HEALTH DISPARITIES BETWEEN WHITES AND JAPANESE IN MEASURES OF DIABETES AND SUBCLINICAL ATHEROSCLEROSIS IN AN INTERNATIONAL POPULATION-BASED STUDY

by

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ABSTRACT

This dissertation includes three manuscripts focusing on health disparities between whites and Japanese with regard to measures of diabetes and subclinical atherosclerosis in the EBCT and Risk Factor Assessment among Japanese and U.S. Men in the Post World War II Birth Cohort (ERA JUMP) study. The first manuscript compares markers of insulin resistance and insulin secretion between white men in the United States and Japanese men in Japan, adjusting for visceral adipose tissue and other covariates. Whites had significantly higher HOMA-IR, HOMA-β%, and disposition index (DI) than Japanese in Japan. The better compensation of insulin resistance by increased insulin secretion in whites as shown by higher DI may partly explain lower susceptibility of whites to developing type 2 diabetes than Japanese in Japan at similar levels of body-mass index.

The second manuscript compares progression of intima-media thickness of the carotid artery (CIMT) between middle-aged Japanese American men and white men, adjusting for baseline cardiovascular risk factors. Japanese Americans had greater progression of CIMT than whites. The third manuscript compares progression of coronary artery calcium (CAC) between Japanese American men and white men. Japanese Americans had similar progression of CAC as whites.

This work contributes uniquely to public health significance. Future studies exploring reasons of poorer compensation of increasing insulin resistance by enhanced insulin secretion in Japanese in Japan may help to prevent earlier onset of type 2 diabetes in Japanese than whites at a given level of BMI. The second and third manuscripts identify increasing subclinical atherosclerosis in Japanese Americans that may translate into increased risk of CHD in Japanese Americans in the future. The second and third manuscripts identify Japanese American as a target group for prevention of CHD.

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1.0 INTRODUCTION

The focus of this work is to examine health disparities between whites and Japanese with regard to measures of diabetes and subclinical atherosclerosis. The chapter that follows begins by describing the epidemiology of cardiovascular disease (CVD). A special section is devoted to atherosclerosis and its assessment using coronary artery calcium (CAC) and intima-media thickness of the carotid artery (CIMT). CAC and CIMT are considered surrogate markers of atherosclerosis. The author also presents a special section on markers of insulin resistance and insulin secretion because the epidemic of obesity places individuals at risk of increased insulin resistance which further enhances the risk of atherosclerosis and CHD.

1.1 CARDIOVASCULAR DISEASE

1.1.1 Epidemiology of Cardiovascular Disease

In 2011, the World Health Organization reported CVD as the leading cause of death worldwide (1). The Global Burden of Disease Study (GBDS) reported that CVD accounted for 11.8% of disability adjusted life years (DALYs) worldwide in 2010. According to GBDS, CHD was the primary cause of DALYs worldwide, accounting for 5.2% of DALYs (2). CVD is the leading cause of death and disability in the United States (US) leading to 1 in every 3 deaths in 2010 (3).

CHD alone caused 1in 6 deaths in the US in 2010 (4). For 2010, the total direct and indirect cost of CVD in the US is estimated to be $315.5 billion dollars (3).

CVD occurs in different geographic patterns. Western countries experience more CHD mortality while Asian countries have higher rates of stroke mortality, this is especially true in comparison to the East Asian countries (5). For example, the recent update on cardiovascular disease by the American Heart Association reported age-adjusted CHD mortality as 132.4 per 100,000 in the US for 2010 compared to 47.0 in Japan for 2011 (4). On the other hand, in the same years, stroke mortality per 100,000 was 21.9 in the US and 49.0 in Japan.

There are also racial/ethnic differences in CVD occurrence in the US. The national Reasons for Geographic and Racial Differences in Stroke (REGARDS) study reported two times higher risk of fatal CHD in blacks than whites, though not statistically higher in multivariable analyses (6). Similarly, the REGARDS study reported higher age-sex-adjusted black/white stroke incidence rate ratio (IRR). The black/white $_{IRR}$ was 1.51 (95% confidence interval [CI], 1.26-1.81), but for ages 45-54 years it was even higher 4.02 (95% CI, 1.23-13.11) (7). Moreover, CVD affects blacks at an earlier age than whites (8).

The traditional risk factors for CVD are: age, gender, family history, total-cholesterol, high blood pressure, diabetes, smoking, obesity, and physical inactivity (4). The population attributable fraction for CVD in the US for 2010 was: 40.6% for high blood pressure, 13.7% for smoking, 13.2% for poor diet, 11.9% for physical inactivity, and 8.8% for abnormal blood glucose (4). Other than age, gender and family history, most CVD risk factors are modifiable. Moreover, premature CVD deaths are largely preventable through effective interventions that target risk factors: tobacco use, unhealthy diet, and regular physical activity.

1.1.2 Insulin Resistance as a Risk Factor for CVD

Type 2 diabetes mellitus (T2D) is an independent risk factor for CVD (9). Insulin resistance, the underlying pathology of T2D, itself has long been debated as an independent risk factor for CVD. Mechanistically, insulin resistance can promote atherosclerosis through increased glucose and insulin, and related dyslipidemia, hypertension, and inflammation (10, 11). Recent meta-analyses have shown that elevated glucose and insulin in participants without diabetes is associated with an increased CVD risk (9, 12). In line with this finding, a recent meta-analysis in subjects without diabetes reported the pooled relative risk of CVD for 1 SD (2.23 units) increase in HOMA-IR as 1.25 (95% CI(7), 1.16-1.35; I^2:0.0%) (13). This meta-analysis reported the pooled relative risk of CHD comparing the highest to the lowest quartile for HOMA-IR as 1.64 (95% CI, 1.35- 2.00; I^2: 0%) and for the 1 SD (2.23 units) increase as 1.46 (95% CI, 1.26-1.69; I^2:0.0%) (13).

1.2 ATHEROSCLEROSIS AND ITS ASSESSMENT

1.2.1 Pathophysiology of CVD

The major underlying pathology of CVD is atherosclerosis (4). Atherosclerosis is a slowly progressing inflammatory disease that begins as early as childhood and has clinical manifestation in middle to late adulthood (14). The lesions of atherosclerosis occur primarily in large and medium sized muscular arteries of the cardiovascular system, mainly the arteries of the heart (the aorta and coronary arteries), brain (cerebral arteries), and lower extremities (femoral, iliac

or popliteal arteries) (14). Advanced lesions of atherosclerosis are referred to as plaque and can lead to impaired circulation in heart, brain, and legs resulting in infarction (14). The resulting infarction may manifest in clinical cardiovascular events in the form of angina, myocardial infarction, transient ischemic attack, stroke, or infarction and intermittent claudication in the lower extremity arteries.

The earliest atherosclerotic lesions begin in infancy and young children as fatty streaks in response to injury to the endothelium (the innermost layer of arteries). The fatty streaks are comprised of lipid-laden macrophages, white blood cells (WBCs), and smooth muscle cells in sub-endothelial space and are accompanied by thrombosis and fibrinolysis on the endothelial layer (**Figure 1-1**). The fatty streaks progress to intermediate and advanced lesions with formation of fibrous cap, which walls off the lesion from the lumen of the artery. The fibrous cap represents a type of healing to injury. The fibrous cap covers the atherosclerotic lesion comprising of WBCs, lipids, and debris, which may form a necrotic core (**Figure 1-2**).

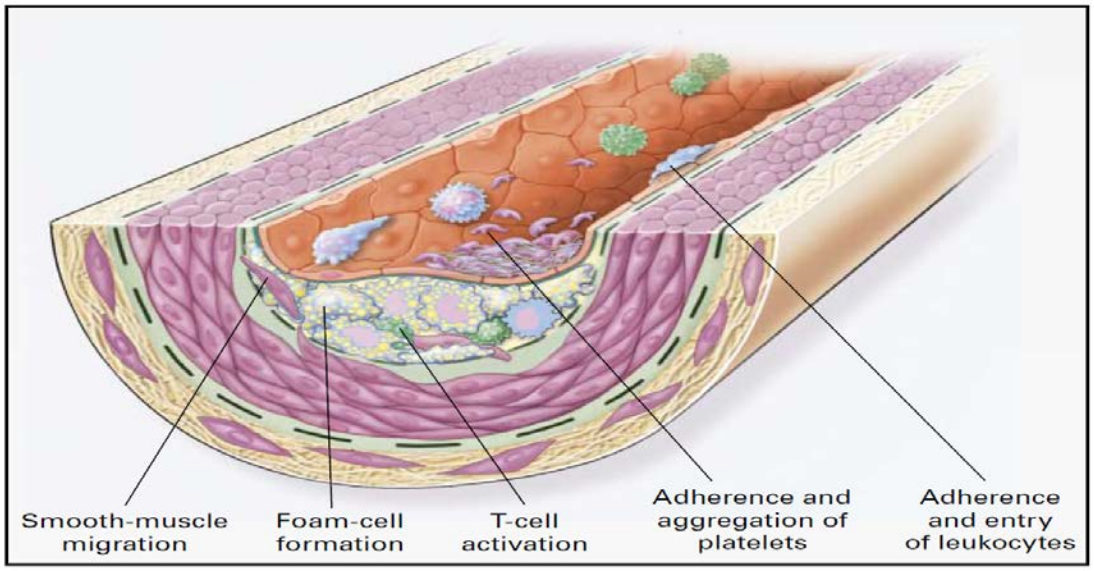

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Figure 1-1 Fatty-streak formation in atherosclerosis

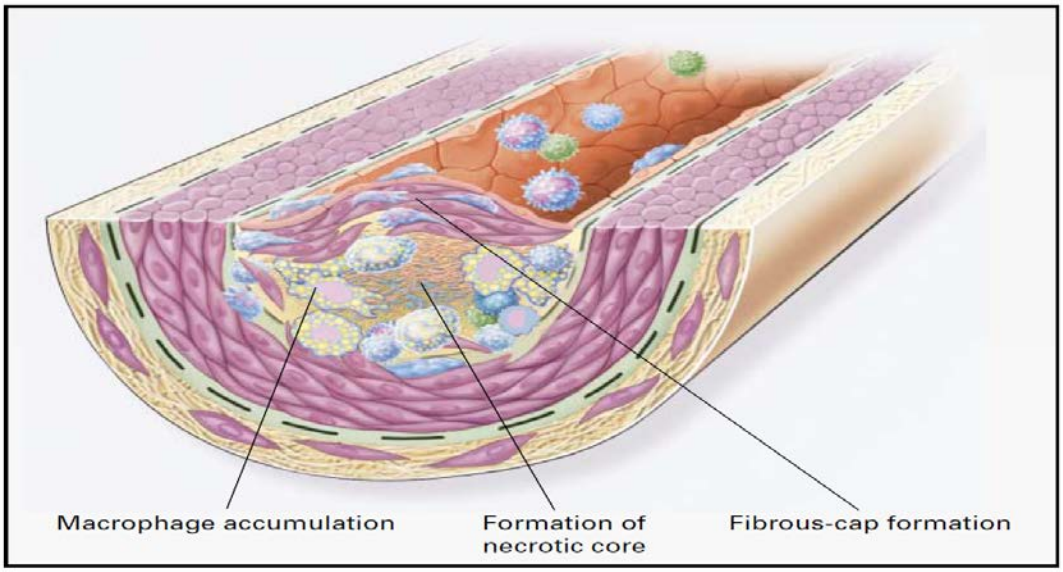

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Figure 1-2 Advance plaque formation in atherosclerosis

1.2.2 Biomarker of atherosclerosis

Risk assessment using risk scores (generated from traditional risk factors) such as the Framingham Risk Score (FRS), the most commonly used score in the US to predict 10-year risk of CVD, is considered an important first step in selecting most appropriate candidates for drug therapy to reduce risk of CVD. However, the FRS has certain limitations in predicting CVD risk (15). Firstly, it classifies around 75% of the individuals into the low-or intermediate risk group; this group presents with around 60% of clinical CVD. Secondly, significant weight is given to the age of an individual. This may not adequately predict risk in younger population, women, or other ethnicities than whites. Therefore, in the past two decades the role of measures of subclinical vascular disease including CAC and CIMT has been explored to improve the prediction of CVD in low-or intermediate risk groups according to FRS. Also, studying

subclinical atherosclerosis as a surrogate marker of clinical events leads to less follow-up in studies thus provides economic benefits (16).

1.2.2.1 Coronary artery Calcium

Coronary artery calcium is a direct measure of atherosclerosis in arteries (14). It occurs in small amount in the early lesions of atherosclerosis appearing in the second and third decade of life (14). CAC is found more frequently in advanced lesions and in older age (14). CAC scoring may be able to globally define a patient's risk of CHD because of its strong association with total coronary atherosclerotic burden (17)

CAC as a surrogate marker of CHD is supported by 1) its association with traditional risk factors (18) (19), 2) its positive and independent association with CHD above and beyond traditional risk factors in asymptomatic individuals (20, 21). Also, individuals with no CAC have very low risk of CHD (22).

CAC is measured using computed tomography: electron beam computed tomography (EBCT) or multi-detector computed tomography (MDCT) (23). EBCT allows faster imaging, has less motion artifacts, and lower radiation exposure. Advantages of MDCT are its higher spatial resolution, less noise, and cheaper cost.

CAC is defined as hyper-attenuating lesion >130 Hounsefield units with an area of ≥ 3 pixels (23). CAC is quantified using Agatson unit (AU) score or calcium volume score (CVS) (24). The AU score is calculated by multiplying the lesion area (mm^2) by a density factor (between 1 and 4). In contrast to AU, the CVS represents an actual volume of CAC (25).

In 2013, CAC was endorsed by the American College of Cardiology /American Heart Association with a class IIb (benefits ≥ risk, additional studies with broad objective required; additional registry data would be useful) recommendation for assessment of cardiovascular risk

in asymptomatic adults with intermediate risk (6-20%) of cardiovascular risk (26). CAC assessment in clinical practice is recommended only when a risk-based treatment decision is still uncertain after quantitative risk assessment using the FRS.

Progression of CAC

Progression of CAC is associated with increased CHD risk and all-cause mortality (27, 28). Progression can be quantified as absolute change in either AU or CVS score or as percentage relative change (23). However, currently there is no standard methodology for assessing CAC progression, or a definition of what clinically meaningful progression is (23). Progression of CAC ranges from 20% to 30% per year in subjects with average Framingham risk (23). The following studies evaluated racial difference in progression of CAC

Table 1-1 Studies comparing progression CAC among races

Study/Follow-up	Author/ Year/ Sample Size	Age	Results
South Bay Heart Watch (29) 7 years	Kawakubo M et al, 2005 (1289 non-population-based participants with ≥ 10% risk of developing coronary heart disease; whites, 1067; blacks, 72; API, 76; Hispanics, 74)	White: 63.9 (7.6) Black: 53.9 (8.0) API: 62.3 (7.2) Hispanics: 60.5 (7.3)	Progression of CAC was significantly greater in whites than in AA and Hispanics: Black, -0.23 (*P*=0.0003); API, -0.02 (*P*=0.73); Hispanic, -0.23 (*P*=0.0003*)*
Multi-Ethnic Study of Atherosclerosis (30) 2.4 years	Kronmal RA et al, 2007 (5756 population-based participants whites, 2302; Chinese= 691; blacks, 1554; Hispanic, 1209)		Among 2948 participants without detectable CAC at baseline, incident CAC was significantly higher in whites than Chinese: Chinese:0.61 (*P*=0.002); black, 0.9 (*P*=0.28); Hispanic, 0.9 (*P*=0.31) Among 2808 participants with detectable CAC at baseline, change of CAC was greatest in whites compared to other races: Chinese:-9.4 (*P*=0.024); black, -6.1 (*P*=0.04); Hispanic, -8.2 (*P*=0.01)
Prospective Army Coronary Calcium Rescan Study (31) 4.2 years	Taylor AJ et al, 2008 (200 Army volunteers; whites, 168; blacks, 32)	White: 47.9 (2.7) Black: 47.3 (2.8)	No significant difference in progression of CAC between the two races
API, Asian/Pacific Islander; CAC, coronary artery calcium			

1.2.2.2 Intima-media thickness of the carotid artery

CIMT is a commonly used image-based measure shown to be an important biomarker of subclinical atherosclerosis and therefore is widely utilized as a surrogate marker of CVD in various observational studies and randomized clinical trials (32). Moreover, it is a non-invasive, safe, painless, quick, relatively inexpensive, and reproducible measure (32). The surrogacy of CIMT as a biomarker for CVD is supported by 1) association of CIMT with cardiovascular risk factors such as age, hypertension, diabetes, and C-reactive protein (33-35) and 2) association of CIMT with risk of CVD independent of cardiovascular risk factors (36, 37). Although CIMT is associated independently with CVD, in the literature it has not consistently lead to incremental prognostic value over traditional risk factors. Therefore, ACC/AHA does not currently recommend routine use of CIMT in clinical practice for risk assessment for the first atherosclerotic cardiovascular disease in asymptomatic adults with intermediate CVD risk (6-20%) by the FRS (26).

Measurement of CIMT

CIMT is commonly measured using B-mode ultrasound. Image are obtained for the distal end of the common carotid artery (CCA), the carotid bulb including the bifurcation (CB), and the internal carotid artery (ICA) or sometimes only the CCA because of ease of measurement and lowest measurement variability. The superficial and stationary course of the carotid artery enables ultrasound examinations to be performed with relative ease (32). Carotid ultrasound imaging is generally performed using a linear-array transducer with a fundamental frequency of 7-10 MHz and typical pixel size of approximately 0.11 mm at 4 cm depth (38). CIMT is measured best with images obtained at the end of diastole, with subject in a supine position with head slightly hyperextended and rotated to the opposite side (38).

CIMT measurement represents the inner two layers of the carotid artery - intima (tunica intima) and media (tunica media). The intima consists of endothelial layer and internal elastic lamina**.** The endothelial layer is a monolayer of cells which regulates vascular tone, inflammation, blood clotting and, absorption of substances from blood (32). The media is the muscular layer mainly consisting of longitudinal smooth muscle cells surrounded by connective tissue; containing elastic lamina that provides elastic property of vessels (32).

In ultrasound, the CIMT appears as 2 parallel echogenic line pattern; consisting of: the lumen-intima and media-adventitia interfaces separated by hypoechoic space. The distance between theses interfaces represent the intima and media (**Figure 1-3**). The ultrasound images are read most frequently using automatic or semi-automatic software (39).

CIMT images are obtained from both the near wall (closest to the ultrasound transducer) and the far wall (furthest from the ultrasound transducer) (39). Historically, the most reported CIMT measure has been mean CIMT. The mean CIMT is the average of all mean CIMTs: the CCA, the ICA, and the CB. The mean maximum CIMT is the average of maximum values of all the segments (39). When plaques are present in a segment, the maximum values are the maximum height of the plaque (39). Therefore, mean maximum measure is more heavily weighted toward plaque (39). Maximum CCA CIMT is being used more because of ease of measurement and reliability of measurement (32). However, loss of potential information can occur on this surrogate marker of atherosclerosis since plaque is more likely to develop in CB/ICA area where vessels bifurcate and area of turbulent flow and low shear stress (32). With well-trained ultra-sonographers and readers, CIMT has been shown to be highly accurate with excellent inter-test and inter-observer reproducibility primarily in research settings (40).

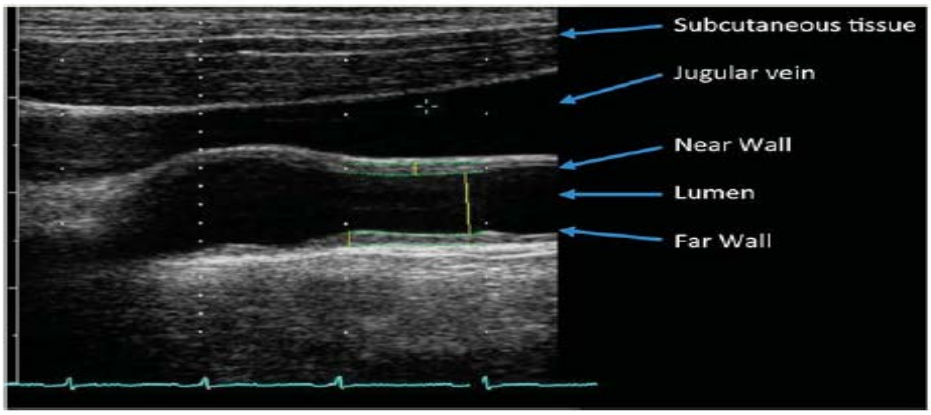

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Figure 1-3 Ultrasound image showing measurement of CIMT

In healthy, middle aged adults, CIMT in CCA generally ranges from 0.6-0.7 mm (41). CIMT in the CB is generally thicker and resembles CIMT of the ICA. CIMT increases with age and is generally thicker in men than in women and in Blacks than in whites (41).

Progression of CIMT

The rate of change of CIMT over time may be considered a reflection of how atherosclerosis develops over time (39). Progression of CIMT is calculated as an absolute yearly rate of progression (42). In health individuals, epidemiological studies demonstrate that typically CIMT progresses from 0.001 to 0.03 mm/year for mean CIMT and 0.001 to 0.065 mm/year for mean maximum CIMT (42). Progression of CIMT is associated negatively with baseline CIMT (43, 44). It is reported to be linear in men and curvilinear in women, due to the hormonal changes in women during or after menopause (45). It is also utilized as surrogate marker of CVD (42). In population-based studies, the association between progression of CIMT and CVD among healthy subjects is still under debate. A recent meta-analysis by the PROG-IMT project (individual progression of carotid intima-media thickness as a surrogate of vascular risk) found a significant

positive association between mean of the two ultrasound measures of CIMT and CVD but not between progression of CIMT and CVD (42). For mean CIMT of the two ultrasound measures on average 7.0 years apart, the hazard ratio (HR) per one SD increase for CHD and stroke was 1.22 (1.14-1.30) and 1.2 (1.09-1.35), respectively. The baseline CIMT is significantly and positively associated with CHD independent of cardiovascular risk factors (36). However, the slow progression of atherosclerosis and difficulty in detecting lower rates of progression in healthy individuals may have led to no association between progression of CIMT and CHD and not between mean of the two ultrasound measures and CHD the PROG-IMT meta-analysis (46). The association between progression of CIMT and CHD in the PROG-IMT meta-analysis may have also been underestimated due to introduction of survivor selection bias because the participants with previous cardiovascular events or death due to cardiovascular disease were excluded. Also, because calculating mean reduces error while the difference or change compounds it, founding no association between progression of CIMT and CHD and not between means of the two ultrasound measures and CHD could have occurred in the PROG-IMT meta-analysis. The use of progression of CIMT for CHD prediction thus requires further validation.

