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Particle Numbers of Lipoprotein Subclasses and Arterial Stiffness among Middle-aged men from the ERA JUMP study

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Abstract

We examined the association between serum lipoprotein subclasses and the three measures of arterial stiffness i.e. (i) carotid-femoral pulse wave velocity (*cf*PWV) which is a gold standard measure of central arterial stiffness, (ii) brachial-ankle PWV (*ba*PWV) which is emerging as a combined measure of central and peripheral arterial stiffness, and (iii) femoral-ankle PWV (*fa*PWV) which is a measure of peripheral arterial stiffness. Among a population-based sample of 701 apparently healthy Caucasian, Japanese American and Korean men aged 40–49 years, concentrations of lipoprotein particles were assessed by nuclear magnetic resonance (NMR) spectroscopy, and PWV was assessed with an automated waveform analyzer (VP2000, Omron, Japan). Multiple linear regressions were performed to analyze the association between each NMR lipoprotein subclasses and PWV measures, after adjusting for cardiovascular risk factors and other confounders. A cut-off of $p<0.01$ was used for determining significance. All PWV measures had significant correlations with total and small low-density lipoprotein particle number (LDL-P) (all $p<0.0001$) but not LDL-cholesterol (LDL-C) (all $p>0.1$), independent of race and age. In

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Conflict of Interest:

Rachel Mackey has been PI of an unrestricted research grant from LipoScience, Inc. to the University of Pittsburgh. LipoScience had no involvement in the design/analysis/conduct of that study or this study and manuscript other than performing the blinded laboratory measurements. Other authors declare no potential conflict of interest.

Supplementary information is available at JHH's website

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multivariate regression analysis, no NMR lipoprotein subclass was significantly associated with *cf*PWV (all p>0.01). However, most NMR lipoprotein subclasses had significant associations with both *ba*PWV and *fa*PWV (p<0.01). In this study of healthy middle-aged men, as compared to *cf*PWV, both *ba*PWV and *fa*PWV had stronger associations with particle numbers of lipoprotein subclasses. Our results may suggest that both *ba*PWV and *fa*PWV are related to arterial stiffness and atherosclerosis, whereas *cf*PWV may represent arterial stiffness alone.

Keywords

lipoproteins; lipoprotein fractions; pulse wave velocity; atherosclerosis

Introduction

Carotid-femoral pulse-wave velocity (*cf*PWV) is established as a gold standard measure of central arterial stiffness, and is known to increase with age and BP.[1] *cf*PWV is an independent predictor of CVD events as well as all-cause mortality.[2] The European Society of Hypertension has recommended *cf*PWV as a marker for subclinical organ damage among patients with hypertension, and in identifying patients with high CVD risk.[3] Brachial-ankle pulse wave velocity (*ba*PWV), which is likely to be a measure of combined central and peripheral arterial stiffness, correlates well with *cf*PWV,[4] and is increasingly being used clinically as a measure of cardiovascular risk.[5] Both *cf*PWV and *ba*PWV predict CVD events and all-cause mortality in the general population as well as among CVD patients, [2,5] and are shown to be markers of therapeutic progress.[6] In contrast, femoral-ankle pulse wave velocity (*fa*PWV), as the name implies, is the velocity at which the pulse wave travels from the femoral artery to the posterior tibial artery at the ankle. Thus, *fa*PWV is likely to be an indicator of arterial stiffness in the peripheral arterial segment.

Nuclear magnetic resonance (NMR) spectroscopy has emerged as a technique for quantifying the size and number of particles within subclasses of serum lipoproteins. While serum total cholesterol and low-density lipoprotein cholesterol (LDL-C) are traditional measures of atherogenic serum lipoproteins,[7] LDL-C as compared to NMR-derived low-density lipoprotein particle number (LDL-P) may be a less accurate determinant of lipemic atherosclerotic risk.[8] For example, in a multi-ethnic cohort of 6,814 US adults, among participants with discordance between LDL-C and LDL-P, LDL-P had a stronger association with atherosclerosis.[8]

Although many studies have not found age-, BP-independent association between central arterial stiffness (*cf*PWV) and serum lipids such as serum total cholesterol, LDL-C or high density lipoprotein cholesterol (HDL-C),[9] to our knowledge, no previous population-based study has examined the association between NMR lipoprotein subclasses and different measures of arterial stiffness. We examined the association of particle numbers of NMR lipoprotein subclasses with *cf*PWV, *ba*PWV and *fa*PWV in the Electron-beam computed tomography, Risk factor Assessment among Japanese and U.S. Men in the Post-World War II birth cohort (ERA JUMP Study), an international study of subclinical atherosclerosis among middle-aged men.

Materials and Methods

Participants

During 2002–2006, a population-based sample of 1,228 men aged 40–49 years, with no clinical CVD or type 1 diabetes, was obtained from 4 centers: 310 whites from Allegheny County, Pennsylvania; 303 Japanese Americans (JA) from Honolulu, Hawaii; 313 Japanese from Kusatsu, Shiga, Japan; and 302 Koreans from Ansan, Gyeonggi-do, South Korea as previously described.[10] Japanese in Japan were excluded from this analysis as *cf*PWV was not examined. Of the original sample, we further excluded those with diabetes (n=82), taking lipid-lowering medications (n=112) or anti-hypertensive medications (n=112). Diabetes was defined as individuals with fasting glucose ≥126 mg/dl or use of medications for diabetes. The final sample consisted of 701 men (248 whites, 191 JA, and 262 Koreans). Written informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of the following institutions: the University of Pittsburgh, Pittsburgh, Pennsylvania; the Kuakini Medical Center, Honolulu, Hawaii; Shiga University of Medical Science, Otsu, Japan; and Korea University, Seoul, South Korea.

