carotid-femoral PWV and brachial-ankle PWV may be differentially influenced by pathology associated with cardiovascular risk factors in different arterial segments. Carotid-femoral PWV is a measure of the stiffness of aorta or proximal aortic arteries having central elastic properties, while brachial-ankle PWV is a measure of stiffness of combination of central elastic and peripheral muscular

arteries. In our findings, brachial-ankle PWV was more likely to be explained by cardiovascular risk factors than carotid-ankle PWV (R^2=0.11 vs. R^2=0.33). A cross-sectional study reported that brachial-ankle PWV was better correlate of early atherosclerosis (i.e., prevalence of coronary calcification) in postmenopausal women than carotid-femoral PWV. However, the study measured the two indices of arterial stiffness by using different methodologies and different devices. Therefore, future research needs to determine if brachial-ankle PWV is a better correlate of early atherosclerosis than carotid-femoral PWV in the same device.

5.2 LIMITATIONS OF STUDY

The ERA-JUMP study provided a proper cohort for the achievement of the three specific aims in the dissertation. However, the study has several limitations. First, a cross-sectional nature of study design has difficulty in inferring a causal relationship between predictor variables and outcome variables in each study. Second, the study population had a certain range of age group (i.e., 40-49 years) and only male population, which limited generalizability to elderly and female population groups. However, the selection of the population aged 40-49 may help provide a more important epidemiologic tool for predicting future coronary risk when comparing two population groups (i.e., Korean and Japanese men) who adopted westernization of lifestyle.

The study on carotid IMT scores between Korean and Japanese men had a limitation on small sample size, especially in Korean men (n=102). Even though the characteristics of the Korean men in the distribution of CHD risk factors and carotid IMT scores were not much different from those in previous studies, our findings on associations between carotid IMT and CHD risk factors showed a weaker pattern for a few risk factors (i.e., systolic blood pressure,

body mass index, or LDL-C) in Korean men compared to Japanese men. Additionally, the Korean and Japanese research sites used different ultrasonography machines (Titan (Sonosite) high-resolution ultrasound system with a 10.5-MHz linear array vs. Toshiba 140A scanner equipped with a 7.5-MHz linear array) for measuring carotid IMT. However, the difference between machines was no greater than the variation between sonographers when evaluating between-machine differences.

The study on the associations between PWV and cardiovascular risk factors had a limitation on the inference of a causal relationship. We found that mean arterial pressure was primary independent correlate of all the segmental PWVs. However, it is uncertain from our study design whether higher mean arterial pressure is the result of higher PWV or the cause of higher PWV. This uncertainty holds for other cardiovascular risk factors. Additionally, the narrow range of the age group may limit the results of the study to elderly groups. We found that carotid-femoral PWV relatively weakly correlated with brachial-ankle PWV compared to findings in previous studies conducting more wide range of aged group.[114] Therefore, future research needs to examine the age-specific correlations between the two PWVs, using general population.

6.0 APPLICATION TO PUBLIC HEALTH

CVD is the leading cause of mortality worldwide, responsible for one-third of all deaths.[1] In the U.S., age-adjusted mortality rates from CHD started to decline during the 1960s and decreased steadily from 1980 through 2002[115]; however, CHD remains the leading cause of death. In most Asian countries (e.g., Japan, Hong Kong, and Singapore), the CHD mortality rates showed an increasing trend until approximately 1970.[116-122] Thereafter, it has shown a decline similar to that observed in Western countries including U.S., England and Wales. In South Korea, aged-adjusted CHD mortality increased until 1990 and thereafter leveled off.[18]