Racial Differences in Progression of IMT

Only a few studies have compared racial differences in progression of IMT; this was limited to white and black races. The Bogalusa Heart study compared progression of IMT between whites and blacks (44, 47). In the BHS, difference in progression of CIMT was not significant between black men (0.027 (0.016, 0.03) and white men 0.019 (0.013-0.025)) aged 29 to 35 years at baseline and followed for around 6 years. The Atherosclerosis Risk in the Community study compared progression of IMT between 15,792 white and black participants aged 45 to 64 years at baseline and who were followed for an average of 9 years. In the ARIC study, the baseline

mean common carotid CIMT and mean progression of CIMT were 688 μm and 655 μm, and 7.4 μm/year and 8.6 μm/year in white men and black men, respectively. The difference in progression of CIMT was not statistically significant between white men and blacks men.

1.3 MARKERS RELATED TO INSULIN RESISTANCE AND INSULIN SECRETION

Insulin is an anabolic hormone secreted by the β-cells of the pancreas. It promotes storage of substrates in fat, muscle, and the liver by stimulating lipid storage, glycogen, and protein synthesis, while inhibiting lipolysis, glycogenolysis, gluconeogenesis, and protein breakdown (48)

Insulin resistance is decrease in insulin-mediated glucose disposal in insulin-sensitive tissues mainly adipose tissue, skeletal muscles, and the liver that is accompanied by increased hepatic glucose production (48). In healthy individuals, increasing insulin resistance is compensated by enhanced insulin secretion by the β-cells in order to maintain glucose concentrations in normal range (49). When insulin secretion is no longer able to maintain glucose in normal range, impaired glucose tolerance develops and eventually, with β-cell exhaustion, T2D manifests (49).

Peripheral insulin resistance is the reduced capacity to utilized glucose mainly in skeletal muscles (48). At the level of adipose tissue, the main feature of insulin resistance is increased lipolysis and at the level of the liver (hepatic insulin resistance) it is the increased glucose production via impaired inhibition of glycogenolysis and stimulation of gluconeogenesis (48).

There is a wide range of normality for insulin resistance **(Figure 1-4)**. No definition of limits of normality with an appropriate measure of dispersion has been formulated so far (50). In

the state of insulin resistance the insulin/glucose disposal curve is shifted to the right, i.e. there is less glucose disposal for any given concentration of insulin **(Figure 1-5)** (50). This is usually accompanied by reduction in maximal velocity (V_{max}) of insulin action **(Figure 1-5)** (50).

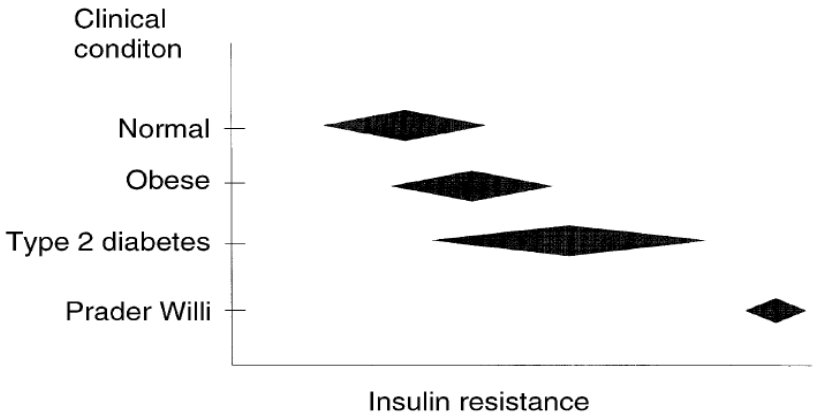

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Figure 1-4 Schematic representation of insulin resistance in humans

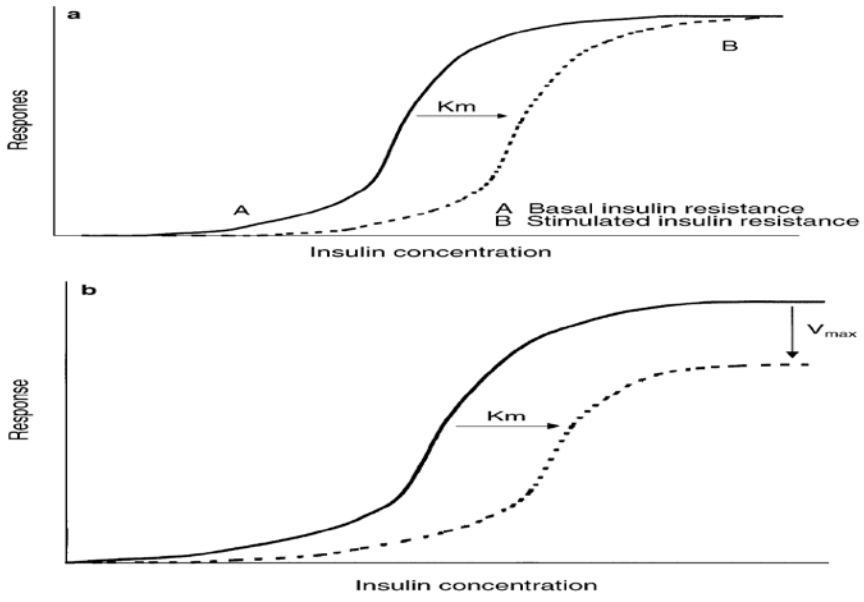

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Figure 1-5 Classical view of insulin-dose response curve

1.3.1 Measurement of Insulin Resistance and Insulin Secretion

Insulin resistance and insulin secretion are measured using the formal tests and surrogate indices. The formal tests include hyperinsulinemic euglycemic clamp, frequently sampled intravenous glucose tolerance test (FSIVGT), and insulin suppression test. The surrogate indices include the measures of insulin resistance and insulin secretion derived from oral glucose tolerance test (OGTT) or fasting glucose and insulin. The fasting glucose and insulin obtained estimation of insulin resistance and insulin secretion -homeostasis model assessment- insulin resistance (HOMA-IR) and HOMA-β%, respectively, are the most widely used markers in population-based epidemiological studies (51). The fasting (basal) and stimulated state tests measure two different aspects of glucose and insulin homeostasis. Though there is a positive relationship between basal and stimulated insulin, there are some differences (e.g., in the basal state there is no influence of activity from recent glucose variation) (52).

Hyperinsulinemic euglycemic clamp, which provides highly reproducible (coefficient of variation [CV] 6-10%) steady-state estimates of insulin-mediated glucose clearance in peripheral tissues (muscles), is considered the gold standard (53). It involves infusion of exogenous insulin at a constant rate along with variable rate of glucose sufficient to maintain blood glucose concentration between 5 and 5.5mmol/l. The infused insulin suppresses hepatic glucose output once a steady state is reached in the last 20-30 minutes of 2-3 hours of the procedure. Once a steady-state is reached, the degree of insulin resistance is inversely related to the amount of glucose necessary to maintain the glucose levels between 5 and 5.5mmol/l. The insulin sensitivity index measured using euglycemic clamp (inverse of insulin resistance) is denoted by metabolic clearance rate or M value and is expressed in mg/kg/min. Using the clamp, The β-cell function is measured using a modified form referred to as hyperglycemic clamp (53).

FSIVGT involves injection of glucose at baseline and insulin or tolbutamide injection at ~20 minutes (54, 55). Samples are taken frequently (12-35 times) over 3 hours. The response of glucose levels to the injection is then modeled using a computer assisted minimal-model program. It provides assessment of hepatic and peripheral insulin sensitivity in form of S_I (min/μU/μl). Further, FSIVGT can be used to obtain both the first-phase (acute insulin response [AIR]) and second-phase of insulin secretion .The CV for FSIGT is 14-30%. However, the above-mentioned tests are cumbersome and impractical to use in large epidemiological studies.

During OGTT a fasting participant is given a 75 g oral dose of glucose dissolved in water. For research purposes, blood glucose concentration is then usually measured at 0, 30, 60, 90, and 120 minutes (53). The glucose concentration in normal subjects reaches a peak after 30-60 min and return to baseline after 120 minutes. The Matsuda index for insulin sensitivity from OGTT is calculated as:

10^4/ [fasting glucose (mg/dl)*(fasting insulin (μU/ml)]*(mean glucose*mean insulin)$^{0.5}$]

The Matsuda index takes into account both the hepatic and peripheral sensitivity. The ratio of change in glucose over the first 30 min of OGTT (Δ30min insulin/ Δ30 min glucose) is a measure of insulin secretion derived from OGTT referred as insulinogenic index. The indices of insulin sensitivity derived from OGTT are influenced by the rate of gastric emptying as well as post-absorptive glucose handling. Therefore, there is a large intra-individual variation in the results obtained using OGTT.

1.3.2 HOMA-IR

The fasting state in healthy subjects is a steady state with glucose levels tightly maintained between normal values as a result of insulin action on hepatic glucose production, which equals whole-body glucose disposal. HOMA-IR is based on the principle that any given combination of β-cell deficiency and hepatic insulin resistance will be associated with characteristic glucose and insulin concentration during fasting state (53). The feedback loop between the liver and the β-cell is central to the model (51). Plasma glucose concentration in the basal state is regulated by hepatic glucose output, which is insulin dependent (**Figure 1-6 B**). Insulin concentration is dependent on the β-cell response to glucose (**Figure 1-6 A**). Insulin signals glucose uptake in fat and muscle (**Figure 1-6 C and D**). Glucose disposal is modeled in the brain (**Figure 1-6 E**) and kidney (**Figure 1-6 F**) as being dependent only on glucose (51). HOMA-IR thus provides an estimation of hepatic insulin resistance. It is estimated from fasting glucose and insulin using the approximated equation given by Matthews et al. as follows (56):

HOMA-IR= fasting insulin (mU/l)*fasting glucose (mmol/l)/22.5

HOMA2-IR or HOMA-%S (inverse of HOMA2-IR), is a computerized model of insulin resistance. However, HOMA2-IR is less popular than HOMA-IR in epidemiological studies. The CV for HOMA-IR was initially reported as 31% using immunoreactive insulin assays(56). Recent studies using specific insulin assays and more number of subjects demonstrated CVs of between 7.8% and 11.7% (57, 58).

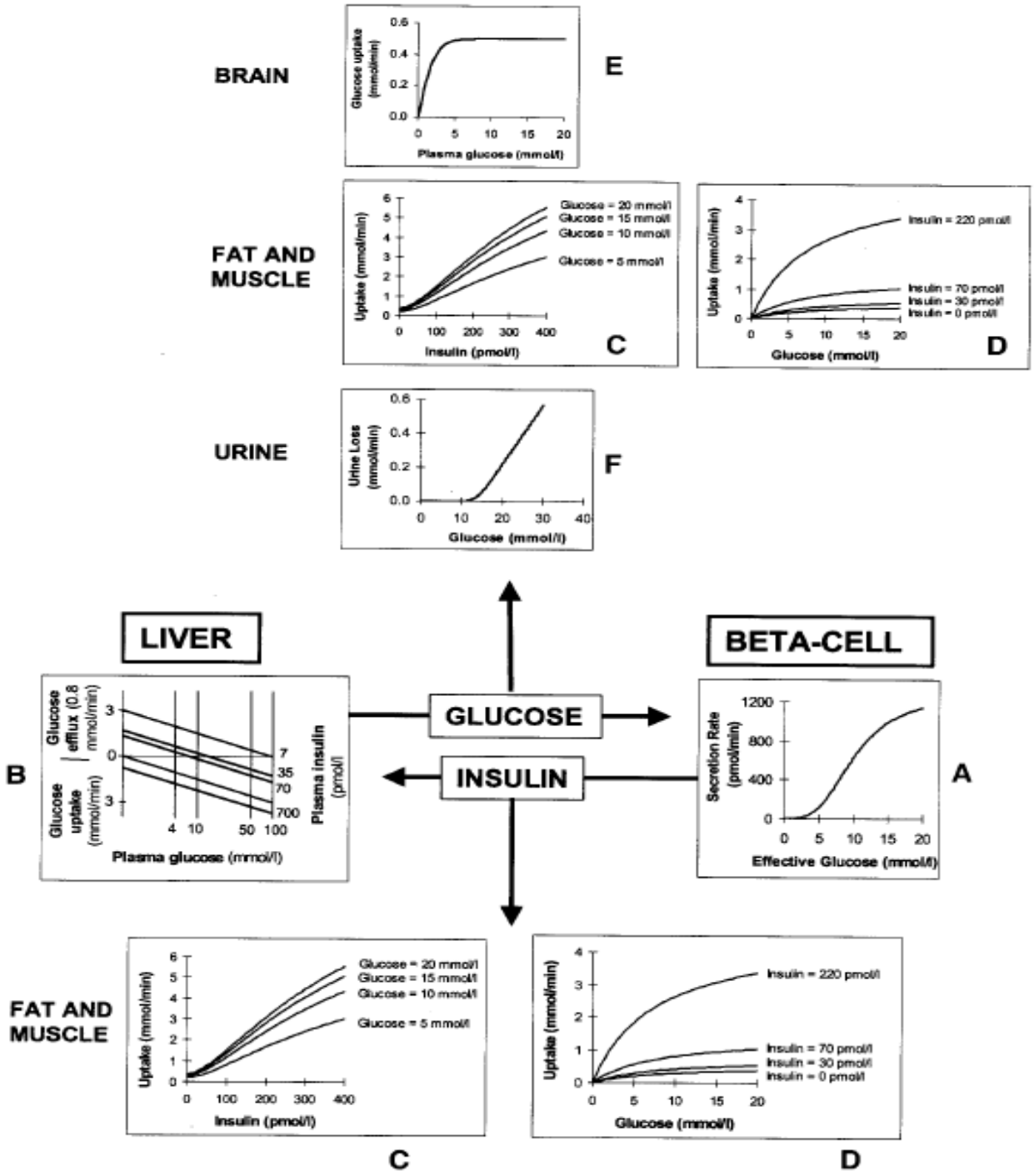

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Figure 1-6 Underlying physiological basis of HOMA model

HOMA-IR provides useful information on risk of developing T2D. The Womens' Health Initiative (WHI) study found a significant positive association between HOMA-IR and risk of T2D (59). The relative risk per SD (1.93 units) increase in HOMA-IR was 3.4 (95% CI, 2.95, 3.92). Similarly, the Mexico Diabetes Study (MDS) reported a significant positive association between HOMA-IR and risk of T2D, odds ratio (OR) 2.76 (95% CI, 1.65, 4.61) (60).

The sampling for HOMA-IR is simple and the results are available without complex computing as soon as fasting glucose and insulin levels are available (50). Because HOMA-IR measures hepatic insulin resistance; it assumes that insulin resistance is common to major sites of insulin action namely liver, muscles, and adipose tissue, which is not generally true. Yet HOMA-IR correlates well with estimates of peripheral (mainly muscle) insulin resistance measured using hyperinsulinemic euglycemic (57). It provides a better assessment of insulin resistance in overweight and obese subjects than normal weight subjects without diabetes (61). Moreover, it does not account for glucose uptake by the brain, which is glucose-dependent process in healthy individuals. Despite its limitations, HOMA-IR is extensively utilized for measuring insulin resistance, comparing insulin resistance among races, and assessing insulin resistance in a group of patients over a long period of times in population-based studies (59, 62, 63).

1.3.3 HOMA-β%

HOMA-β% is a complementary formula of HOMA-IR to estimate insulin secretion (β-cell function). The CV for HOMA-β% using specific insulin assays is reported as 7.7% (64). It is also estimated from fasting glucose and insulin using the approximated equation given by Matthews et al. as follows (56): HOMA-β% = 20*fasting insulin (mU/l)/fasting glucose (mmol/l) – 3.5. HOMA-β% provides useful information on risk of developing T2D. The WHI study found

a significant negative association between HOMA-β% and risk of T2DM, 0.57 (95% CI, 0.51, 0.63) (59). Similarly, the MDS reported a significant negative association, OR, 0.05 (95% CI, 0.03, 0.85) (60). In the Cardiovascular Health Study, a 20% lower in HOMA-β% was weakly associated with increased odds of incident cardiovascular events, OR, 1.1 (95% CI, 1.07, 1.14) (65).

Insulin secretion measured using HOMA-β% reflects a single point on the complex glucose-insulin dose-response curve and thus does not measure the ability of β-cells to respond to rising and falling glucose concentration that typically occur after a meal (66, 67). Furthermore, HOMA-β% underestimates insulin secretion especially in the subjects with impaired fasting glucose (IFG)* or impaired glucose tolerance (IGT)$^{\int}$. (68). Despite its limitations, HOMA-β% is extensively utilized for estimating insulin secretion, comparing insulin secretion among races, and assessing insulin secretion in a group of patients over a long period of times in population-based studies (59, 62, 63).

*Fasting glucose level above normal levels and below T2D levels, i.e., between 5.9 mmol/l and 6.9 mmol/l. ∫ -2-hours glucose levels after OGGT between 7.8 mmol/l and 11.1 mmol/l

1.3.4 Disposition Index

Disposition index (DI) is a β-cell function index which measures the ability of β-cell function to compensate for insulin resistance. DI is given by: measure of insulin secretion/measure of insulin resistance (or measure of insulin secretion*measure of insulin sensitivity). For FSIGT, it is estimated as: AIR*S_I (66). For fasting measures, it is estimated as HOMA-β%/HOMAIR. DI is assumed to be approximately hyperbolic so that the product of insulin secretion and insulin sensitivity is constant **(Figure 1-7)** for individuals with the same degree of glucose tolerance (69). The solid line in **Figure 1-7** represents the relationship in individuals with normal glucose

tolerance (NGT). In glucose intolerance (IGT) and type 2 diabetes (T2D) the curve is shifted down left. In NGT individuals, insulin resistance is accompanied by a concomitant increase in insulin secretion (from point A to B along the black arrow), so that NGT is maintained (**Figure 1-7)**. If the increase in insulin secretion is insufficient (from point A to C along the gray arrow), hyperglycemia (from IGT to T2D) develops **(Figure 1-7)**.

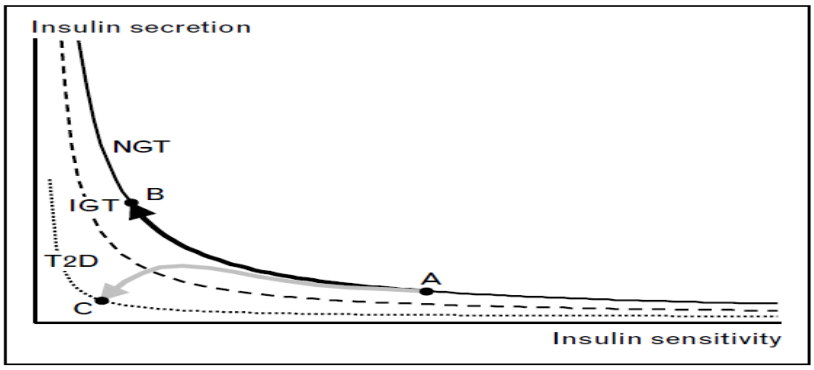

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Figure 1-7 The relationship between insulin sensitivity and insulin secretion

The concept of constant DI (hyperbolic relationship between insulin sensitivity and insulin secretion) is depicted in **Figure 1-8** by the effect of 48-hour lipid infusion in NGT subjects (70). Before lipid infusion, insulin sensitivity (solid downward arrow) and insulin secretion (solid horizontal arrow) levels are depicted by Black Square (**Figure 1-8)**. After lipid infusion, insulin sensitivity decreased (dotted downward arrow) and insulin secretion increased (dotted horizontal arrow) depicted by Black Circle, keeping DI constant (**Figure 1-8)**.

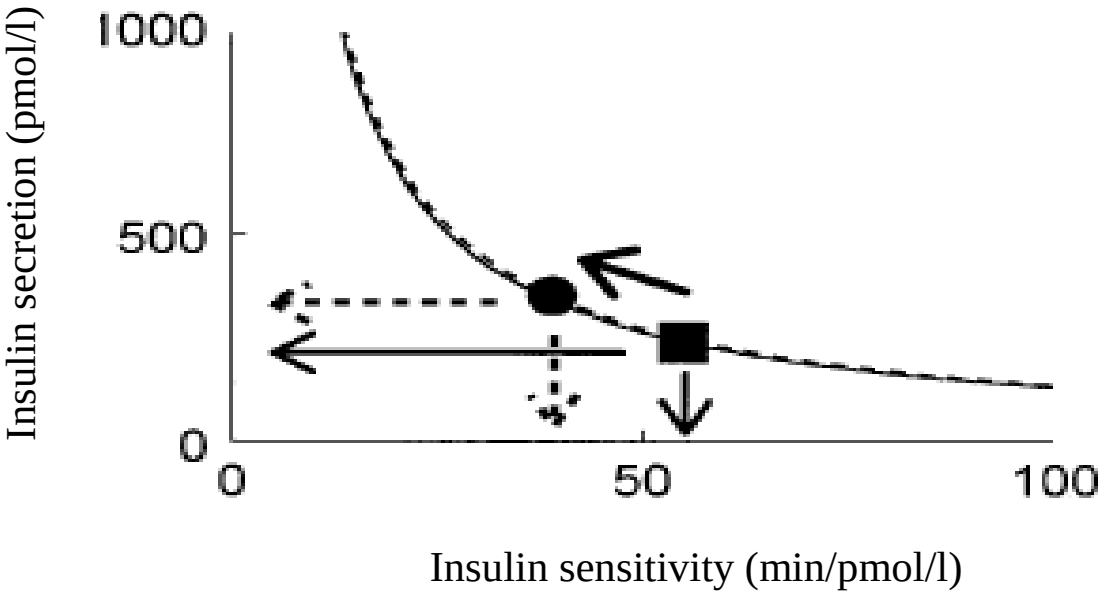

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Figure 1-8 The effect of 48-Hour lipid infusion in normal glucose tolerant participants

Failure to account for the hyperbolic relationship may lead to misinterpretation of β-cell function in population-based studies. This is shown by **Table 1-2** based on the preliminary results of the Insulin Resistance and Atherosclerosis Study (IRAS) in which insulin sensitivity and insulin secretion were measured in 1,524 subjects with NGT, IGT, or T2D (71).

The relative defect in β-cell function (insulin secretion) in subjects with IGT was 35%, measured using AIR. This can well interpreted as a mild defect. However, it is notable that subjects with IGT were also insulin resistance: SI was 43% reduced. If a subject with normally functioning β-cell were insulin resistant to this extent, one would expect an increase in AIR similar to the finding of Boden et al. with lipid infusion. However, the reduction in AIR in the face of insulin resistance suggests a much more substantial β-cell health as depicted by almost 70% reduction in DI. Therefore, it is important to assess insulin secretion correcting for insulin resistance to avoid misinterpretation of β-cell function.

Table 1-2 Preliminary results from the Insulin Resistance and Atherosclerosis Study (n= 1,524)

	Relative Defect		
	NGT	IGT	Type 2 diabetes
Insulin sensitivity (S_I)	0	43%	67%
Insulin response ($AIR_{glucose}$)	0	35%	92%
Disposition index (β-cell function)	0	67%	98%

NGT, normal glucose tolerance.

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Moreover, β-cell dysfunction is an important component in glucose intolerance associated with aging. The misinterpretation of β-cell function without accounting for insulin resistance in case of lipid infusion study by Boden et al., the IRAS, and aging is given by **Figure 1-9**.

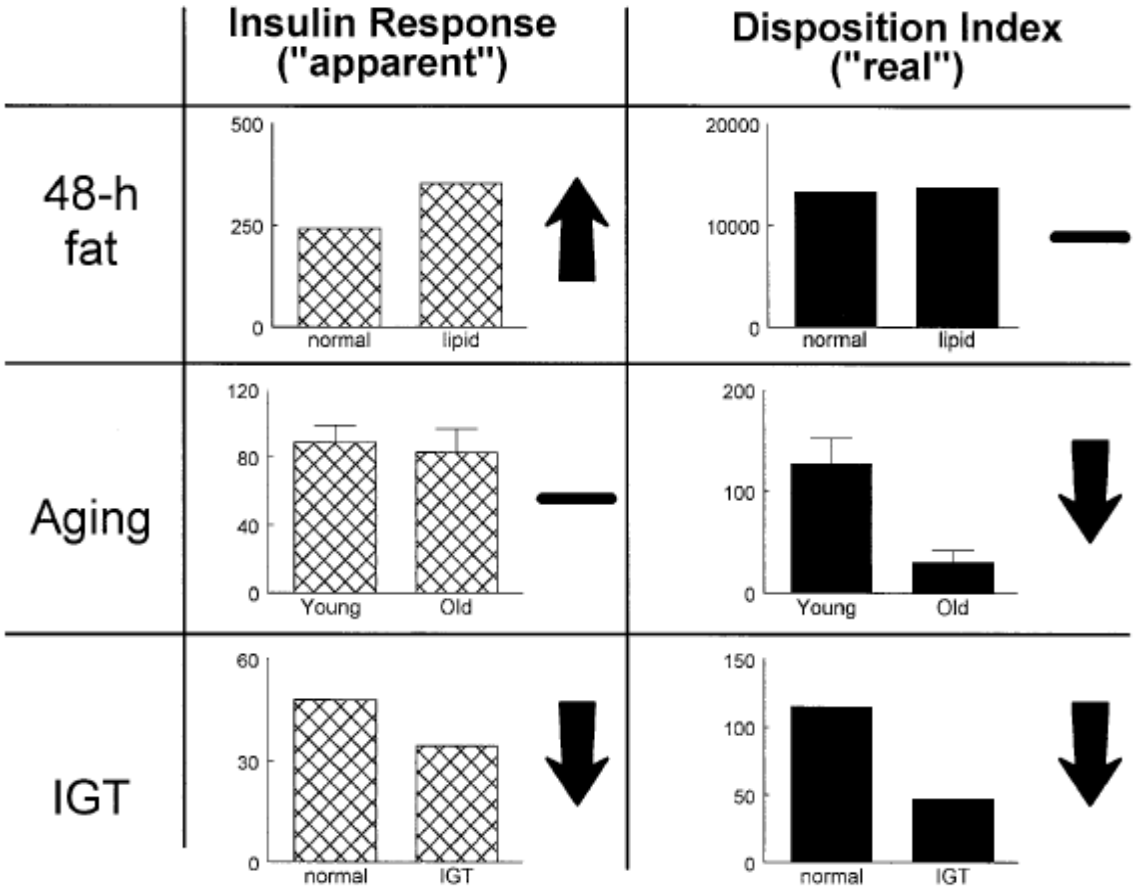

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Figure 1-9 Possible interpretation of insulin secretory function if the hyperbolic relationship between secretion and sensitivity is not considered

1.3.5 Racial Differences in Markers Related to Insulin Resistance and Insulin Secretion

The IRAS assessed insulin resistance and insulin secretion in 1089 (288 blacks, 363 Hispanics, and 435 non-Hispanic whites) participants without diabetes using FSIVGTT (71). The IRAS data showed higher insulin resistance and insulin secretion in blacks and Hispanics than non-Hispanic whites. The insulin resistance and insulin secretion remained higher in blacks and Hispanics than non-Hispanic whites even after adjusting for age, sex, clinic, body-mass index, waist-hip ratio, and reported physical activity. The San Antonio Heart Study (n= 2,456) also

reported higher HOMA-model assessed insulin resistance and insulin secretion in Hispanics compared to non-Hispanic whites (72).

In regard to comparison of insulin indices between whites and Japanese, three studies have evaluated these differences between whites and Japanese Americans and one study between Denmark whites and Japanese in Japan. However, no previous population-based studies have evaluated differences in insulin resistance and insulin secretion between whites in the US and Japanese in Japan in a standardized manner. Below mentioned (**Table 1-3)** are the results of the studies comparing insulin resistance and insulin secretion between whites and Japanese:

Table 1-3 Differences in markers related to insulin resistance and insulin secretion between whites and Japanese

Author, year Population (sample size)	**Age (years, mean ± SD)**	**Method of assessing insulin resistance / secretion**	**Results**
Chiu et al, 2000 77 health glucose-tolerant volunteers (18 JA, 9 AA, 34 whites, and 16 Hispanics) (73)	Whites- 23 ± 2.0 JA- 27 ± 2.0	Clamp	Higher insulin resistance in JA than whites, 6.9 (5.7-8.3) vs. 4.2 (3.1-5.6), respectively No difference in DI
Jensen et al, 2002 531 volunteers who were first-degree relatives of subjects with T2D with no previous history of diabetes (55 AA, 66 JA, 217 whites, and 193 Hispanics) (74)	Whites- 45.4 ± 1.1 JA- 53.4 ± 2.0	HOMA-IR / insulinogenic index and DI	Higher insulin resistance and insulin secretion in whites than JA No difference in DI
Torréns et al, 2004 2,789 premenopausal	Whites- 46 ± 2.7 JA- 47 ± 2.7	HOMA % S / HOMA % β	Higher insulin resistance in JA than whites, 116.5 vs. 104

Table 1-3 continued

and early peri-menopausal community-based samples (1,359 whites, 746 AA, 291 Hispanics, 210 CA, and 255 JA) (75)			($p < 0.01$) and 91.2 vs. 84 ($p < 0.01$), respectively
Møller et al, 2014 270 hospital-based participants (150 whites and 120 JA) (76)	Whites- 55 ± 7.0 JA- 54 ± 8.0	HOMA-IR / insulinogenic index and DI	No difference in insulin resistance, insulin secretion and DI
SD, standard deviation; JA, Japanese Americans; AA, African Americans; DI, disposition index; HOMA-IR, homeostasis model-assessment- insulin resistance; T2D, type 2 diabetes mellitus; CA, Chinese-Americans			

1.4 THE ERA JUMP STUDY

This dissertation uses data from the Electron-Beam Computed Tomography and Risk Factor Assessment among Japanese and US Men in the Post World War II Birth Cohort (ERA JUMP) study. The ERA JUMP is an international population-based study to determine prevalence and progression of subclinical cardiovascular disease in four centers in three countries, the US, Japan, and Korea. It comprises whites (23%), blacks (8%), Japanese American (23%), Japanese (23%), and Korean (23%) participants. The participants were 40-49 year old men at a baseline exam in 2004-07. Briefly, at baseline, 310 whites and 100 blacks from Allegheny County, Pennsylvania, US; 303 Japanese Americans from Honolulu, Hawaii, US; 313 Japanese from Kusatsu, Shiga, Japan; and 302 Koreans from Ansan, Gyeonggi-do, South Korea were randomly selected(77). The Japanese Americans were a representative sample of offspring of fathers who participated in the Honolulu Heart Program and were third or fourth generation Japanese Americans without ethnic admixture (78, 79). The participants were without clinical

cardiovascular disease, type 1 diabetes, or other chronic disease at baseline (77). The follow-up exam was performed from 2008-13.

1.5 SUMMARY

The proposed research will produce three unique manuscripts with principle focus on improving our knowledge of health disparities between whites and Japanese. This introduction gives an overview of epidemiology of CVD, atherosclerosis and its assessment using CAC and CIMT, and markers of insulin resistance and secretion, focusing on racial disparities. The specific aims of the three manuscripts are outlined in the next section.

1.6 SPECIFIC AIMS

Specific Aim 1 To compare HOMA-IR, HOMA-β%, and DI between population-based samples of US white men and Japanese men in Japan aged 40-49 years in the ERA JUMP study

Specific Aim 2 To compare progression of CIMT between population-based samples of Japanese American men and white men aged 40-49 years in the ERA JUMP study

Specific Aim 3 To compare progression of CAC between population-based samples of Japanese American men and white men aged 40-49 years in the ERA JUMP study

2.0 MANUSCRIPT 1: COMPARISON OF HOMA-IR, HOMA-B%, AND DISPOSITION INDEX BETWEEN U.S. WHITE MEN AND JAPANESE MEN IN JAPAN: THE ERA JUMP STUDY

Diabetologia

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2.1 ABSTRACT

Aims/hypothesis At the same level of BMI, white people have less visceral adipose tissue (VAT) and are less susceptible to developing type 2 diabetes than Japanese people. No previous population-based studies have compared insulin resistance and insulin secretion between these two races in a standardised manner that accounts for VAT. We compared HOMA-IR, HOMA of beta cell function (HOMA-β%) and disposition index (DI) in US white men and Japanese men in Japan.

Methods We conducted a population-based, cross-sectional study, comprising 298 white men and 294 Japanese men aged 40–49 years without diabetes. Insulin, glucose, VAT and other measurements were performed at the University of Pittsburgh. We used ANCOVA to compare geometric means of HOMA-IR, HOMA-β% and DI, adjusting for VAT and other covariates.

Results White men had higher HOMA-IR, HOMA-β% and DI than Japanese men, and the difference remained significant ($p<0.01$) after adjusting for VAT (geometric mean [95% CI]): 3.1 (2.9, 3.2) vs 2.5 (2.4, 2.6), 130.8 (124.6, 137.3) vs 86.7 (82.5, 91.0), and 42.4 (41.0, 44.0) vs 34.8 (33.6, 36.0), respectively. Moreover, HOMA-IR, HOMA-β% and DI were significantly higher in white men even after further adjustment for BMI, impaired fasting glucose and other risk factors.

Conclusions/interpretation The higher VAT-adjusted DI in white men than Japanese men may partly explain lower susceptibility of white people than Japanese people to developing type 2 diabetes. The results, however, should be interpreted with caution because the assessment of insulin indices was made using fasting samples and adjustment was not made for baseline glucose tolerance. Further studies using formal methods to evaluate insulin indices are warranted.

2.2 INTRODUCTION

White people are less susceptible to developing type 2 diabetes than Asians (including Japanese) for any given level of BMI or waist circumference (80). Moreover, white people have less visceral adipose tissue (VAT) than Japanese people in Japan for any given BMI or waist circumference (81). In terms of pathophysiology, VAT is more strongly associated with increased insulin resistance than is BMI, because VAT is associated with excess fat deposition in the liver and enhanced pro-inflammatory cytokine production (82). However, no previous population-based studies have compared insulin resistance or insulin secretion in white people in the USA and Japanese people in Japan after adjusting for VAT. A few volunteer-based studies have compared white and Japanese Americans (73-75) and one study compared white people in Denmark and Japanese people in Japan (83), after adjusting for BMI. However, the results of these studies were inconsistent. Most of these studies used HOMA indices for measuring insulin resistance and insulin secretion (74, 75, 83). Although assessment of HOMA-IR and HOMA of beta cell function (HOMA-β%) is performed from fasting samples, HOMA-IR and HOMA-β% are the most extensively used methods for evaluating insulin resistance and insulin secretion in epidemiological studies (51).

A recent meta-analysis indicated a similar disposition index (DI), i.e. insulin secretion and insulin resistance, among healthy glucose-tolerant participants of several races, including white people and East Asians (including Japanese) (84). According to this meta-analysis, white people have higher insulin resistance and insulin secretion than East Asians that leads to a similar DI in the two races (84). However, the sample sizes of the studies in East Asians were very small (84). In this study, we hypothesise that white men are more insulin resistant, secrete more insulin and have higher DI than Japanese men at the same level of VAT. These differences

may partly explain the lower susceptibility of white than Japanese people to type 2 diabetes at a given BMI. To test this hypothesis, we compared HOMA-IR, HOMA-β% and DI in population-based samples from 298 US white men and 294 Japanese men in Japan aged 40-49 years who were participants in the EBCT and Risk Factor Assessment among Japanese and U.S. Men in the Post World War II Birth Cohort (ERA JUMP) study.

2.3 METHODS

Study population

We have previously described the method of participant selection in detail elsewhere (77). Briefly, we randomly selected 310 white men from Allegheny County, Pennsylvania, USA and 313 Japanese men from Kusatsu, Shiga, Japan from 2002 to 2006. Exclusion criteria included coronary heart disease, stroke, type 1 diabetes and other severe diseases. Informed consent was obtained from all the participants. Our study was designed to examine differences in subclinical atherosclerosis measures, such as intima-media thickness of the carotid artery and coronary artery calcium, between white men and Japanese men, with adequate power. For the current analyses, we excluded 31 participants (12 white and 19 Japanese men) with type 2 diabetes, defined as fasting glucose levels ≥7 mmol/l or use of medications for type 2 diabetes (85). These individuals were excluded because HOMA-IR does not provide an accurate measure of insulin resistance among individuals with type 2 diabetes (86). The final sample consisted of 592 men: 298 white men and 294 Japanese men. Of the 592 men, 348 (147 white men and 201 Japanese men) had impaired fasting glucose (IFG), defined as fasting glucose levels ≥5.6 mmol/l (85). The

study was approved by the Institutional Review Boards of the University of Pittsburgh, Pittsburgh, USA and the Shiga University of Medical Science, Otsu, Japan.

Study protocol

All participants underwent a physical examination and a laboratory assessment, and completed a self-administered questionnaire, as described previously (77). Body weight and height were measured while the participants were wearing light clothing without shoes. BMI was calculated as weight (kg)/height squared (m^2). Blood pressure was measured in the right arm of the seated participants after they emptied their bladder and sat quietly for 5 min, using an automated sphygmomanometer (BP-8800; Colin Medical Technology, Komaki, Japan) and an appropriate sized cuff. The average of two measurements was used in the analyses. Participants were considered smokers if they reported current use of cigarettes or having stopped smoking within the past 30 days. Participants were considered alcohol drinkers if they consumed alcohol ≥2 days per week. Physical activity was defined as participants exercising for ≥1 h per week. Participants were considered to have a family history of type 2 diabetes if either their father or mother had self-reported type 2 diabetes. Venipuncture was performed early in the clinic visit after a 12 h fast. Serum and plasma samples were stored at -80ºC and shipped to the University of Pittsburgh. Serum/plasma samples were assayed for glucose, insulin, lipids (including triacylglycerol, LDL-cholesterol and HDL-cholesterol), C-reactive protein (CRP) and adiponectin as described previously (77).

VAT was determined at the level between the fourth and fifth lumbar vertebrae, using CT images obtained with the same apparatus at each site using a GE-Imatron C150 scanner (GE Medical System, South San Francisco, CA) (87). All CT images were analysed at the University of Pittsburgh by one trained reader using image analysis software (AccuImage Diagnostic, San

Francisco, California). To determine the respective area of VAT and subcutaneous adipose tissue, a separation line was drawn manually using a cursor along the abdominal wall musculature in continuity with fascia of the paraspinal muscle. The reproducibility of the scans had an intra-class correlation of 0.90.

Insulin resistance was estimated from fasting samples as HOMA-IR using the approximated equation of Matthews et al (56). Insulin secretion (beta cell function) was also obtained from fasting samples, as HOMA-β% using the approximated equation of Matthews et al (56). At any given time, insulin secretion depends on the level of insulin resistance; therefore, DI, estimated as HOMA-β%/HOMA-IR, provides a better assessment of insulin secretion than only considering HOMA-β% (88).

Statistical analyses

We calculated race-specific means (±SD), medians (interquartile range) or geometric means (95% CI) for continuous variables based on their distributions. Proportions were estimated for categorical variables. Means, geometric means, medians and proportions of the variables were then compared between white and Japanese men using *t* tests, Mann-Whitney *U* tests or χ^2 tests as appropriate. CRP and adiponectin were log converted for non-normal distributions. We used ANCOVA to compare geometric means of HOMA-IR, HOMA-β% and DI between white and Japanese men, adjusting for VAT. Insulin resistance is determined by factors other than VAT, such as smoking (89), alcohol consumption (89), physical activity (89), lipid medications (90), and levels of CRP (91) and adiponectin (92). Moreover, individuals with IFG have higher insulin resistance and insulin secretion than individuals without IFG (93). Therefore, we compared HOMA-IR, HOMA-β% and DI between white and Japanese men after further adjustment for BMI, smoking, alcohol, physical activity, lipid medications, IFG, and levels of CRP and

adiponectin. We also performed sensitivity analyses on participants with normoglycaemia and IFG to examine how HOMA-IR, HOMA-β% and DI differ between the two races in these subgroups. All p values were two tailed. P values of <0.01 were considered significant. All statistical analyses were performed using SAS software version 9.3 (Cary, NC, USA).

2.4 RESULTS

Table 2-1 shows the characteristics of the study population. White men had significantly higher BMI, VAT, fasting insulin levels and CRP levels than Japanese men. White men were significantly more physically active, had higher adiponectin levels and lipid medication use than Japanese men. In addition, white men had a significantly lower prevalence of smoking, alcohol consumption, hypertension and IFG, and lower levels of glucose than Japanese men. Supplementary tables and figures, Table 2-3 shows the characteristics of the participants with normoglycaemia and supplementary tables and figures, Table 2-4 shows the characteristics of the participants with IFG.

White men had significantly higher HOMA-IR (Fig. 2-1. a, b), HOMA-β% (Fig. 2-1c, d) and DI (Fig. 1 e, f) than Japanese men, both before and after adjusting for VAT. Even after further adjustment for BMI, smoking, alcohol, physical activity, lipid medications, IFG and levels of CRP and adiponectin, white men had higher HOMA-IR, HOMA-β% and DI than Japanese men (Table 2-2).

In sensitivity analyses, HOMA-IR, HOMA-β% and DI were significantly higher in white than Japanese men in both subgroups (normoglycaemia and IFG) before (Supplementary tables and figures**,** Fig. 2-2 a, c, e**)** and after adjusting for VAT **(**Supplementary tables and figures**,** Fig.

2-2 b, d, f). After further adjustment for BMI, smoking, alcohol, physical activity, lipid medications, and levels of CRP and adiponectin, HOMA-IR, HOMA-β% and DI were still higher in white men than Japanese men in both subgroups, but the difference was not statistically significant **(**Supplementary tables and figures**,** Tables 2-5 and 2-6**)**.

2.5 DISCUSSION

This population-based study is the first to compare VAT-adjusted HOMA-IR, HOMA-β% and DI in US white men and Japanese men in Japan in a standardised manner. White men were more insulin resistant than Japanese men, with a significantly higher HOMA-IR both before and after adjusting for VAT. White men also had higher insulin secretion with a significantly higher HOMA-β% and higher insulin secretion relative to insulin resistance with a significantly higher DI than Japanese men, both before and after adjusting for VAT.

Most of the studies evaluating differences in insulin indices between white and Japanese individuals have examined differences between white people from the USA and Japanese Americans (73-75). However, it is important to make a distinction between Japanese Americans and Japanese in Japan because insulin resistance is determined not only by genetics (94) but also by metabolic factors such as obesity (95) and abdominal fat deposition (82). Japanese Americans have a more Westernised lifestyle and thus have much higher BMI, VAT and prevalence of type 2 diabetes than Japanese living in Japan (96, 97).