Age-adjusted mortality rates may reflect neither trends in CHD mortality in young and middle-aged adults, nor recent trends in CHD mortality. In fact, mortality rates from CHD among U.S. adults age 35 to 54 years decreased from 1980 until 2000, but slightly increased from 2000 until 2002.[115] CHD mortality among Korean men age 35-44 years since the late 1980s showed a rapidly increasing trend.[4] Higher carotid IMT in Korean men than in Japanese men (aged 40 to 49) is likely to reflect a recent trend of the risk for CHD or stroke in South Korea. In this regard, our findings of the first article have important implications for public health: 1) we can provide scientific information imperative to addressing a health-related problem (i.e., an increasing risk for CVD) in the prevention of CVD in South Korea. 2) we can address the necessity of efforts to set up a surveillance system for the incidence of CVD. In addition, according to the U.S. health statistics, leveled-off or slightly increasing trend in mortality rates

from CHD among young or middle aged adults may be in accordance with considerable increases in several risk factors, including obesity, diabetes and hypertension in younger adults.[115] Since a major goal of epidemiology is to identify subgroups in the population who are at high risk for disease,[123] a high-risk group of Korean middle-aged men may be those having obesity, diabetes, and hypertension among middle-aged men based on the U.S. statistics. Therefore, efforts on the modification of those unfavorable risk factors in the high risk group may serve as a potential strategy for reducing CHD risk in the future in South Korea.

The quality of dietary fat, but not the quantity, matters. Many epidemiological and clinical studies delineated cardioprotective effects of n-3 and n-6 fatty acids, including antiarrhythmic, hypolipidemic, and antithrombotic effects.[124] With regard to hypolipidemic effects, the n-3 fatty acids, especially EPA and DHA, are well-known as having hypotriglyceridemic effects. Instead, they were reported to slightly increase LDL-C.[124, 125] Several clinical trials confirmed the beneficial effects of EPA and DHA on small LDL particle concentration that is another feature for determining CHD risk. Our finding, the linear inverse trend of the association between DHA (or DHA+EPA+DPA) and total/small LDL particle concentration in U.S. white men, indicates that U.S. people can still benefit through increasing fish intake in the U.S. diet because they consume less than a suggested amount of EPA and DHA in a population level. Current mean estimated intake of marine-derived n-3 PUFA in the U.S. is $\leq$ 0.1 % of total energy that is much lower than a recommended intake of 0.25% of total energy (0.55 g/day at 2000 kcal).[38] Therefore, it is needed to promote an enriched diet in fish or fish oil supplements in the U.S in the purpose of lipoprotein modification. On the other hand, a primary index of n-6 PUFA, LA is the predominant dietary fatty acids regulating LDL-C metabolism when saturated fatty acids were replaced with LA. Our finding, the linear trend of the inverse

association between LA proportion and atherogenic lipoproteins (i.e., total/small LDL particles and large VLDL particles), indicates that, regardless of marine-derived n-3, a relative increase in LA proportion (%) of total fat to a decrease in saturated fatty acid proportion, can contribute to a reduction in atherogenic lipoproteins. In the U.S., a mean intake of LA is estimated to be 16 g/day for adult men (corresponding to approximately 6.4% of total energy) that is close to the adequate intake level (17g/day for adult men), based on the U.S. Department of Agriculture's Nationwide Food Consumption Survey.[126] Therefore, the substitution of saturated fatty acids for enriched LA within a total energy should be continuously promoted in the U.S. diet as well as in Japanese diet, which can lead to the reduction in atherogenic lipoproteins.

We have shown for the first time that the correlates of PWV with cardiovascular risk factors differed by arterial segments. *ba*PWV was a combined stiffness of the central and peripheral arteries, explained by cardiovascular risk factors to a great extent. These findings indicate that *ba*PWV may be another useful index of arterial stiffness. A cross-sectional study reported that *ba*PWV was a better correlate of subclinical cardiovascular disease than *cf*PWV.[91] In this regard, *ba*PWV may be a useful tool in screening a high risk group of cardiovascular disease. This may have important implications for the potential utility of *ba*PWV measured by very simple methodology for clinical practice or large-scaled population-based epidemiological studies.

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