Previous comparisons of insulin resistance between white and Japanese individuals have produced inconsistent results. In accordance with our findings, one study reported higher insulin resistance in US white people than Japanese Americans, measured using HOMA-IR among first

degree relatives of individuals with type 2 diabetes, both before and after adjusting for BMI (74). Similarly, a recent hospital-based study found higher insulin resistance in white people from Denmark than Japanese in Japan, as estimated by HOMA-IR and the Matsuda index in the unadjusted analyses (83). However, after adjusting for BMI in this study, the significant difference in insulin resistance between the two races attenuated and became nonsignificant. In contrast to our findings, two studies reported lower insulin resistance in US white people than Japanese Americans. One study reported lower insulin resistance in white than Japanese American women without diabetes, as measured by the HOMA index (75). Similarly, another study reported lower insulin resistance in white people than Japanese Americans, measured by the hyperglycaemic clamp among normal glucose tolerant participants (73). However, all the above-mentioned studies were volunteer-based; most of the studies were small, and adjusted for only BMI in the comparison of insulin resistance between the races. The interpretations of these studies are thus limited.

We observed higher HOMA-β% (beta cell response or insulin secretion) in white than Japanese men both before and after adjusting for VAT. Our finding of higher insulin secretion is in accordance with higher insulin secretion in white than Japanese Americans in two earlier studies (74, 75). In disagreement to our study findings, two studies found similar levels of insulin secretion in white and Japanese people (73, 83). Smoking has been inversely associated with impaired insulin secretion in Japanese individuals in a prospective study (98). Similarly, we found a significant inverse association of pack years of smoking with HOMA-β% in Japanese but not in white men (data not shown). Furthermore, in contrast to our study, previous studies did not find any difference in DI between white and Japanese people (73, 74, 83). Nevertheless, as mentioned earlier, these studies were volunteer-based.

After adjusting for VAT and other covariates, the higher DI in white than Japanese men suggests that insulin resistance is better compensated for in white individuals. This difference may partly explain the lower susceptibility of white than Japanese people to type 2 diabetes. However, higher DI in white than Japanese people suggests that white people have longer periods of exposure to hyperinsulinaemia before onset of diabetes than Japanese people. As hyperinsulinaemia is associated with increased atherosclerosis (99), longer exposure to hyperinsulinaemia in white than Japanese people before onset of type 2 diabetes would translate into higher incidence of CHD in white than Japanese individuals. In line with this reasoning, white people with diabetes have a higher incidence of CHD than Japanese people with type 2 diabetes who live in Japan (100, 101).

The strengths of our study include that it is the first population-based international study that compared VAT-adjusted insulin resistance and secretion between white people in the USA and Japanese in Japan in a standardised manner. Our study has some limitations. The participants were men aged 40-49 years. Therefore, it is unknown whether our results can be generalised to other age groups or women. As HOMA-IR and HOMA-β% are determined from fasting samples, these essentially provide an estimate of hepatic insulin resistance and nonstimulated insulin secretion. Moreover, HOMA-IR provides a better assessment of insulin resistance in overweight and obese participants than normal-weight participants without diabetes (61). Insulin secretion measured using HOMA-β% does not account for factors other than glucose that determine beta cell function, such as amino acids, nonesterified fatty acids, cortisol, growth hormone, etc. (102). Furthermore, HOMA-β% underestimates insulin secretion, especially in individuals with IFG or impaired glucose tolerance (IGT) (68). Despite the limitations, HOMA-IR and HOMA-β% are the most extensively used markers of insulin resistance and beta cell function in epidemiological

studies and are widely used for comparing insulin resistance and insulin secretion among various races in population-based studies (59, 62, 72).

DI is assumed to be hyperbolic (the constant product of insulin sensitivity and insulin secretion) for individuals with the same degree of glucose tolerance (103). However, we are unable to comment on the glucose tolerance state of the participants, as we did not perform the OGTT. Thus, it is possible that some of the participants with normoglycaemia may have had IGT, which would not be accounted for by adjusting for IFG (93). Furthermore, the estimation of DI using HOMA-β% and HOMA-IR provides a less credible index of beta cell function than DI obtained using dynamic methods because both HOMA-β% and HOMA-IR are estimated from fasting samples and thus are interdependent (103). Therefore, the results should be interpreted with caution.

We did not measure autoimmune antibodies against pancreatic beta cells. These antibodies reduce insulin secretion by destroying beta cells and may be purported as one of the reasons for significantly different DI between white and Japanese people. However, the estimated prevalence of these antibodies is similar among patients with type 2 diabetes in these races (104, 105). We did not evaluate genetic differences related to insulin resistance or insulin secretion between white and Japanese individuals. To date, there is no conclusive evidence in regard to genetic differences between the two races (83, 94).

In summary, white men appear to have higher insulin resistance, insulin secretion and DI than Japanese men at the same level of VAT and on the basis of fasting levels of glucose and insulin. The higher DI in white than Japanese people may partly explain the lower susceptibility of white than Japanese individuals to type 2 diabetes at a given BMI. The reason for the difference in DI between white and Japanese individuals warrants further investigation. These

findings need to be confirmed using formal methods for assessing insulin resistance and insulin secretion, such as the hyperinsulinaemic clamp and the intravenous glucose tolerance test.

2.6 TABLES AND FIGURES

Table 2-1 Baseline characteristics of the ERA JUMP participants, 2004-2007

Characteristic	White men	Japanese men	*P* value
Participants (n)	298	294	
Age (years)	45.0 (2.8)	45.1 (2.8)	NS
BMI (kg/m2)	27.8 (4.3)	23.5 (2.9)	<0.01
VAT (cm2)	171.0 (73.5)	131.6 (51.2)	<0.01
Systolic BP (mmHg)	122.5 (11.3)	124.6 (16.0)	NS
Current smokers [a] (%)	7.4	50.3	<0.01
Alcohol drinkers [b] (%)	44.6	67.0	<0.01
Hypertension [c] (%)	14.4	24.5	<0.01
Glucose (mmol/l)	5.5 (5.2, 5.8)	5.7 (5.4, 6.1)	<0.01
Insulin (pmol/l)	88.9 (71.5, 120.8)	65.3 (50.0, 84.7)	<0.01
Triacylglycerol (mmol/l)	1.4 (1.0, 2.1)	1.6 (1.1, 2.1)	NS
LDL-cholesterol (mmol/l)	3.5 (0.87)	3.4 (0.93)	NS
HDL-cholesterol (mmol/l)	1.2 (0.33)	1.4 (0.33)	<0.01
CRP (nmol/l)	9.2 (8.2, 10.3)	3.7 (3.3, 4.1)	<0.01
Adiponectin (mg/l)	10.1 (9.6, 10.7)	6.1 (5.6, 6.5)	<0.01
IFG [d] (%)	49.3	68.4	<0.01
Hypertension medication (%)	7.7	5.1	NS
Lipid medication (%)	12.1	3.1	<0.01
Physical activity [e] (%)	72.8	26.1	<0.01
Family history of type 2 diabetes [f] (%)	12.8	11.6	NS

Values are means (SD) unless specified otherwise

[a]Current smokers were defined as having reported current use of cigarettes or having stopped smoking within the past 30 days

[b]Alcohol drinkers were defined as those who consumed alcohol ≥2 times/week

[c]Hypertension was defined as systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg, or use of antihypertensive medications

[d]IFG was defined as fasting serum glucose level ≥5.6 mmol/l

[e]Physical activity was defined as exercise ≥1 h in a week

[f]Family history of type 2 diabetes was defined as either father or mother of participant having type 2 diabetes

NS, not significant

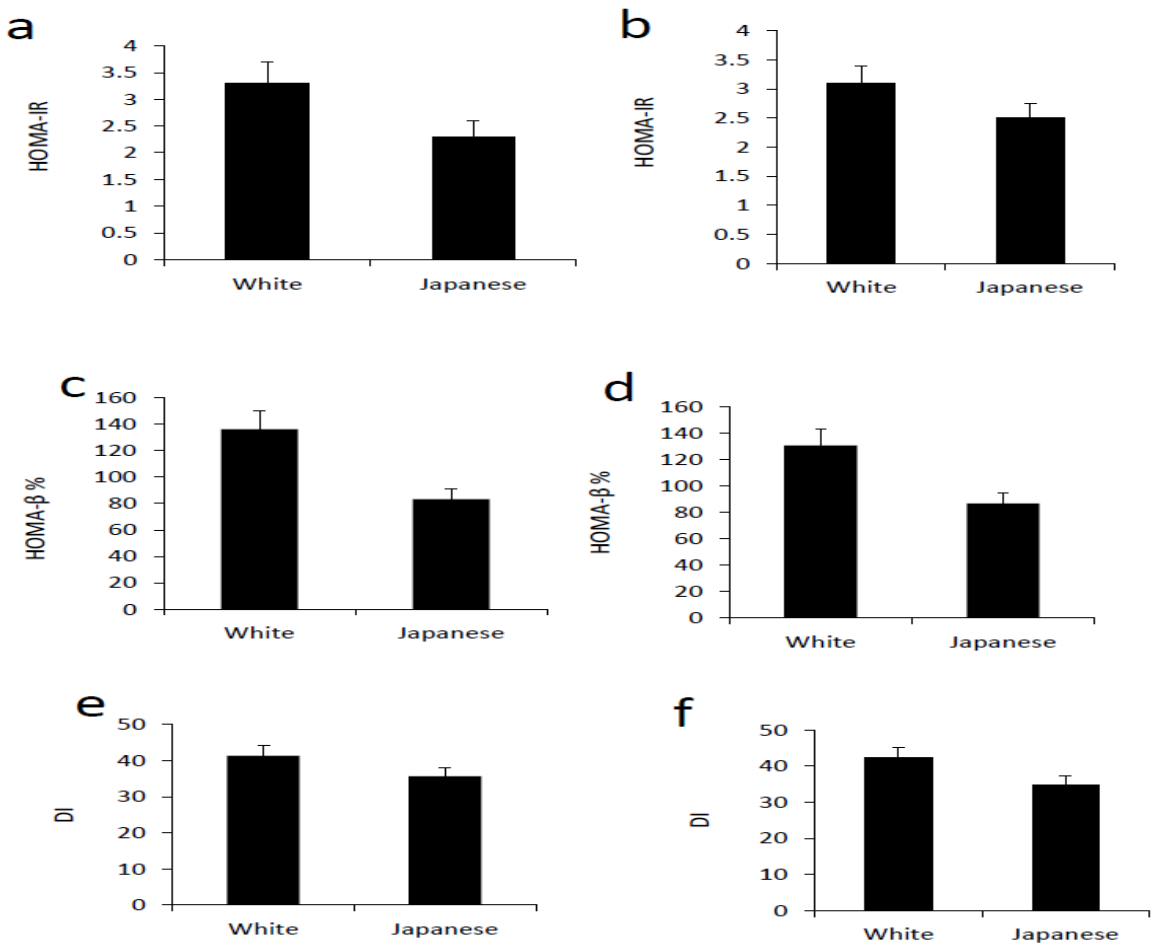

Figure 2-1 HOMA-IR, HOMA-β%, and disposition index (DI) by race without (a, c and e, respectively) and with (b, d and f, respectively) adjustment for VAT in the ERA JUMP study. Data are geometric means ± 95% CI. HOMA-IR, HOMA-β%, and DI were significantly different (*p* <0.01) between white men and Japanese men before and after adjustment for VAT

Table 2-2 Comparison of HOMA-IR, HOMA-β% and DI between white and Japanese men in the ERA JUMP study

	White men (n=298)	Japanese men (n=294)	*P* value
Insulin resistance marker			
HOMA-IR	3.1 (2.9, 3.2)	2.5 (2.4, 2.7)	<0.01
Insulin secretion markers			
HOMA-β%	121.9 (114.9, 129.3)	93.5 (88.0, 99.3)	<0.01
DI	40.0 (38.8, 41.1)	37.0 (35.9, 38.1)	<0.01

Values are geometric means (95% CI)
Adjusted for visceral adipose tissue, BMI, current smokers, alcohol drinkers, physical activity, lipid medication, IFG, C-reactive protein and adiponectin

2.7 SUPPLEMENTARY TABLES AND FIGURES

Table 2-3 Characteristics of the ERA JUMP participants with normoglycaemia

	White men (n=151)	**Japanese men** (n=93)	***P* value**
Age (years)	44.8 (2.8)	44.8 (2.8)	NS
BMI (kg/m^2)	27.0 (3.8)	22.4 (2.9)	<0.01
VAT (cm^2)	158.8 (65.8)	108.1 (49.3)	<0.01
SBP (mmHg)	120.9 (10.7)	122.5 (15.3)	NS
Current Smokers[a] (%)	5.3	52.7	<0.01
Alcohol Drinkers[b] (%)	45.0	63.4	<0.01
Hypertension[c] (%)	10.6	17.2	NS
Glucose (mmol/l)	5.2 (5.0, 5.4)	5.3 (5.1, 5.4)	NS
Insulin (pmol/l)	82.7 (70.8, 05.6)	57.6 (40.3, 68.1)	<0.01
Triacylglycerol (mmol/l)	1.4 (0.99, 1.9)	1.4 (1.1, 1.9)	NS
LDL-C (mmol/l)	3.5 (0.82)	3.3 (0.98)	NS
HDL-C (mmol/l)	1.3 (0.32)	1.5 (0.38)	<0.01
CRP (nmol/l)	8.3 (4.2, 18.1)	3.1 (1.4, 5.7)	<0.01
Adiponectin[d] (mg/dl)	11.1 (8.7, 13.8)	7.4 (5.1, 8.9)	<0.01
Hypertension Medication (%)	6.6	3.2	NS
Lipid Medication (%)	9.3	2.2	NS
Physical Activity[e] (%)	78.2	21.5	<0.01
Family History of T2DM[f] (%)	20.5	21.5	NS

Values are mean (standard deviation) unless specified otherwise

[a] Current smokers were defined as having reported current use of cigarettes or having stopped smoking within the last 30 days

[b] Alcohol drinkers were defined as those who consumed alcohol ≥2times/week

[c] Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medications

[d] SI unit not available

[e] Physical activity was defined as exercise ≥1 hour in a week

[f] Family history of T2DM was defined as either father or mother of participant having T2DM

NS, non-significant; BMI, body-mass index; VAT, visceral adipose tissue; SBP, systolic blood pressure; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; CRP, C- reactive protein; T2DM, type 2 diabetes mellitus

Table 2-4 Characteristics of the ERA JUMP participants with impaired fasting glucose

	Whites (n=147)	Japanese (n=201)	*P* value
Age (years)	45.2 (2.8)	45.2 (2.8)	NS
BMI (kg/m2)	28.7 (4.5)	24.0 (2.8)	<0.01
VAT (cm2)	183.8 (79.0)	142.4 (48.5)	<0.01
SBP (mmHg)	124.1 (11.6)	125.5 (16.2)	NS
Current Smokers (%)	9.5	49.3	<0.01
Alcohol Drinkers (%)	44.2	68.7	<0.01
Hypertension (%)	18.4	27.9	NS
Glucose (mmol/l)	5.8 (5.7, 6.1)	5.9 (5.7, 6.2)	<0.01
Insulin (pmol/l)	101.4 (72.9, 137.5)	69.5 (55.6, 88.2)	<0.01
Triacylglycerol (mmol/l)	1.5 (1.0, 2.2)	1.6 (1.2, 2.1)	NS
LDL-C (mmol/l)	3.5 (0.93)	3.5 (0.90)	NS
HDL-C (mmol/l)	1.2 (0.34)	1.4 (0.33)	<0.01
CRP (nmol/l)	9.2 (5.5, 15.4)	3.1 (1.4, 6.8)	<0.01
Adiponectin (mg/dl)	9.3 (7.2, 12.6)	5.8 (4.4, 7.9)	<0.01
Hypertension Medication (%)	8.8	6.0	NS
Lipid Medication (%)	15.0	3.5	<0.01
Physical Activity (%)	67.4	28.4	<0.01
Family History of T2DM (%)	30.6	24.4	NS

Values are mean (standard deviation) unless specified otherwise
Refer table 1 for definitions and abbreviations

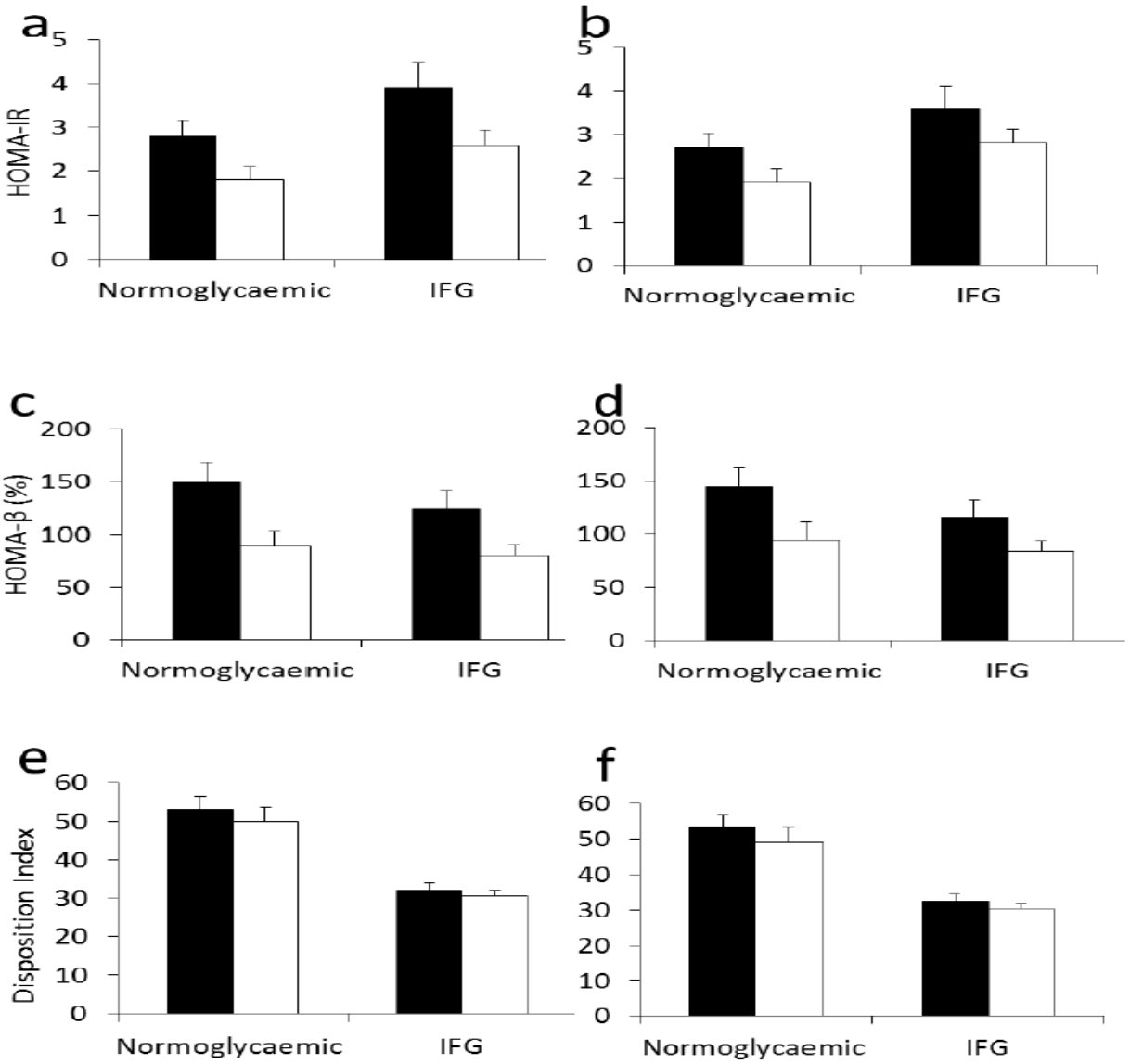

Figure 2-2 HOMA-IR, HOMA-β %, and disposition index (DI) by race and fasting-glucose state without (a, c, e respectively) and with (b, d, f respectively) adjustment for VAT in the ERA JUMP study. Data are geometric mean ± 95% confidence interval. HOMA-IR, HOMA-β %, and DI were significantly different before and after adjustment for VAT at p<0.01. IFG, impaired fasting glucose; black bars Whites; white bars, Japanese.

Table 2-5 Comparison of HOMA-IR, HOMA-β%, and disposition index between white men and Japanese men with normoglycaemia in the ERA JUMP study

	Whites (n=151)	Japanese (n=93)	*P* value
Insulin Resistance Marker			
HOMA-IR	2.6 (2.4-2.8)	2.1 (1.9-2.3)	<0.01
Insulin Secretion Markers			
HOMA-β%	139.1 (128.5-150.6)	101.9 (90.8-114.4)	<0.01
DI	53.0 (50.8-55.3)	49.2 (46.3-52.3)	0.10

Values are Geometric means ± 95% confidence intervals

Adjusted for visceral adipose tissue , BMI, current smokers, alcohol drinkers, physical activity, lipid medication, C-reactive protein , and adiponectin

DI=disposition index

Table 2-6 Comparison of HOMA-IR, HOMA-β%, and disposition index between white men and Japanese men with impaired fasting glucose in the ERA JUMP study

	Whites (n=147)	Japanese (n=201)	*P* value
Insulin Resistance Marker			
HOMA-IR	3.4 (3.1-3.7)	2.9 (2.7-3.1)	0.03
Insulin Secretion Markers			
HOMA-β%	109.2 (100.3-118.9)	88.0 (82.1-94.3)	<0.01
DI	32.6 (31.3-34.0)	30.2 (29.2-31.2)	0.02

Values are Geometric means ± 95% confidence intervals
Adjusted for visceral adipose tissue , BMI, current smokers, alcohol drinkers, physical activity, lipid medication, C-reactive protein , and adiponectin
DI=disposition index

3.0 MANUSCRIPT 2: SIGNIFICANTLY GREATER PROGRESSION OF INTIMA-MEDIA THICKNESS OF THE CAROTID ARTERY IN MIDDLE-AGED JAPANESE AMERICAN MEN THAN WHITE MEN- THE ERA JUMP STUDY

3.1 ABSTRACT

Aims: Progression of intima-media thickness of the carotid artery (CIMT) is hypothesized as an important predictor of coronary heart disease (CHD). Only a few studies have compared racial difference in progression of CIMT. We aimed to compare annual progression rate of CIMT between Japanese Americans and whites.

Methods: Population-based samples of 473 men (Japanese Americans=227 and whites=246) aged 40-49 years at baseline and free of clinical cardiovascular disease were examined for CIMT at baseline (2002-06) and follow-up (2007-13) exams. The average CIMT was read centrally at the University of Pittsburgh. Analysis of covariance (ANCOVA) was used to compare annualized progression of CIMT between Japanese Americans and whites, adjusting for cardiovascular risk factors and baseline CIMT.

Results: Progression of CIMT was significantly greater in Japanese Americans than whites both before and after adjusting for covariates (mean in μm/year [95% confidence interval]): 14.4 (12.3, 16.4) vs. 9.8 (7.8, 11.7), $P<0.01$ and 14.5 (12.4, 16.5) vs. 9.9 (9.8, 11.7), $P<0.05$, respectively. Age, total cholesterol/high-density lipoprotein-cholesterol, and diabetes in

Japanese Americans and age, hypertension, and lipid medication in whites were significantly associated with CIMT progression.

Conclusion: The significantly greater progression of CIMT in Japanese Americans than whites may suggest a higher burden of CHD in Japanese Americans than whites in the future. The current finding highlights the importance of examining racial disparities in subclinical vascular disease and may be utilized in CHD prevention.

3.2 INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in the United States (US) (4). The major underlying cause of CHD is atherosclerosis (4). Subclinical atherosclerosis assessed non-invasively as intima-media thickness (CIMT) of the carotid artery and its progression are proposed to be predictors of CHD (16, 36, 106, 107). Progression of CIMT is widely studied in observational studies (42). However, only a few studies have been able to compare racial differences in progression of CIMT (43, 44, 47, 108)

In 2009, the President of the US issued an Executive Order demanding to improve health and to obtain data on the health disparities in Asian Americans subgroups as data on this group are sparse and many health disparities remain ambiguous (109). In response to this Executive Order, the American Heart Association issued an advisory recommending changes in data collection procedures so as to disaggregate Asian Americans rather than combining them in a single group, development of better standards to measure diet and acculturation, and new research studies to improve health disparities among Asian Americans (110). The Electron-Beam Computed Tomography and Risk Factor Assessment among Japanese and US Men in the

Post World War II Birth Cohort (ERA JUMP) study is an international population-based cohort designed to determine prevalence and progression of subclinical cardiovascular disease in Japanese American men and white men in the US (77). Therefore, the ERA JUMP study provides an opportunity to better describe risk profile of CHD in Japanese Americans compared to whites.

We aimed to compare progression of CIMT between 40-49 year old Japanese American men and white men in the ERA JUMP study. From our cross-sectional study, we previously reported significantly higher baseline CIMT in Japanese Americans than whites independent of traditional cardiovascular risk factors (77). Progression of CIMT is reported to be inversely associated with baseline CIMT (44, 47). We therefore hypothesized that progression of CIMT is slower in Japanese Americans than whites. We also examined which risk factors were associated with progression of CIMT in Japanese Americans and whites.

3.3 METHODS

Study population

We have described the methods for subject selection previously (77). Briefly, during 2002 to 2006, 613 men were randomly selected: 310 white men from the Allegheny County, Pennsylvania (77) and 303 Japanese American men from a representative sample of offspring of fathers who participated in the Honolulu Heart Program, Honolulu, Hawaii (78, 79). These offspring were third or fourth generation of Japanese Americans without ethnic admixture (78). The participants were without clinical cardiovascular disease, type 1 diabetes, or other chronic disease (77). The current analyses include 473 participants (227 Japanese Americans and 246

whites) who underwent carotid-ultrasound at baseline and during the follow-up from 2007 to 2013. Informed consent was obtained from all the participants.

Study protocol

All participants underwent a physical examination, lifestyle questionnaire, and laboratory examination as described previously (77, 78). Body weight and height were measured while participants were wearing light clothing without shoes. Body-mass index (BMI) was calculated as weight (kg)/ height squared (m^2). Blood pressure was measured in the right arm of the seated participants after they emptied their bladder and sat quietly for five minutes, using an automated sphygmomanometer (BP-8800; Colin Medical Technology, Komaki Japan), and an appropriate sized cuff. The average of two measurements was used in the analyses. Venipuncture was performed early in the clinic visit after a 12 hour fast. Plasma and serum samples were stored at -80ºC, shipped to the University of Pittsburgh, and were assayed for glucose, insulin, and lipids including total cholesterol, triglycerides, low-density lipoprotein-cholesterol, and high-density lipoprotein-cholesterol (HDL-C), and C-reactive protein (CRP).

Participants were considered current smokers if they reported current use of cigarettes or having stopped smoking within the last 30 days. Alcohol drinkers were defined as those who consumed alcohol ≥2 days per week. Hypertension and diabetes were defined using the criteria by the 7th Report of the Joint National Committee (111) and the 2013 American Diabetes Association guidelines respectively (85).

Intima-media thickness of the carotid artery

The scanning procedures have been described in detail elsewhere (77, 112). Briefly, before the study initiated, sonographers were trained for carotid ultrasound scanning provided by the Ultrasound Research Laboratory, University of Pittsburgh. A Toshiba 140A scanner at

Pittsburgh and a Siemens Acuson Cypress scanner at Honolulu were used. The right and left common carotid arteries (CCA), the carotid bulbs (CB), and the internal carotid arteries (ICA) were scanned. A total of 8 segments were scanned: the far and near wall of the CCAs 1 cm proximal to the CB, the far wall of the CBs and the ICAs. The scans were recorded on videotape and sent to the Ultrasound Research Laboratory for scoring. Trained readers digitized the best image for scoring and then using semi-automated edge detection software measured the average CIMT across all the 8 segments. Continuous quality improvement procedures were employed to assure the accuracy of measurements (113). The same protocol and ultrasound machines were utilized for measuring CIMT at baseline and repeated scans 4-6 years later. The intra-class correlation coefficients for repeated scans between machines, sonographers, and readers for average CIMT were 0.90, 0.96, and 0.98, respectively.

Statistical analyses

We calculated mean (median) and standard deviation (interquartile range) for continuous variables and proportions for categorical variables. For comparing continuous and categorical variables between Japanese Americans and whites, we used t-tests or Wilcoxon rank-sum tests and chi-square tests, respectively. We defined annualized progression of CIMT as: (follow-up average CIMT – baseline average CIMT)/follow-up (years). We performed analysis of covariance (ANCOVA) to compare annualized mean progression of CIMT between the two races, adjusting for the following covariates from the baseline visit: age (years), BMI (kg/m^2), current smoking (yes or no), hypertension (yes or no), diabetes (yes or no), total cholesterol/HDL-C ratio, lipid medication (yes or no), and CRP. We also compared unadjusted and covariate-adjusted segment-specific progression of CIMT in the CCA, the CB, and the ICA in Japanese Americans and whites. We built race-specific models using multiple linear

regressions to examine risk factor associated with progression of CIMT in each racial subgroup, adjusting for age, BMI, current smoking, hypertension, diabetes, total cholesterol/HDL-C ratio, lipid medication, and CRP. We log transformed total cholesterol/HDL-C and CRP because their distributions were non-normal. Total cholesterol/HDL-C ratio was used because it demonstrated the strongest association with CIMT among lipids in our study and earlier studies (44). Further, we adjusted all the models for baseline CIMT because it was significantly and inversely associated with progression of CIMT in the whole sample (-0.26, P <0.05) and each race (Japanese Americans (-0.35, P <0.05) and whites (-0.19, P <0.05)). P-values<0.05 of were considered significant. In secondary analyses, in order to assess whether progression of risk factors affected progression of CIMT between the two races, we calculated annual change in risk factors such as BMI, systolic blood pressure (SBP), total cholesterol/HDL-C ratio, and glucose considered these as covariates in addition to baseline variables (age, BMI, SBP, glucose, total cholesterol/HDL-C ratio, CRP, medication for hypertension, lipid medication, and medication for diabetes). Annual change in risk factors were calculated in the same manner as mentioned above for annual change in CIMT, i.e., (follow-up levels – baseline levels)/follow-up (years). For multivariable analyses, we selected all risk factors associated at $P \leq 0.25$ with outcome in the univariable analyses. In multivariable analyses, we entered covariates with small P-values followed by covariates with large P-values using forward stepwise regression. Covariates were dropped from the final model with $P \geq 0.1$. We forced baseline age, baseline CIMT, hypertension medication, and lipid medication in all models. All P-values were 2-tailed. All statistical analyses were performed using SAS software, version 9.3, Cary, NC, US.

3.4 RESULTS

Table 3-1 describes the characteristics of the study population at baseline. Japanese Americans were significantly older than whites. Japanese Americans had significantly higher systolic blood pressure, and glucose. Japanese Americans had higher prevalence of hypertension, use of medication for hypertension, diabetes, use of medication for diabetes, and use of lipid medication than whites. On the other hand, Japanese Americans had significantly lower low-density lipoprotein-cholesterol, total cholesterol/HDL-C ratio, and CRP than whites. The baseline characteristics of the participants who attended the follow-up (n=473) did not differ from the baseline characteristics of the total participants (n=613) with respect to any of the biomarkers (Supplementary tables and figures 3-4).

Table 3-2 presents the baseline CIMT, follow-up CIMT, and follow-up duration in Japanese Americans and whites. The baseline CIMT, follow-up CIMT, and follow-up duration were greater in Japanese American than whites. Progression of CIMT was significantly greater in Japanese Americans than whites in the unadjusted analyses, (mean in μm/year [95% confidence interval]): 14.4 (12.3, 16.4) vs. 9.8 (7.8, 11.7), P <0.05, respectively (Figure 3-1A). Moreover, progression of CIMT remained significantly greater in Japanese Americans than whites even after adjusting for age, BMI, current smoking, hypertension, diabetes, total cholesterol/HDL-C ratio, lipid medication, CRP, and baseline CIMT, 14.5 (12.4, 16.5) vs. 9.9 (9.8, 11.7), P <0.05, respectively (Figure 3-1B).

Table 3-3 shows the risk factors associated with progression of CIMT in Japanese Americans and whites. In Japanese Americans, age and total cholesterol/ HDL-C were positively associated and diabetes was negatively associated with progression of CIMT. Furthermore, BMI was marginally positively associated with progression of CIMT in Japanese Americans. In

whites, age and hypertension were positively associated and lipid medication was negatively associated with progression of CIMT. Additionally, current smoking was marginally positively associated with progression of CIMT in whites. There was a significant interaction between diabetes and use of lipid medication in Japanese Americans (b=-0.01, p=0.06).

In the secondary analyses where we considered annual change in risk factors as covariates in addition to baseline risk factors, progression of CIMT was nevertheless greater in Japanese Americans than whites (Supplementary tables and figures, Table 3-5). Only Japanese American race and medication for diabetes was associated with progression of CIMT in multivariable model.

3.5 DISCUSSION

Contrary to our hypothesis, annualized progression of CIMT was significantly greater in middle-aged Japanese American men than in white men. The greater progression of CIMT in Japanese Americans than whites was not explained by differences in cardiovascular risk factors. Although age was associated with progression of CIMT in both races, associations with other risk factors were different by race, i.e., total cholesterol/HDL-C and diabetes in Japanese Americans and hypertension and lipid medication in whites.

Recently, the Multi-Ethnic Study of Atherosclerosis (MESA) found a slower progression of CIMT in Chinese and Hispanics than whites in participants with a mean age (± SD) age 60.3 (9) years and followed for 9.5 (0.5) years (108). In contrast to our study, in the MESA, progression of CIMT was slower in Asians (Chinese) than whites; this may be due to fact that more than 90 percent of Chinese participants in the MESA were not born in the US as opposed to

third or fourth generation Japanese Americans in our study (114). The Bogalusa Heart Study (age range 29-35 year and followed for 6 years) and the Atherosclerosis Risk in Communities study (45-64 years and followed for 9 years) examined racial differences in progression of CIMT between whites and blacks (43, 44, 47); these studies did not find any racial difference in progression of CIMT.

In our study, diabetes was negatively associated with progression of CIMT in Japanese Americans as opposed to the previously reported positive association (43, 115). This negative association of diabetes with progression of CIMT in Japanese Americans may be due to widespread use of lipid medications in participants with diabetes (54% of participants with diabetes were on lipid-lowering medications compared to 15% of the participants without diabetes); lipid medications lead to slowed progression of CIMT (116). The negative association between diabetes and progression of CIMT may also be explained by negative interaction between diabetes and lipid medication indicating that the effect of diabetes on progression of CIMT was not deleterious in participants with diabetes on lipid medication compared to participants with diabetes not on lipid medication. After excluding participants on lipid medications, diabetes was not associated with progression of CIMT in Japanese Americans (data not shown).

Progression of CIMT remained greater in Japanese Americans than whites after accounting for education, a surrogate marker of socio-economic status (data not shown). The significantly greater progression of CIMT in Japanese Americans than whites was driven by greater progression in the CB and ICA (Supplementary tables and figures, Figure 1 A-F); CIMT in the CB and ICA is associated with risk factors of atherosclerosis (117). Furthermore, progression of CIMT remained greater in Japanese Americans than whites even among

participants without diabetes and hypertension (Supplementary tables and figures, Figure 2 A and B). The propensity of significantly greater progression of CIMT in Japanese Americans than whites in the CB and ICA and greater progression in participants without diabetes and hypertension may suggest that Japanese Americans are more vulnerable than whites to accelerated atherosclerosis. This may be due to susceptibility of Japanese Americans than whites of developing metabolic derangements at lower BMI levels (118, 119). In this context, it is important to observe that BMI was marginally significantly associated with progression of CIMT in Japanese Americans but not in whites and significantly associated among participants without hypertension and diabetes (Supplementary tables and figures, Table 3). The susceptibility to developing metabolic derangements at lower levels of BMI in Japanese Americans may also explain the association of total cholesterol/HDL-C ratio to progression of CIMT.

Japanese Americans were followed for an average of two more years than whites. Although in our study the outcome was annualized progression of CIMT, biologically a non-linear progression in these two years may be suggested as a reason for greater progression in Japanese Americans than whites. The longer period of follow-up may be ruled out as an explanation for greater progression in Japanese Americans than whites in our study by the observation that the distribution of progression of CIMT was normal in both Japanese Americans and whites (Supplementary tables and figures, Figure 3 A and B).

Among Chinese, blacks, and Hispanic immigrants, the MESA reported an association of acculturation (incorporation into Western culture) assessed by place of birth and generation of US residence with greater CIMT independent of cardiovascular risk factors (114, 120). In the same line, acculturation may also be one of the explanations for greater progression of CIMT in these third or fourth generation Japanese Americans in our study.

In the past, CHD was reported to be lower among Japanese Americans than whites. A study based on the vital statistics of the 1951 reported lower CHD mortality in Japanese Americans than whites (121). Two recent studies reported lower prevalence or mortality of CHD in Japanese Americans than whites (122, 123). However, in these two studies a majority of the subjects were either first or second generation Japanese Americans (110, 121). It is highly likely that the first and second generation immigrants had retained a traditional Japanese lifestyle compared to the third and fourth generation Japanese Americans in our study who adopted Western lifestyle since birth. In our study, significantly greater progression of IMT in Japanese Americans than whites, therefore, may suggest a higher burden of CHD in these Japanese Americans than whites in the future.

The significance of progression of CIMT in predicting CHD is currently under debate. A recent meta-analysis by the PROG-IMT project (individual progression of carotid intima-media thickness as a surrogate of vascular risk) did not find a significant positive association between progression of CIMT and CHD (42). The baseline CIMT is significantly and positively associated with CHD independent of cardiovascular risk factors (36). However, slow progression of atherosclerosis and difficulty in detecting lower rates of progression in healthy individuals may have led to the null association between progression of CIMT and CHD in the PROG-IMT meta-analysis (42, 46). The association between progression of CIMT and CHD in this meta-analysis may have also been underestimated due to introduction of survivor selection bias because the participants with previous cardiovascular events or death due to cardiovascular disease were excluded. The use of progression of CIMT in predicting CHD requires further validation.

This study must be interpreted in the context of its known limitations. The sample only included men, thus the results cannot be generalized to women. We did not evaluate differences in physical activity levels between the two races, which slow progression of CIMT (124). There are methodological issues pertaining to progression of CIMT, especially the technique of correction for potential measurement error. However, the laboratory that performed the CIMT measurement for our study is very experienced and continuous quality control measures were employed to ensure accuracy of measurements (113). Moreover, progression of CIMT was greater in Japanese Americans than whites even without adjusting for the baseline CIMT (data not shown).

In conclusion, we found a greater progression of CIMT in Japanese American men than white men that was not explained by difference in baseline cardiovascular risk factors. The greater progression of CIMT in Japanese American men than white men may suggest a higher burden of CHD in Japanese American men in the future than white men. The current finding accentuates the importance of racial disparities in subclinical cardiovascular disease and may be utilized to target prevention and treatment of CHD.

3.6 TABLES AND FIGURES

Table 3-1 Baseline characteristic of participants in the ERA JUMP study, 2004-2007

	Japanese American (n=227)	**White (n=246)**	***P* Value**
Age (years)	46.3 (2.8)	45.0 (2.9)	<0.05
BMI (kg/m2)	27.7 (4.5)	27.9 (4.2)	0.52
Current Smokers (%)*	11.9	6.9	0.08
Alcohol Drinkers (%)†	36.6	44.7	0.08
SBP (mmHg)	126.4 (11.7)	122.6 (10.9)	<0.05
Hypertension (%)‡	29.1	13.8	<0.05
LDL-C (mmol/L)	3.1 (0.80)	3.5 (0.82)	<0.05
Total Cholesterol/HDL-C	4.0 (3.4, 5.0)	4.7 (3.9, 5.5)	<0.05
Lipid Medication (%)	24.2	13.1	<0.05
Glucose (mmol/L)	5.9 (5.6, 6.3)	5.6 (5.2, 5.9)	<0.05
Diabetes (%)§	13.2	3.7	<0.05
CRP (nmol/L)	5.6 (3.1, 11.6)	9.5 (5.1, 17.8)	<0.05

Categorical variables are expressed as (%) and continuous variables as mean (SD) or median (interquartile range)

BMI, indicates body-mass index; NS, not significant; SBP, systolic blood pressure; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; CRP, C-reactive protein

* Current smokers were considered if participants reported current use of cigarettes or having stopped smoking within the last 30 days

† Alcohol drinkers were considered if participants consumed alcohol≥2 days per week

‡Hypertension was defined as systolic blood pressure≥140 mm Hg, diastolic blood pressure≥90 mm Hg, or use of antihypertensive medications

§Diabetes was defined as fasting serum glucose level≥126 mg/dl or use of medications for type 2 diabetes

Table 3-2 Baseline CIMT, follow-up CIMT, and follow-up duration in Japanese Americans and whites in the ERA JUMP Study

	Japanese American (n=227)	**White (n=246)**	***P* Value**
Baseline Average CIMT (μm)	724.0 (122.2)	676.6 (103.7)	<0.05
Follow-up Average CIMT (μm)	820.3 (131.7)	722.4 (114.0)	<0.05
Follow-up time (years)	6.5 (0.50)	4.6 (0.21)	<0.05

mean (SD) are presented
CIMT indicates, intima-media thickness of the carotid artery

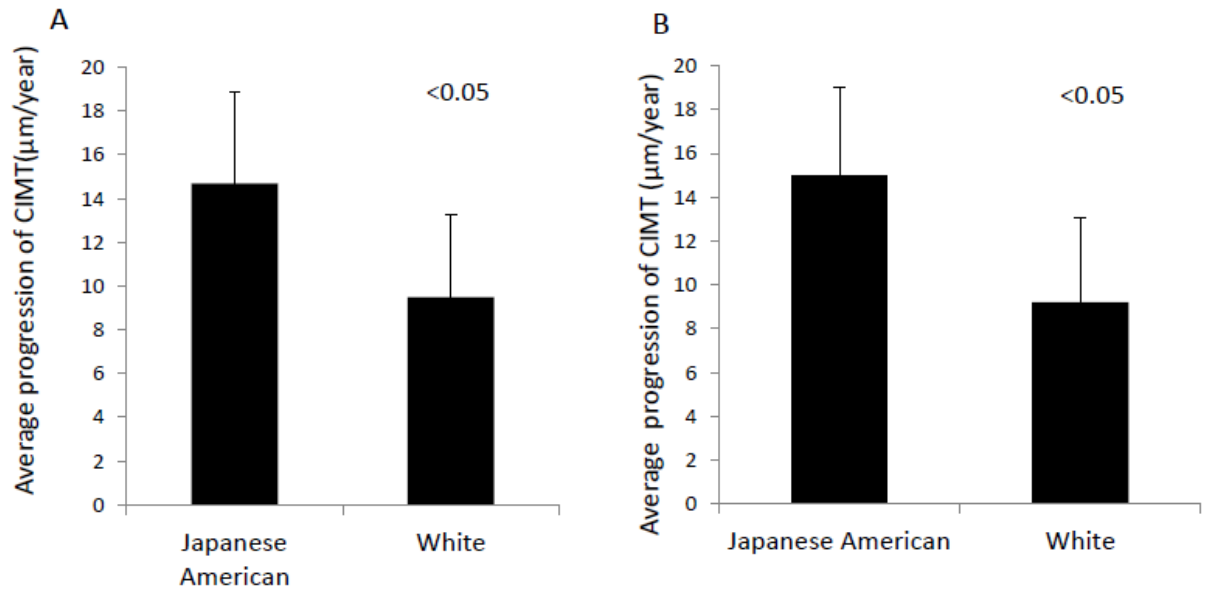

Figure 3-1 Progression (mean (95% confidence interval) of intima-media thickness of the carotid artery (CIMT) in Japanese American men and white men in the ERA JUMP Study, 2004-2007, unadjusted (A) and adjusted (B)

Table 3-3 Regression coefficients of progression of CIMT on cardiovascular risk factors (adjusted for baseline CIMT)

	Japanese Americans (n=227)		Whites (n=246)	
	B	*P*	B	*P*
Continuous Variables (Standardized Coefficients)				
Age	0.15	<0.05	0.23	<0.05
BMI	0.14	0.06	0.03	0.66
Log-total Cholesterol/HDL-C	0.15	<0.05	-0.04	0.53
Log-CRP	0.002	0.98	-0.07	0.30
Binary Variables (Unstandardized Coefficients (Standard Error))	b	*P*	b	*P*
Current Smoking	0.0003 (0.003)	0.92	0.007 (0.004)	0.06
Alcohol Drinkers	0.0002 (0.002)	0.94	-0.0008 (0.002)	0.68
Hypertension	0.002 (0.003)	0.41	0.006 (0.003)	<0.05
Diabetes	-0.008 (0.003)	<0.05	-0.005 (0.005)	0.37
Lipid medication	-0.002 (0.003)	0.52	-0.006 (0.003)	<0.05

Refer table 1 for definition and abbreviations

3.7 SUPLEMENTARY TABLES AND FIGURES

Table 3-4 Baseline (2004-07 characteristics of the participants who participated in the follow-up (2008-13) compared to the whole sample at baseline

	Whole Sample (n=613)	**Participants in follow-up (n=473)**	***P* Value**
Age (years)	45.6 (2.9)	45.6 (2.9)	0.87
Caucasians (%)	50.6	51.8	0.66
BMI (kg/m2)	28.0 (4.5)	27.8 (4.3)	0.47
Current Smokers (%)*	10.3	9.3	0.61
Alcohol Drinkers (%)†	40.8	40.6	0.99
SBP (mmHg)	125.1 (12.1)	124.5 (11.6)	0.33
Hypertension (%)‡	24.0	21.5	0.27
LDL-C (mmol/L)	3.3 (0.87)	3.3 (0.83)	0.83
Total Cholesterol/HDL-C	4.0 (3.4, 5.0)	4.7 (3.9, 5.5)	0.93
Lipid Medication (%)	17.6	18.5	0.75
Glucose (mmol/L)	5.7 (5.4, 6.1)	5.7 (5.4, 6.1)	0.78
Diabetes (%)§	8.7	8.2	0.83
CRP (pmol/L)	7.7 (3.8, 14.3)	7.6 (3.8, 14.0)	0.78
Baseline Average IMT (μm/y)	700.0 (120.0)	700.0 (120.0)	0.75

Categorical variables are expressed as (%) and continuous variables as mean (SD) or median (interquartile range)

BMI, indicates body-mass index; NS, not significant; SBP, systolic blood pressure; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; CRP, C-reactive protein; IMT, intima-media thickness of the carotid artery

*Current smokers were considered if participants reported current use of cigarettes or having stopped smoking within the last 30 days

† Alcohol drinkers were considered if participants consumed alcohol≥2 days per week

‡ Hypertension was defined as systolic blood pressure≥140 mm Hg, diastolic blood pressure≥90 mm Hg, or use of antihypertensive medications

§ Diabetes was defined as fasting serum glucose level≥126 mg/dl or use of medications for type 2 diabetes

Table 3-5 Regression coefficients of progression of CIMT on cardiovascular risk factors (baseline risk factors and change in risk factors) in the ERA JUMP Study, 2004-2007

	b (SE)	*P*
Variables		
Japanese American	0.005 (0.001)	<0.01
Medication for diabetes	-0.008 (0.003)	0.04

Model built using forward selection. Baseline age, baseline CIMT, medication for hypertension, and medication for lipid were forced in the model
CIMT indicates, intima-media thickness of the carotid artery

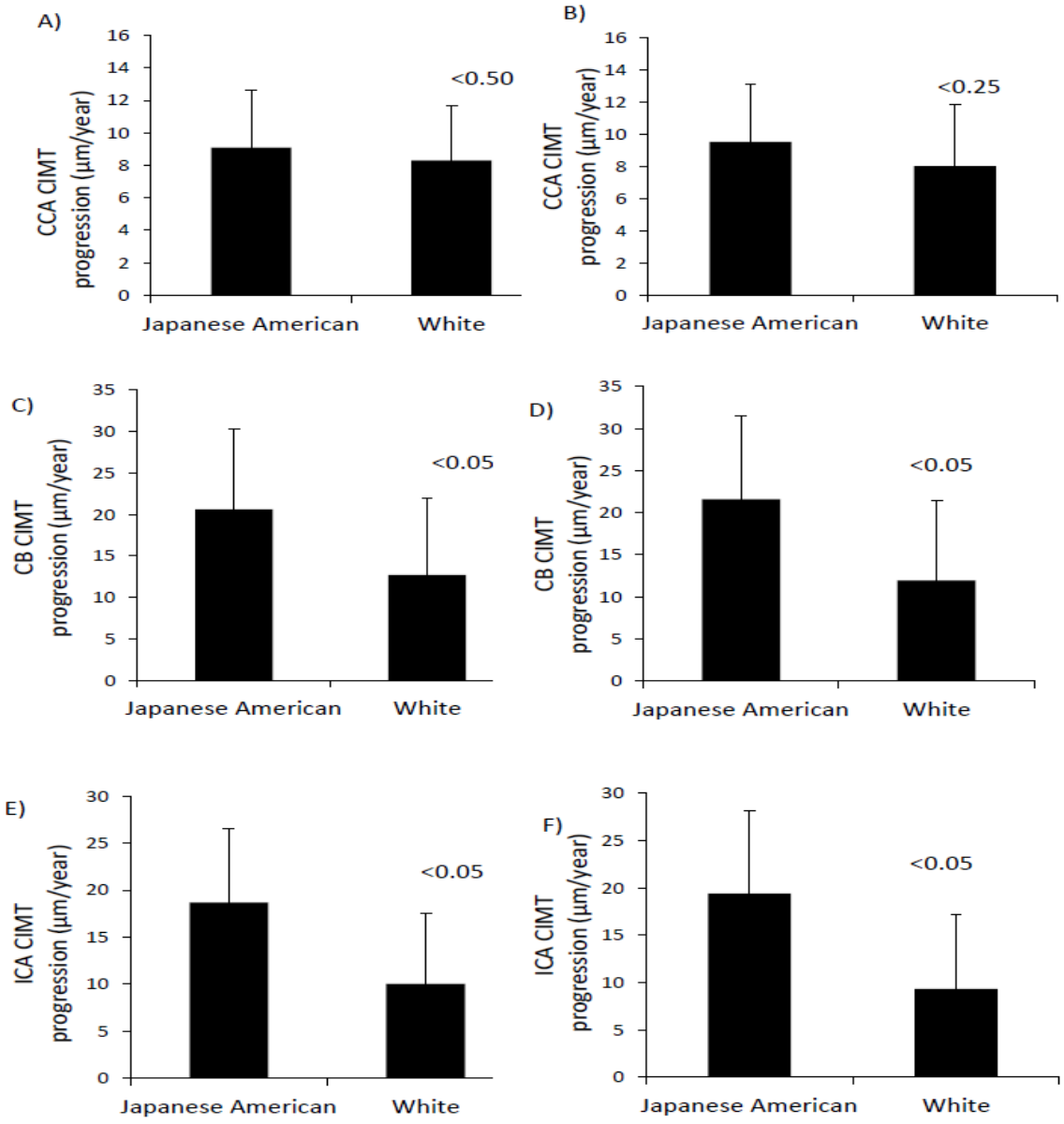

Figure 3-2 Unadjusted (A, C, and E) and adjusted (B, D, and F) progression (mean [95% confidence interval]) of the common carotid-artery (CCA), the carotid bulb (CB), and the internal carotid-artery (ICA), intima-media thickness of the carotid artery (CIMT) in the ERA JUMP study, 2004-2007

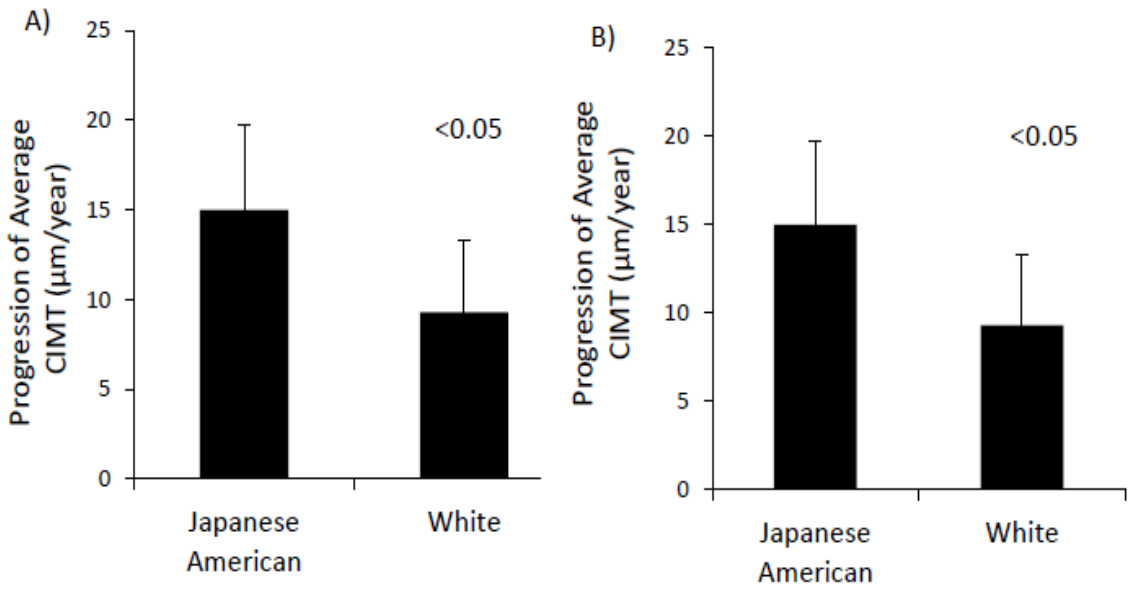

Figure 3-3 Progression (mean [95% confidence interval]) of intima-media thickness of the carotid artery (CIMT) in participants without hypertension and diabetes in the ERA JUMP study, 2004-2007, unadjusted (A) and adjusted (B)

Table 3-6 Regression coefficients of progression of CIMT on cardiovascular risk factors among participants without hypertension and diabetes in the ERA JUMP Study, 2004-2007 (adjusted for baseline CIMT)

	Japanese Americans (n=147)		Whites (n=204)	
	B	*P*	B	*P*
Continuous Variables (Standardized Coefficients)				
Age (years)	0.19	<0.05	0.22	<0.05
BMI (kg/m2)	0.30	<0.05	0.01	0.90
Log-total Cholesterol/HDL-C	0.04	0.61	-0.04	0.52
Log-CRP (mg/L)	-0.02	0.75	-0.04	0.56
Binary Variables (Unstandardized Coefficients (Standard Error))	b	*P*	b	*P*
Current smoking*	0.001 (0.003)	0.68	0.008 (0.004)	<0.05
Lipid medication	0.0003 (0.003)	0.93	-0.004 (0.003)	0.22

BMI, indicates body-mass index; NS, not significant; HDL-C, high-density lipoprotein-cholesterol; CRP, C-reactive protein

* Current smokers were considered as participants having current use of cigarettes or having stopped smoking within the last 30 days

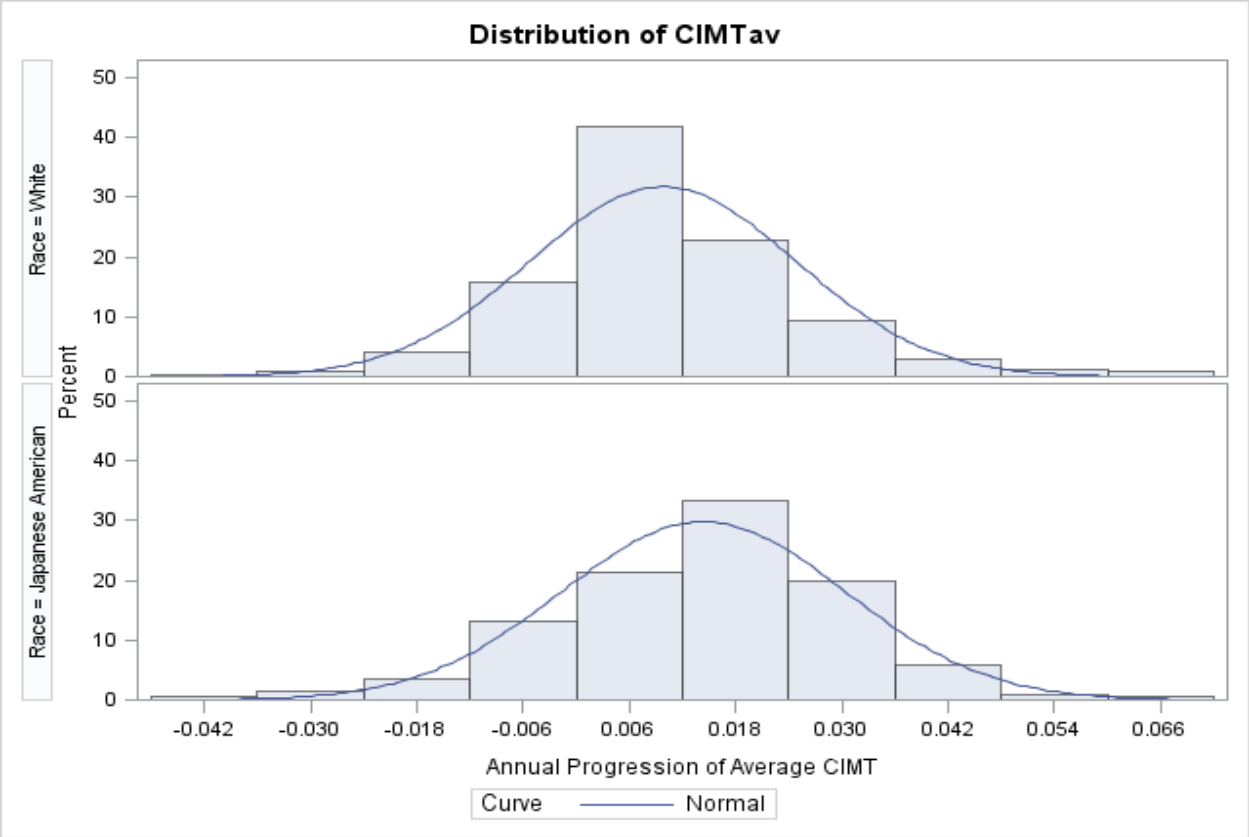

Figure 3-4 The distribution of annual progression of average intima-media thickness of the carotid artery (CIMTav) in Japanese Americans and whites

4.0 MANUSCRIPT 3: PROGRESSION OF CORONARY ARTERY CALCIUM IN MIDDLE-AGED JAPANESE AMERICAN MEN AND WHITE MEN: THE ERA JUMP STUDY

4.1 ABSTRACT

Aims: Progression of CAC is associated with higher risk of CHD. Progression of CAC is reported to be greater in whites than blacks, Hispanics, and Chinese in the US. Our objective was to compare progression of CAC between Japanese Americans and whites.

Methods: Population-based samples of 613 men (303 Japanese Americans [third or fourth generation without ethnic admixture] and 310 whites) aged 40-49 years and free of clinical cardiovascular disease were examined for CAC at baseline (2004-07) and follow-up (2008-2013). CAC was read centrally at the University of Pittsburgh. Progression of CAC (change in CCSs in participants with baseline CCSs>0 and incident CAC in participants with baseline CAC=0) was compared between the two races using multiple linear regression and relative risk regression adjusting for baseline cardiovascular risk factors and follow-up.

Results: Japanese Americans (11.3 Agatston units [1.4, 24.9], median [interquartile range]) had significantly greater annual change in CCSs than whites (2.5 [-0.22, 14.5]) in the unadjusted analyses. In the adjusted analyses, change in CCSs was not significantly different between

Japanese Americans and whites. In the adjusted analyses, the incidence rate ratio of CAC was similar (0.87 [95% CI, 0.20, 3.9]) between Japanese Americans and whites.

Conclusions: In contrast to previously reported greater progression of CAC in whites than other races, we found similar progression of CAC in Japanese Americans and whites. This may suggest an increasing burden of CHD in these Japanese Americans in the future.

4.2 INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in the United States (US) (125). The major underlying cause of CHD is atherosclerosis (125). Atherosclerotic burden of coronary arteries is highly and positively correlated with coronary artery calcium (CAC), which is determined non-invasively using computed tomography (17). Moreover, increased baseline CAC is a strong independent predictor of incident CHD above and beyond traditional risk factors , even among asymptomatic individuals (20). Prevalence of CAC differs among racial/ethnic groups independent of traditional risk factors (18, 126).

Serial scannings using computed tomography enables measurement of progression of CAC (23). Progression of CAC is shown to be associated with risk of CHD and all-cause mortality (27, 28, 127). Therefore, examining racial differences in progression of CAC may have a promising clinical advantage in assessing risk of CHD among races. However, only a few studies have examined racial differences in progression of CAC (29-31). No study has examined progression of CAC in Japanese Americans compared to other races in the US.

In this study, we aimed to compare progression of CAC between Japanese American men and white men in the Electron-Beam Computed Tomography and Risk Factor Assessment

among Japanese and US Men in the Post World War II Birth Cohort (ERA JUMP) study. Progression of CAC is reported to be greater in whites than other races in the US (30). Therefore, we hypothesize that progression of CAC is greater in white men than Japanese American men in the ERA JUMP study.

4.3 METHODS

Study Participants

We have described the methods for subject selection previously (77). Briefly, during 2002 to 2006, 613 men aged 40-49 years were randomly selected: 310 white men from the Allegheny County, Pennsylvania (77) and 303 Japanese American men from a representative sample of offspring of fathers who participated in the Honolulu Heart Program, Honolulu, Hawaii (78, 79). These offspring were third or fourth generation Japanese Americans without ethnic admixture (78). The participants were without clinical cardiovascular disease, type 1 diabetes, or other chronic disease (77). Informed consent was obtained from all participants. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Institutional Review Boards of the University of Pittsburgh, Pennsylvania and the Kuakini Medical Center, Honolulu, Hawaii. This study includes participants with both baseline (2002- 2006) and follow-up (2007-2013) CAC scans.

Measurement of coronary artery calcium

CAC was measured using electron beam computed tomography (EBCT) (Imatron C150, GE Medical Systems, South San Francisco, USA) at baseline both in Pittsburgh and Honululu, and follow-up in Pittsburgh (128). At the follow-up in Honolulu, CAC was measured using a 64 slice

multi-detector computed tomography (MDCT) (Toshiba Medical System Corporation, Tochigi Japan). Scanners were calibrated regularly by technicians using standardized protocol. A total of 40 contiguous three mm thick transverse images were obtained from the level of the aortic root to the apex of the heart. Images were recorded during a maximal breath hold using ECG guided triggering. CAC was considered to be present with three contiguous pixels (area=1mm^2) ≥130 Hounsfield units. One trained reader at the Cardiovascular Institute, University of Pittsburgh, read the images using a Digital-Imaging-and-Communication-in-Medicine workstation and software by the AccuImage Diagnostic Corporation, San Francisco that calculated coronary calcium scores with Agatston scoring method (24). The reader was blinded to participants' characteristics and the study centers. The intra-reader reproducibility of non-zero coronary calcium score had an intra-class correlation of 0.98.

Measurement of covariates

All participants underwent a physical examination, lifestyle questionnaire, and laboratory examination as described previously (77, 78). Body weight and height were measured while participants were wearing light clothing without shoes. Body-mass index (BMI) was calculated as weight (kg)/ height squared (m^2). Blood pressure was measured in the right arm of the seated participants after they emptied their bladder and sat quietly for five minutes, using an automated sphygmomanometer (BP-8800; Colin Medical Technology, Komaki Japan), and an appropriate sized cuff. The average of two measurements was used in the analyses. Venipuncture was performed early in the clinic visit after a 12 hour fast. Plasma and serum samples were stored at -80ºC, shipped to the University of Pittsburgh, and were assayed for glucose, and lipids including total cholesterol, triglycerides, low-density lipoprotein-cholesterol, and high-density lipoprotein-cholesterol (HDL-C).

Participants were considered current smokers if they reported current use of cigarettes or having stopped smoking within the last 30 days. Alcohol drinkers were defined as those who consumed alcohol ≥2 days per week. Hypertension and diabetes were defined using the criteria by the 7th Report of the Joint National Committee (111) and the 2013 American Diabetes Association guidelines respectively (85).

Statistical analysis

To compare baseline characteristics between Japanese Americans and whites, we used t-tests, Wilcoxon rank-sum tests or chi-square tests as appropriate. Progression of CAC was defined as following: change in coronary calcium scores (CCSs) in participants with baseline CCSs > 0 and incident CAC defined as CCSs ≥ 10 at the follow-up examination in participants with CCSs = 0 at baseline. We defined incident CAC as CCSs ≥ 10 because (19/67) 28 percent of Japanese Americans and (27/50) 28 percent of whites with CCSs >0 and <10 at baseline reverted to CCSs=0 at follow-up (supplementary material, table 4-6). These two end-points, change in CCSs and incident CAC, were modeled separately. The following covariates were adjusted in the multivariable models to examine whether change in CCSs and incidence of CAC differed between Japanese Americans and whites: age, BMI, systolic blood pressure (SBP), pack-years, diabetes (yes/no), total cholesterol/HDL-c ratio, hypertension medication (yes/no), lipid medication (yes/no), and follow-up time based on previous literature (30).

Among 303 participants with CCSs >0 at baseline (Japanese Americans = 148 and whites = 155), we defined change in CCSs using the Multi-Ethnic Study of Atherosclerosis (MESA) method: ln ($CCSs_{Follow\text{-}up}$ +25) - ln ($CCSs_{Baseline}$ +25) (30). This outcome was reasonably normally distributed; multiple linear regression was used to model this outcome adjusting for

covariates. In our secondary analysis, we modeled change in CCSs adjusting for the above-mentioned covariates and baseline CCSs.

Among 305 (Japanese Americans = 153 and whites = 152) participants free of CAC at baseline, we calculated incidence as the number of incident cases divided by the number of participants exposed to risk between baseline and follow-up (per 100 person-years). We used relative risk regression to model the probability of incident CAC using generalized linear model with log link and binomial error distribution, adjusting for covariates. Whenever these models did not converge, we used Poisson link with robust error variance. We used relative risk regression rather than logistic regression because the incidence of CAC was not rare in our study (occurred in 16 percent of the participants during the follow-up); in such cases odds ratio overestimates relative risk (129). *P*-values < 0.05 were considered significant. All *P* -values were 2-tailed. All statistical analyses were performed using SAS software, version 9.3, Cary, NC, US.

4.4 RESULTS

Change in coronary calcium scores

Table 1 describes the baseline characteristics of participants with baseline CCSs> 0. Japanese Americans were significantly older, had higher pack-years of smoking, SBP, and glucose than whites. Japanese Americans had significantly higher prevalence of current smoking, hypertension, diabetes, use of hypertension medication, and lipid medication than whites. On the other hand, Japanese Americans had significantly lower low-density lipoprotein-cholesterol and total cholesterol/HDL-C ratio than whites. Baseline characteristics of participants who attended

the follow-up exam were not significantly different from baseline characteristics of the whole group both for Japanese-Americans and whites (supplementary tables Table 4-7 and Table 4-8).

After a mean follow-up period of 5.5 years (±1.0), participants had a median rate of change in CCSs of 22.4 percent per year. Table 2 presents unadjusted comparison between Japanese Americans and whites in baseline CCSs, follow-up CCSs, and change in CCSs. In the unadjusted analyses, Japanese Americans had significantly higher median baseline CCSs, follow-up CCSs, and annual change in CCSs. Table 3 shows the regression models for change in CCSs over time among those having baseline CCSs > 0. There was no significant difference in change in CCSs between Japanese Americans and whites, after adjusting for cardiovascular risk factors and follow-up. The results for regression model remained similar after adjusting for baseline CAC (data not shown).

Incidence of coronary artery calcium

Table 4 describes the baseline characteristics of participants with baseline CCSs = 0 and unadjusted incidence rate of CAC in Japanese Americans and white. Japanese Americans were significantly older, had higher SBP, and glucose than whites. Japanese Americans had significantly higher prevalence of hypertension, diabetes, and use of lipid medication than whites. On the other hand, Japanese Americans had significantly lower low-density lipoprotein-cholesterol and marginally lower total cholesterol/HDL-C ratio than whites. Although incidence rate of CAC was lower in Japanese Americans was lower than whites, it was not significantly different. Baseline characteristics of participants who participated in follow-up were not significantly different from baseline characteristics of the whole group both for Japanese-Americans and whites (supplementary material, table 4-9 and table 4-10). Table 5 shows the

adjusted incidence rate ratio of CAC between Japanese Americans and white (0.87 [95% CI, 0.20, 3.9]); no significant difference was observed between Japanese Americans and white.

4.5 DISCUSSION

In this study, we aimed to compare progression of CAC between Japanese Americans and whites. We found baseline CCSs, follow-up CCSs, and annualized change in CCSs to be significantly higher in Japanese Americans than whites. However, we did not find a significant difference in change in CCSs between Japanese Americans and whites after adjusting for cardiovascular risk factors. Similarly, we found no significant difference in incident CAC between Japanese Americans and whites both in unadjusted and adjusted analyses. This is in contrast to our hypothesized greater progression of CAC in white men than Japanese American men.

Recently we reported higher incidence of CAC in whites than Japanese in Japan (130). Finding a similar incidence of CAC and change in CCSs in Japanese American and whites, in contrast to the findings in Japanese in Japan, may indicate increasing susceptibility to atherosclerosis in these third or fourth generation Japanese Americans. Acculturation (incorporation into Western culture) is a likely explanation for increasing atherosclerosis in these third or fourth generation Japanese Americans. This is in line with the positive association of acculturation, defined by the years in the US, and prevalence of CAC in Chinese, black, and Hispanic immigrants by the Multi-Ethnic Study of Atherosclerosis (MESA) (114). Moreover, Westernized lifestyle in Japanese Americans is reported to be associated with increased

metabolic derangements that may increase susceptibility to subclinical atherosclerosis (131, 132).

In contrast to our study, the MESA reported a greater change in CCSs over time and greater incidence in whites compared to blacks, Chinese, and Hispanics (30). In the MESA, 5756 participants aged 45 to 84 years were scanned after an average 2.4 years. In the MESA, the majority of Chinese and Hispanics were first generation or less acculturated in contrast to the third or fourth generation Japanese Americans in our study that may explain greater progression of CAC in whites compared to Chinese and Hispanics in the MESA. Two other studies have compared racial difference in progression of CAC between whites and other races, but these studies were limited by non-population-based specific samples (29, 31).

In the past, the incidence of CHD was lower in Japanese Americans than whites (121). Although some recent studies have reported lower prevalence of CHD or CHD mortality in Japanese Americans than whites; the majority of the participants in these studies were either first or second generation Japanese Americans in contrast to the third or fourth generation Japanese Americans in our study (122, 123, 133). It is likely that first and second generation immigrants had retained their traditional lifestyle unlike the third or fourth generation immigrants in our study who adopted Westernized lifestyle since birth. Therefore, finding similar progression of CAC in Japanese Americans as whites indicates an increasing burden of atherosclerosis in these third or fourth generation Japanese Americans that may translate into increasing risk of CHD in the future.

The calculation for change in CCSs is challenging because different analytical strategies such as change in raw scores, annual rate of change in CCSs, percent change, log-transformed change, which may lead to different conclusions. Therefore, it is difficult to draw comparison

among studies, many of which used different methods for calculating change. Moreover, certain standard analytical techniques, for e.g., modeling change in raw scores or percent change are highly influenced by outliers (30). The influence of outliers in analysis of CCSs is overcome by addition of constant in the MESA method that prevents very small baseline CCSs from leading to unreasonably large relative increase and down-weights exceptionally large follow-up CCSs (30).

We defined incident cases of CAC as CCSs ≥ 10 at follow-up in participants with CCSs=0 at baseline. Firstly, 32 percent (34/107) of participants having baseline CCSs >0 and < 10 reverted to CCSs= 0 at follow-up. Secondly, inter-scan variability of CCSs >0 and < 10 is much higher than CCSs ≥ 10 and therefore there is a possibility that CCSs between1-9 are noise (134). To avoid the possibility of noise due to inter-scan variability, the Dallas Heart Study defined presence of CAC as CCSs ≥ 10 (134). However, the MESA and Coronary Artery Risk Development in Young Adults (CARDIA) study defined incident CAC as follow-up CAC > 0 in participants with baseline CAC =0 (135). The possibility of a noise caused by inter-scan variability in the MESA and CARDIA studies is less because these studies scanned twice both at baseline and follow-up; whereas our study only scanned once at both the time points.

This study must be interpreted in the context of its known limitations. The sample only included healthy men, thus the results cannot be generalized to women and other populations. Our sample only included Japanese Americans and whites. Further studies are warranted to include other immigrant populations such as blacks and Hispanics. CAC was measured using the EBCT at baseline and MDCT at follow-up at Honolulu site. However, the inter-scan variability of 64-slice MDCT is reported to be similar to EBCT (136). Also, change in CCSs is a

complicated variable to handle because of error in measurement and lack of consensus in the ideal analytical strategies. Our study is also limited by a small sample size.

In conclusion, although we found a significant difference in annual change in CCSs between Japanese Americans and whites in unadjusted analyses, we did not find a significant difference in change in CCSs over time in the adjusted analyses. Also, we did not find a significant difference in incidence of CAC between Japanese Americans and whites. This is contrast to previously reported greater progression of CAC in whites than other races in the US. Finding similar progression of CAC in Japanese Americans and whites may indicate an increasing burden of atherosclerosis that may translate into increased risk of CHD in these third or fourth generation Japanese Americans in the future. Further studies with larger sample sizes are warranted to better evaluate the risk of CHD in this Japanese American population.

4.6 TABLES

Table 4-1 Characteristic of participants with coronary calcium scores greater than zero at baseline in the ERA JUMP study, 2004-2007

	Japanese Americans (n=148)	Whites (n=155)	*P* Value
Age (years)	46.5 (2.6)	45.5 (2.8)	<0.05
BMI (kg/m2)	29.0 (4.8)	29.3 (4.6)	0.24
Smoking			
Current (%)	17.0	7.1	<0.05
Past (%)	23.0	20.7	0.68
Pack- years	0.0 (0.0, 9.0)	0.0 (0.0, 3.0)	<0.05
Alcohol			
Drinkers (≥2 drinks/week, %)	37.8	39.4	0.81
Ethanol consumption (g/day)	0.7 (0.0, 29.8)	4.1 (0.41, 12.3)	0.38
SBP (mmHg)	129.4 (13.4)	124.2 (11.2)	<0.05
Hypertension (%) ‡	43.2	18.7	<0.05
Medication for hypertension (%)	28.4	11.0	<0.05
LDL-C (mmol/L)	3.2 (0.88)	3.6 (0.83)	<0.05
Total cholesterol/HDL-C	4.2 (3.6, 5.2)	4.8 (4.2, 5.6)	<0.05
Lipid medication (%)	29.7	16.8	<0.05
Glucose (mmol/L)	6.0 (5.7, 6.6)	5.5 (5.2, 5.9)	<0.05
Diabetes (%) §	17.0	5.8	<0.05

Categorical variables are expressed as (%) and continuous variables as mean (SD) or median (interquartile range)

BMI, indicates body-mass index; SBP, systolic blood pressure; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol

* Current smokers were considered if participants reported current use of cigarettes or having stopped smoking within the last 30 days

‡Hypertension was defined as systolic blood pressure≥140 mm Hg, diastolic blood pressure≥90 mm Hg, or use of antihypertensive medications

§Diabetes was defined as fasting serum glucose level≥126 mg/dl or use of medications for type 2 diabetes

Table 4-2 Comparison between Japanese Americans and whites in baseline CCSs, follow-up CCSs, and annual change in CCSs

	Japanese Americans	Whites	*P* Value
CCSs at baseline (n=303)	34.2 (4.4, 110.8)	12.0 (3.3, 41.4)	<0.05
CCSs at follow-up (n=230)	108.3 (19.0, 268.4)	25.6 (2.8, 112.6)	<0.05
Annual change in CCSs* (n=230)	11.3 (1.4, 24.9)	2.5 (-0.22, 14.5)	<0.05
Follow-up time (years)	6.6 (0.48)	4.7 (0.2)	<0.5

Median (interquartile range)
CCSs indicates, coronary calcium scores
* Annual change in CCSs was defined as: [(CCSs Follow-up - CCSs Baseline)/Follow-up)]

Table 4-3 Regression models for change in CCSs over time among those having greater than zero CCSs at baseline*

	MESA method‡ (n=230)	*P* value
	b (SE)	
Japanese American	-0.07 (0.24)	0.77
Age	0.04 (0.01)	<0.05
BMI	-0.01 (0.01)	0.34
SBP	0.006 (0.003)	0.09
Pack-years	0.0002 (0.004)	0.93
Log-total cholesterol/HDL-C	-0.04 (0.16)	0.61
Diabetes	0.08 (0.15)	0.58
Hypertension medications	0.01 (0.12)	0.92
Lipid medication	0.04 (0.11)	0.73

*Adjusted for follow-up duration
‡MESA method: [ln (CCSs Follow-up +25) - ln (CCSs Baseline +25)]
§Significant at <0.05
CCSs indicates coronary calcium scores; BMI, body-mass index; SBP, systolic blood pressure; HDL-C, high-density lipoprotein-cholesterol

Table 4-4 Characteristic of participants with zero CAC at baseline, incidence rate of CAC, and follow-up duration in the ERA JUMP study, 2004-2007

	Japanese Americans (n=152)	Whites (n=153)	*P* Value
Age (years)	45.7 (2.9)	44.6 (2.8)	<0.05
BMI (kg/m2)	26.7 (3.5)	26.4 (3.1)	0.37
Smoking			
Current (%)	8.6	7.8	0.83
Past (%)	20.4	18.3	0.67
Pack- years	0.0 (0.0, 0.53)	0.0 (0.0, 1.0)	0.87
Alcohol			
Drinkers (≥2 drinks/week, %)	37.8	39.4	<0.05
Ethanol consumption (g/day)	1.2 (0.0, 24.7)	6.2 (1.0, 16.5)	0.05
SBP (mmHg)	125.9 (11.5)	120.8 (10.8)	<0.05
Hypertension (%) ‡	22.4	11.1	<0.05
Medication for hypertension (%)	12.5	6.5	0.08
LDL-C (mmol/L)	3.1 (0.82)	3.4 (0.91)	<0.05
Total cholesterol/HDL-C	4.0 (3.4, 4.8)	4.4 (3.6, 5.2)	0.06
Lipid medication (%)	16.5	7.8	<0.05
Glucose (mmol/L)	5.9 (5.6, 6.3)	5.5 (5.2, 5.8)	<0.05
Diabetes (%) §	9.9	1.3	<0.05
CAC incidence rate (/100 person year)	1.4	2.2	0.55
Follow-up (years)	6.5 (0.49)	4.6 (0.22)	<0.05

Categorical variables are expressed as (%) and continuous variables as mean (SD) or median (interquartile range)

CAC indicates, coronary artery calcium; BMI, body-mass index; SBP, systolic blood pressure; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol

* Current smokers were considered if participants reported current use of cigarettes or having stopped smoking within the last 30 days

‡Hypertension was defined as systolic blood pressure≥140 mm Hg, diastolic blood pressure≥90 mm Hg, or use of antihypertensive medications

§Diabetes was defined as fasting serum glucose level≥126 mg/dl or use of medications for type 2 diabetes

Table 4-5 Relative risk regression for incident CAC among those having zero CAC at baseline* (n=231)

	IRR (95% CI)	*P* Value
Japanese American	0.87 (0.20, 3.9)	0.86
Age	1.0 (0.9, 1.1)	0.84
BMI	1.1 (1.0, 1.2)	0.07
SBP	0.98 (0.96, 1.0)	0.23
Pack-years	1.0 (1.0, 1.1)	0.04
Log-total cholesterol/HDL-C	0.98 (0.71, 1.3)	0.89
Diabetes	0.56 (0.14, 2.3)	0.42
Hypertension medication	1.4 (0.59, 3.2)	0.47
Lipid medication	1.0 (0.44, 2.4)	0.96

*Adjusted for follow-up duration

[§]Significant at <0.05

CAC indicates coronary artery calcium; IRR, incidence rate ratio; CI, confidence interval; BMI, body-mass index; SBP, systolic blood pressure; HDL-C, high-density lipoprotein-cholesterol

4.7 SUPPLEMENTARY TABLES

Table 4-6 The number of participants by coronary calcium scores at baseline and follow-up in Japanese American and white

Japanese American

CCSs at baseline	CCSs at follow-up				
	0	0 < & <10	10 ≤ & <100	100 ≤	Total
0	86	11	19	2	118
0 < & <10	15	4	17	4	40
10 ≤ & <100	1	1	9	24	35
100 ≤	0	0	0	25	25
Total	102	16	45	55	218

White

CCSs at baseline	CCSs at follow-up				
	0	0 < & <10	10 ≤ & <100	100 ≤	Total
0	72	26	14	1	113
0 < & <10	19	29	16	3	67
10 ≤ & <100	2	2	27	20	51
100 ≤	0	0	0	12	12
Total	93	57	57	36	243

CCSs, indicates coronary calcium scores

Table 4-7 Comparison of baseline (2004-07) characteristics of Japanese American participants with CCSs >0, whole sample vs participants in the follow-up (2008-13)

	Whole Sample (n=148)	Follow-up Participants (n=100)	P Value
Age (years)	46.5 (2.6)	46.7(2.5)	0.78
BMI (kg/m2)	29.0 (4.8)	28.6 (4.8)	0.92
Smoking			
Current (%)	17.0	16.0	1.0
Past (%)	23.0	26.0	0.65
Pack- years	0.0 (0.0, 9.0)	0.0 (0.0, 10.2)	0.55
Alcohol			
Drinkers (≥2 drinks/week, %)	37.8	34.0	0.59
Ethanol consumption (g/day)	0.7 (0.0, 29.8)	0.5 (0.0, 24.7)	0.76
SBP (mmHg)	129.4 (13.4)	126.9 (11.8)	0.18
Hypertension (%) ‡	43.2	38.0	0.43
Medication for hypertension (%)	28.4	26.0	0.77
LDL-C (mmol/L)	3.2 (0.88)	3.1 (0.75)	0.08
Total cholesterol/HDL-C	4.2 (3.6, 5.2)	4.0 (3.5, 5.0)	0.78
Lipid medication (%)	29.7	31.0	0.89
Glucose (mmol/L)	6.0 (5.7, 6.6)	6.0 (5.7, 6.7)	0.76
Diabetes (%) §	17.0	17.0	1.0
Baseline CCSs	34.2 (4.4, 110.8)	33.0 (3.3, 102.9)	0.59

Categorical variables are expressed as (%) and continuous variables as mean (SD) or median (interquartile range)

CCSs indicates, coronary calcium scores; NS, indicates not significant; BMI, body-mass index; SBP, systolic blood pressure; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol

* Current smokers were considered if participants reported current use of cigarettes or having stopped smoking within the last 30 days

‡Hypertension was defined as systolic blood pressure≥140 mm Hg, diastolic blood pressure≥90 mm Hg, or use of antihypertensive medications

§Diabetes was defined as fasting serum glucose level≥126 mg/dl or use of medications for type 2 diabetes

Table 4-8 Comparison of baseline (2004-07) characteristics of white participants with CCSs >0, whole sample vs participants in the follow-up (2008-13)

	Whole Sample (n=155)	Follow-up Participants (n=130)	*P* Value
Age (years)	45.5(2.8)	45.4 (2.8)	0.92
BMI (kg/m2)	29.3 (4.6)	29.1 (4.4)	0.52
Smoking			
Current (%)	7.1	6.2	0.82
Past (%)	20.7	18.5	0.66
Pack- years	0.0 (0.0, 3.0)	0.0 (0.0, 0.0)	0.69
Alcohol			
Drinkers (≥2 drinks/week, %)	39.4		0.71
Ethanol consumption (g/day)	4.1 (0.41, 12.3)	36.9 3.3 (0.41, 12.3)	0.80
SBP (mmHg)	124.2 (11.2)	123. 4 (10.9)	0.72
Hypertension (%) ‡	18.7	16.2	0.64
Medication for hypertension (%)	11.0	10.8	1.0
LDL-C (mmol/L)	3.6 (0.83)	3.6 (0.83)	0.80
Total cholesterol/HDL-C	4.8 (4.2, 5.6)	5.0 (4.2, 5.6)	0.75
Lipid medication (%)	16.8	17.7	0.88
Glucose (mmol/L)	5.5 (5.2, 5.9)	5.6 (5.2, 5.9)	0.88
Diabetes (%) §	5.8	5.4	1.0
Baseline CCSs	12.0 (3.3, 41.4)	9.6 (3.1, 32.9)	0.80

Refer to table 4-7 for definitions and abbreviations

Table 4-9 Comparison of baseline (2004-07) characteristics of Japanese American participants with CCSs = 0, whole sample vs participants in the follow-up (2008-13)

	Whole Sample (n=152)	Follow-up Participants (n=118)	*P* Value
Age (years)	45.7 (2.9)	45.8(2.9)	0.72
BMI (kg/m2)	26.7 (3.5)	26.7 (3.5)	0.95
Smoking			
Current (%)	8.6	8.5	1.0
Past (%)	20.1	21.2	0.88
Pack- years	0.0 (0.0, 0.53)	0.0 (0.0, 0.55)	0.99
Alcohol			
Drinkers (≥2 drinks/week, %)	37.7	36.4	0.90
Ethanol consumption (g/day)	1.2 (0.0, 24.7)	0.75 (0.0, 21.4)	0.81
SBP (mmHg)	125.9 (11.5)	125.9 (11.7)	0.98
Hypertension (%)	22.4	23.7	0.88
Medication for hypertension (%)	12.5	13.6	0.86
LDL-C (mmol/L)	3.1 (0.82)	3.1 (0.79)	0.83
Total cholesterol/HDL-C	4.0 (3.4, 4.8)	4.0 (3.4, 4.8)	1.0
Lipid medication (%)	16.5	19.5	0.53
Glucose (mmol/L)	5.9 (5.6, 6.3)	5.9 (5.6, 6.3)	0.97
Diabetes (%)	9.9	10.2	1.0

Refer to table 4-7 for definitions and abbreviations

Table 4-10 Comparison of baseline (2004-07) characteristics of white participants with CCSs = 0, whole sample vs participants in the follow-up (2008-13)

	Whole Sample (n=153)	Follow-up Participants (n=113)	*P* Value
Age (years)	44.6(2.8)	44.7 (2.9)	1.0
BMI (kg/m2)	26.4 (3.1)	26.4 (3.0)	0.95
Smoking			
Current (%)	7.8		1.0
Past (%)	18.3	8.0	1.0
Pack- years	0.0 (0.0, 1.1)	18.6 0.0 (0.0, 1.1)	0.94
Alcohol			
Drinkers (≥2 drinks/week, %)	49.7		0.46
Ethanol consumption (g/day)	6.2 (1.0, 16.5)	54.9 8.2 (1.7, 18.5)	0.71
SBP (mmHg)	120.8 (10.8)	121. 6 (11.0)	0.57
Hypertension (%)	11.1	11.5	1.0
Medication for hypertension (%)	6.5	5.3	0.80
LDL-C (mmol/L)	3.4 (0.91)	3.5 (0.81)	0.78
Total cholesterol/HDL-C	4.4 (3.6, 5.2)	4.4 (3.6, 5.2)	1.0
Lipid medication (%)	7.8	8.0	1.0
Glucose (mmol/L)	5.5 (5.2, 5.8)	5.5 (5.2, 5.8)	0.88
Diabetes (%)	1.3	1.8	1.0

Refer to table 4-7 for definitions and abbreviations

5.0 DISCUSSION AND PUBLIC HEALTH SIGNIFICANCE

This work focuses on health disparities between white men and Japanese men with regard to measures of diabetes and subclinical atherosclerosis. Health disparities research seeks to identify, understand, and eliminate differences in health status between groups of individuals defined by racial or ethnic background, socioeconomic position, age, gender, or sexual orientation. This work identified significant differences in health status between white men and Japanese men which are of public health importance.

White men are less susceptible to developing type 2 diabetes than Japanese men in Japan at a any given BMI level (137). The first manuscript therefore delves into pathophysiological differences in insulin resistance and insulin secretion between white men and Japanese men to explore whether these differences contribute to less susceptibility of white men to developing type 2 diabetes than Japanese men.

The first manuscript compares markers of insulin resistance and insulin secretion between US white men and Japanese men in Japan without diabetes in the ERA JUMP study. HOMA-IR, the marker of insulin resistance and HOMA-β% and DI (insulin secretion relative to insulin resistance), the markers of insulin secretion were compared between the two races. In the adjusted analyses, at p <0.01, white men had significantly higher HOMA-IR, HOMA-β%, and DI than Japanese men (geometric mean [95% CI]): 3.1 (2.9, 3.2) vs 2.5 (2.4, 2.7), 121.9 (114.9, 129.3) vs 93.5 (88.0, 99.3), and 40.0 (38.8, 41.1) vs 37.0 (35.9, 38.1), respectively. The higher

VAT and other risk factor adjusted DI in white men compared to Japanese men suggests that insulin resistance is better compensated for in white men than Japanese men; this may partly explain lower susceptibility of white people than Japanese people to developing type 2 diabetes at a given level of BMI.

There are several areas of future research related to comparison of markers of insulin resistance and insulin secretion between whites and Japanese. First, we obtained measures of insulin resistance and insulin secretion using fasting insulin and glucose. Although commonly used and widely acceptable in population-based studies, HOMA-IR, HOMA-β%, and DI do not provide accurate estimate of insulin resistance and insulin secretion, respectively. Therefore, future studies should be performed comparing these markers in whites and Japanese using formal methods for measuring insulin resistance and insulin secretion. Second, further studies should examine the reasons for difference in insulin secretion between these two races. Finding the reasons for poorer compensation of insulin resistance by increased insulin secretion in Japanese men in Japan than white men in the US may help in preventing earlier onset of type 2 diabetes in Japanese men than white men at a given level of BMI. Third, our study only included middle-age men. Future work should be performed on women and participants of other age groups.

The second and third manuscripts have Japanese Americans as the focus. Japanese Americans are a special group and different from Japanese in Japan because after migrating to the US, levels of BMI and prevalence and incidence of type 2 diabetes increase in them compared to native Japanese (96, 97). This increases the future risk of CHD in Japanese Americans compared to native Japanese. Several studies reported increased prevalence, incidence, and mortality of CHD in Japanese Americans than native Japanese (79, 138). Some recent studies, including ours, have reported increased subclinical atherosclerosis in Japanese

Americans than native Japanese (77, 131, 139). In the past, CHD mortality was reported to be lower in Japanese Americans than whites (121). However, with increased BMI and type 2 diabetes levels in Japanese Americans, it is important to study where Japanese Americans currently stand in terms of subclinical atherosclerosis compared to whites. This will provide an estimate of CHD risk in Japanese Americans compared to whites.

The second manuscript compared progression of CIMT, a measure of subclinical atherosclerosis, between Japanese Americans and whites in the ERA JUMP study. Although still currently debated, progression of intima-media thickness of the carotid artery is proposed to be a predictor of coronary heart disease. Progression of CIMT was significantly greater in Japanese Americans than whites both before and after adjusting for covariates (mean in μm/year [95% confidence interval]): 14.4 (12.3, 16.4) vs. 9.8 (7.8, 11.7), $P<0.01$ and 14.5 (12.4, 16.5) vs. 9.9 (9.8, 11.7), $P<0.05$, respectively. The significantly greater progression of CIMT in Japanese Americans than whites suggests a potentially higher burden of CHD in Japanese Americans than whites in the future.

The third manuscript compared progression of CAC, another measure of subclinical atherosclerosis, between Japanese Americans and whites in the ERA JUMP study. Progression of CAC is positively associated with CHD. In the adjusted analyses, change in CCSs and incidence of CAC were not significantly different between Japanese Americans and whites. Finding similar progression of CAC may indicate an increasing risk of CHD in these Japanese Americans. The public health significance of the second and third manuscripts is that these identify Japanese Americans as a target group for prevention of CHD. However, future population-based studies with large sample sizes are warranted to confirm these findings.

It is interesting to observe that subclinical atherosclerosis levels in Japanese Americans are higher or similar to that of whites. This is especially notable given that Japan has one of the lowest risks of CHD among developed countries (125). So, the question is how the risk of atherosclerosis increases after Japanese move to the US? Some studies have shown that acculturation, defined by the country of birth and number of years in the US is reported to be positively associated with subclinical atherosclerosis in other immigrant populations (114). Therefore, acculturation is one likely explanation for increasing subclinical atherosclerosis in these third or fourth generation Japanese Americans in our study. Moreover, acculturation may serve as a proxy measure of adoption of Americanized lifestyle by these Japanese Americans. Studies have shown that adoption of American lifestyle by Japanese Americans compared to Japanese in Japan in terms of increased animal protein in diet, increased saturated fat, and decreased physical activity is associated with increased CIMT and progression of CIMT in Japanese Americans than Japanese in Japan (131, 139).

There are several strengths of this research work. The participants of the ERA JUMP study includes healthy population-based sample of middle-aged men. Studying measures of diabetes and biomarkers of atherosclerosis is especially useful in this group for prevention of future CHD. Several established research laboratory handled data process of the ERA JUMP study. The Heinz laboratory at the University of Pittsburgh systematically examined serum samples from all sites. The Ultrasound Research Laboratory at the University of Pittsburgh examined and kept quality control check on assessment of CIMT. This Ultrasound Research Laboratory is one of the pioneers in establishing validity and reproducibility for some of the subclinical vascular measures (113). The Cardiovascular Institute at the University of Pittsburgh

Medical Center examined CAC from all the study centers. One trained reader examined CAC with high intra-class correlation.

APPENDIX: ADDITIONAL ANALYSIS CHAPTER 2: MANUSCRIPT 1-ANALYSIS STRATIFIED BY BODY MASS INDEX

Table A-1 Characteristics of normal weight participants in the ERA JUMP study, 2004-2007

Characteristic	White men	Japanese men	*P* value
Normal weight /Total (n, %) [a]	82 (27.5)	129 (43.9)	
Age (years)	45.2 (2.8)	45.0 (2.8)	NS
BMI (kg/m^2)	29.4 (3.9)	25.5 (2.2)	<0.01
VAT (cm^2)	192.2 (73.5)	157.1 (45.3)	<0.01
Systolic BP (mmHg)	125.0 (10.6)	127.8(16.1)	NS
Current smokers (%)[b]	6.1	59.7	<0.01
Alcohol drinkers (%)[c]	43.9	68.2	<0.01
Hypertension (%)[d]	4.9	17.1	<0.01
Glucose (mmol/l)	5.6 (5.2, 5.9)	5.8 (5.6, 6.1)	<0.01
Insulin (pmol/l)	95.2 (76.1, 135.1)	72.2 (59.0, 91.7)	<0.01
Triacylglycerol (mmol/l)	1.5 (1.1, 2.2)	1.7 (1.3, 2.3)	NS
LDL-cholesterol (mmol/l)	3.6 (0.86)	3.4 (0.93)	NS
HDL-cholesterol (mmol/l)	1.2 (0.31)	1.4 (0.33)	<0.01
CRP (nmol/l)	10.9 (6.1, 18.0)	3.8 (1.8, 7.1)	<0.01
Adiponectin (mg/l)	9.8 (7.4, 12.6)	5.5 (4.2, 7.7)	<0.01
IFG (%)[e]	42.7	55.8	NS
Hypertension medication (%)	1.2	5.4	NS
Lipid medication (%)	8.5	3.1	NS
Physical activity(%)[f]	81.7	24.8	<0.01
Family history of type 2 diabetes(%)[g]	14.6	19.4	NS

Values are means (SD) unless specified otherwise

[a] Normal weight was defined as BMI < 25 kg/m2 for whites and BMI < 23 kg/m2 for Japanese

[b]Current smokers were defined as having reported current use of cigarettes or having stopped smoking within the past 30 days

[c]Alcohol drinkers were defined as those who consumed alcohol ≥2 times/week

[d]Hypertension was defined as systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg, or use of antihypertensive medications

[e]IFG was defined as fasting serum glucose level ≥5.6 mmol/l

[f]Physical activity was defined as exercise ≥1 h in a week

[g]Family history of type 2 diabetes was defined as either father or mother of participant having type 2 diabetes

NS, not significant

Table A-2 Characteristics of overweight participants in the ERA JUMP study, 2004-2007

Characteristic	White men	Japanese men	*P* value
Overweight/Total (n, %)[a]	216 (72.5)	165 (56.1)	
Age (years)	45.1 (2.8)	45.0 (2.8)	NS
BMI (kg/m^2)	29.4 (3.9)	25.5 (2.2)	<0.01
VAT (cm^2)	192.2 (73.5)	157.1 (45.3)	<0.01
Systolic BP (mmHg)	125.0 (10.6)	127.8(16.1)	NS
Current smokers (%)[b]	7.9	43.0	<0.01
Alcohol drinkers (%)[c]	45.0	66.1	<0.01
Hypertension (%)[d]	18.1	30.3	<0.01
Glucose (mmol/l)	5.6 (5.2, 5.9)	5.8 (5.6, 6.1)	<0.01
Insulin (pmol/l)	95.2 (76.1, 135.1)	72.2 (59.0, 91.7)	<0.01
Triacylglycerol (mmol/l)	1.5 (1.1, 2.2)	1.7 (1.3, 2.3)	NS
LDL-cholesterol (mmol/l)	3.6 (0.86)	3.4 (0.93)	NS
HDL-cholesterol (mmol/l)	1.2 (0.31)	1.4 (0.33)	<0.01
CRP (nmol/l)	10.9 (6.1, 18.0)	3.8 (1.8, 7.1)	<0.01
Adiponectin (mg/l)	9.8 (7.4, 12.6)	5.5 (4.2, 7.7)	<0.01
IFG (%)[e]	51.9	78.2	<0.01
Hypertension medication (%)	10.2	4.9	NS
Lipid medication (%)	13.4	3.0	<0.01
Physical activity(%)[f]	69.4	27.3	<0.01
Family history of type 2 diabetes(%)[g]	29.6	26.7	NS

Values are means (SD) unless specified otherwise

[a] Overweight was defined as BMI≥25 kg/m^2 for whites and BMI≥23 kg/m^2 for Japanese

[b]Current smokers were defined as having reported current use of cigarettes or having stopped smoking within the past 30 days

[c]Alcohol drinkers were defined as those who consumed alcohol ≥2 times/week

[d]Hypertension was defined as systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg, or use of antihypertensive medications

[e]IFG was defined as fasting serum glucose level ≥5.6 mmol/l

[f]Physical activity was defined as exercise ≥1 h in a week

[g]Family history of type 2 diabetes was defined as either father or mother of participant having type 2 diabetes

NS, not significant

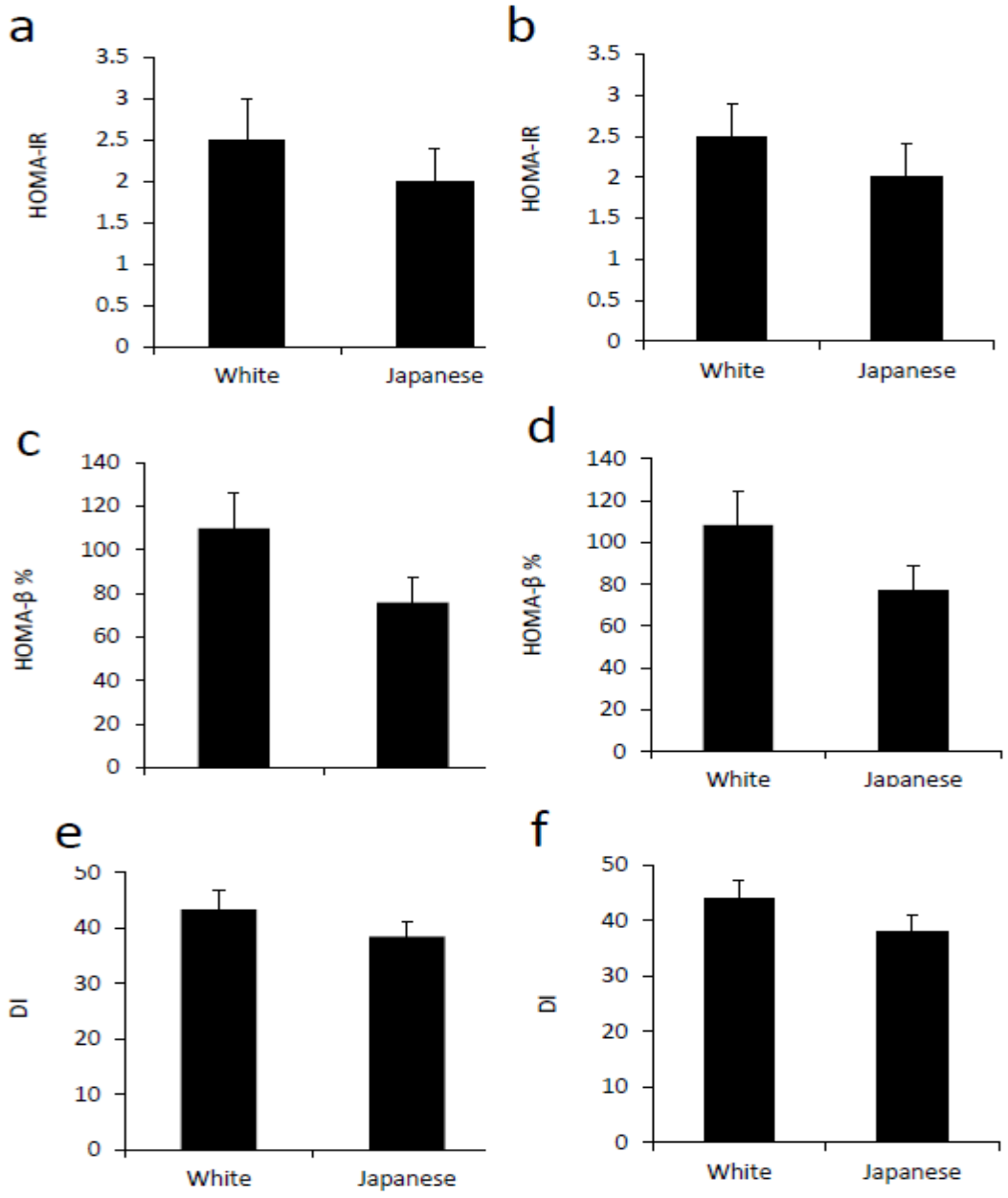

Figure A-1 HOMA-IR, HOMA-β %, and disposition index (DI) by race in normal weight participants without (a, c, e respectively) and with (b, d, f respectively) adjustment for VAT in the ERA JUMP study. Data are geometric mean ± confidence interval. HOMA-IR, HOMA-β %, and DI were significantly different before and after adjustment for VAT at *P* <0.01

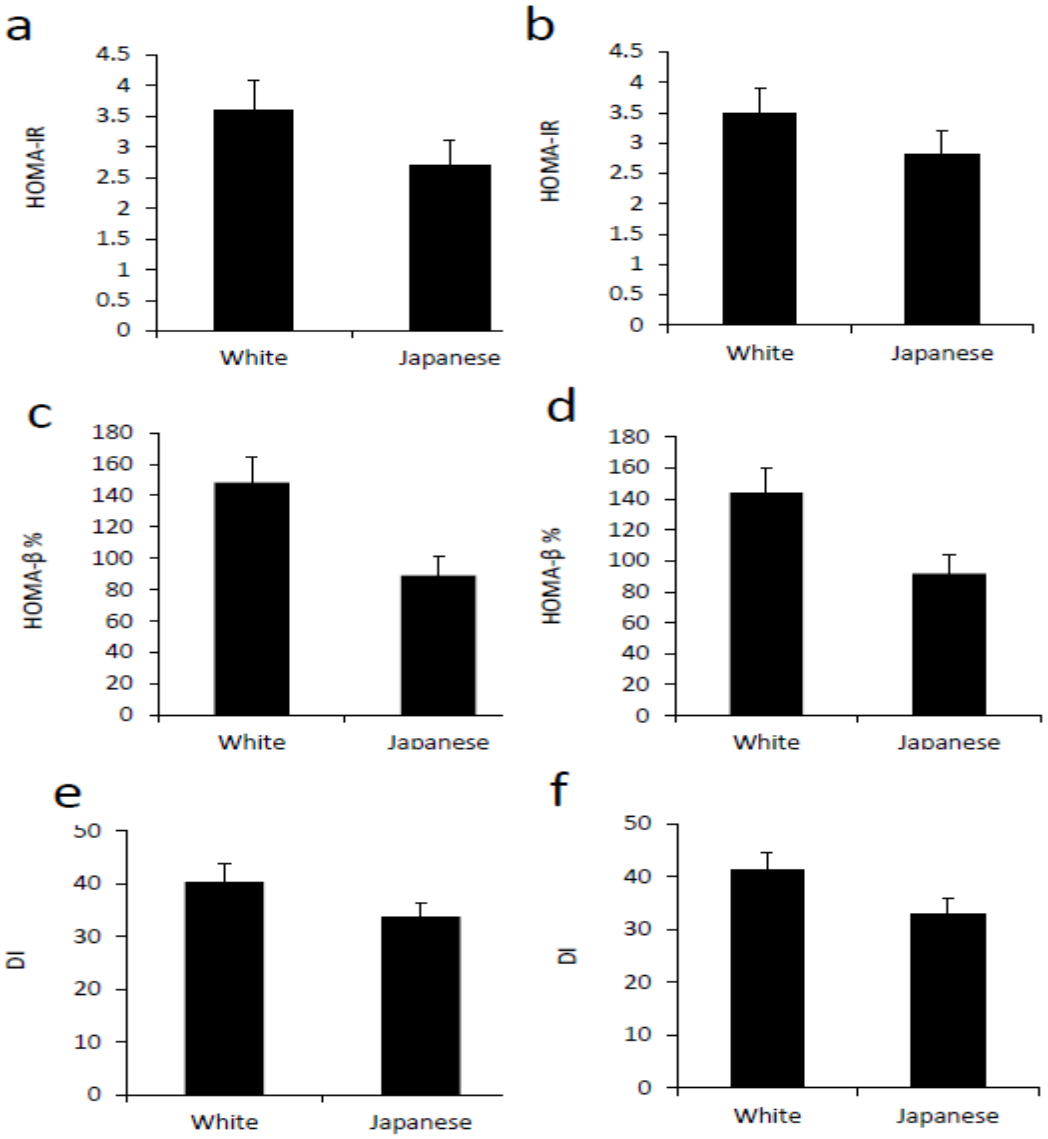

Figure A-2 HOMA-IR, HOMA-β %, and disposition index (DI) by race in overweight participants without (a, c, e respectively) and with (b, d, f respectively) adjustment for VAT in the ERA JUMP study. Data are geometric mean ± confidence interval. HOMA-IR, HOMA-β %, and DI were significantly different before and after adjustment for VAT at *P* <0.01

Table A-3 Comparison of HOMA-IR, HOMA-β% and DI in normal weight[a] white men and Japanese men in the ERA JUMP study[b]

	White men (n=82)	Japanese men (n=129)	*P* value
Insulin resistance marker			
HOMA-IR	2.4 (2.2, 2.7)	2.1 (1.9, 2.2)	<0.05
Insulin secretion markers			
HOMA-β%	102.0 (91.4, 113.7)	80.5 (74.3, 87.3)	<0.01
DI	42.2 (39.8, 44.7)	38.8 (37.2, 40.6)	0.06

Values are geometric means (95% CI)

[a] Noraml weight was defined as BMI < 25 kg/m^2 for whites and BMI < 23 kg/m^2 for Japanese

[b]Adjusted for visceral adipose tissue, current smokers, alcohol drinkers, physical activity, lipid medication, IFG, C-reactive protein and adiponectin

Table A-4 Comparison of HOMA-IR, HOMA-β% and DI in overweight[a] white men and Japanese men in the ERA JUMP study[b]

	White men (n=216)	Japanese men (n=165)	*P* value
Insulin resistance marker			
HOMA-IR	3.7 (3.4, 4.0)	2.6 (2.4, 2.8)	<0.01
Insulin secretion markers			
HOMA-β%	141.5 (132.2, 151.4)	94.1 (86.6, 102.1)	<0.01
DI	38.3 (37.2, 39.5)	36.6 (35.2, 38.0)	0.12

Values are geometric means (95% CI)

[a] Overweight was defined as BMI ≥ 25 kg/m^2 for whites and BMI ≥ 23 kg/m^2 for Japanese

[b]Adjusted for visceral adipose tissue, current smokers, alcohol drinkers, physical activity, lipid medication, IFG, C-reactive protein and adiponectin

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