All participants underwent a physical examination, completed a lifestyle questionnaire and a laboratory assessment as described previously.[10–12] Body weight and height were measured while the participant was wearing light clothing without shoes. Body-mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. An Alcohol drinker was defined as intake ≥ twice/week. Smoking was determined by pack years of cigarette smoking. Blood pressure and heart rate were measured after the participant emptied his bladder and sat quietly for 5 min. Blood pressure was measured twice on both arms with an automated sphygmomanometer (BP-8800, Colin Medical Technology, Komaki, Japan). The average of the two measurements was used. Hypertension was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg. Venipuncture was performed early in the clinic visit after a 12-h fast. Blood samples were stored at −80°C and shipped on dry ice to the University of Pittsburgh. Serum lipids were determined using CDC-standardized methods.[10] Serum glucose was determined using hexokinase–glucose-6-phosphate-dehydrogenase enzymatic assay. Data collection was standardized across all centers.

Nuclear magnetic resonance lipoprotein measurements

Serum samples obtained from study participants were shipped from the University of Pittsburgh to LipoScience (LipoScience Inc., Raleigh, NC) for NMR spectroscopy. NMR spectroscopy using LipoProfile-3 algorithm was performed to quantify the particle numbers of very low-density lipoprotein (VLDL-P), LDL-P, and high-density lipoprotein (HDL-P).[13, 14] Particle concentrations were determined for 3 VLDL subclasses (large, >60 nm; medium, 35–60 nm; and small, 29–35 nm), 3 LDL subclasses (intermediate-density lipoprotein (IDL), 23–29 nm; large, 20.5–23 nm; small, 18–20.5 nm), and 3 HDL subclasses (large, 9.4–14 nm; medium, 8.2–9.4 nm; and small, 7.3–8.2 nm).

Measurement of PWV

At the start of the study, staff from the University of Pittsburgh's Ultrasound Research Laboratory visited the Honolulu site to train the sonographers in Honolulu and from South

Korea for PWV measurements. PWV measurements were automatically generated using a noninvasive and automated waveform analyzer (VP2000, Omron, Japan).[4] This device recorded the carotid and femoral pulse waveform using multi-array tonometer, and provided automated measures of *cf*PWV, *fa*PWV, and *ba*PWV. Following 10 min of rest in a supine position, occlusion and monitoring cuffs were placed around both arms and both ankles of the participant. The arm cuffs were placed on skin or over light clothing, and the ankle cuffs were directly placed over the skin. ECG electrodes were placed on both wrists and a phonocardiogram i.e. a microphone for detecting heart sounds was placed on the left edge of the sternum. For *cf*PWV path length, the distance between the carotid and femoral sites was measured over the surface of the body with a tape measure. The path lengths for *ba*PWV and *fa*PWV were calculated using height-based formulae.[15] Sonographers palpated the left femoral artery and the left carotid artery, and placed the handheld tonometer over these two pulse areas to obtain femoral and carotid pulse waveforms simultaneously. A foot-pedal was used to start the recording. Data was collected two times for each participant and the observed values were averaged. *cf*PWV was calculated by time-phase analysis using volume waveforms of the respective arteries (carotid and femoral arteries). PWV was calculated as distance between arterial sites divided by the time between the feet of the respective waveforms. Intra-class correlations (ICC) for re-examination were 0.76 (*cf*PWV), 0.97 (*ba*PWV), and 0.96 (*fa*PWV) within technicians. Similarly, ICCs of 0.73 (*cf*PWV), 0.87(*fa*PWV), and 0.91 (*ba*PWV) were achieved between technicians.

Statistical analysis

We first calculated overall and race-specific mean (standard deviation) or median (interquartile range) values of the study population, as appropriate. Frequencies and proportions were calculated for categorical variables. Analysis of variance and chi-square tests were used to examine significant differences between individual racial groups. Two-tailed cut-off p-value for significance was kept at 0.05 for these analyses.

We then used Spearman rank statistics to test the age-, race-adjusted correlation between each of individual lipoprotein subclasses and the different measures of PWV i.e. (i) *cf*PWV, (ii) *ba*PWV, and (iii) *fa*PWV. To determine its statistical significance, we used a more conservative two-tailed p-value of 0.01 to compensate for multiple comparisons. Multiple linear regressions were then used to calculate standardized regression coefficients and their significance, keeping individual NMR lipoprotein subclasses and serum lipids as predictor variables, and each PWV measure as an outcome variable. For the multiple linear regressions, other covariates adjusted for in the model were age, BMI, race, glucose, SBP, heart rate, pack years of smoking, and frequency of alcohol intake. Similar to previous analysis, we used a two-tailed p-value of 0.01 as cut-off for significance.

To test the presence of effect modification of lipoprotein subclasses by race, we tested for statistical interaction between race and predictor variable i.e. each of the lipoprotein subclasses in a regression model for the outcome variable i.e. *cf*PWV, *ba*PWV and *fa*PWV. Regression models testing for interactions were unadjusted for other covariates. We used the p-value of <0.05 for determining significant interaction in the regression model. All data

analysis was performed using SAS/STAT® software, Version 9.3 of the SAS System, Cary, NC, USA.

Results

Table 1 presents descriptive characteristics of the study population overall, and by race. About 7.7% of the study population had SBP ≥140 mmHg; 45% were ever-smokers and the median value of pack-years of smoking among ever-smokers was 14 (interquartile range: 7, 24.5). More than two-fifths of the study participants were alcohol drinkers. Koreans, as compared to whites and JA, had significantly less BMI, SBP, total cholesterol, HDL-C and LDL-C, although they had significantly higher pack-years of smoking.

Table 2 presents the three measures of PWVs in the three racial groups. Koreans had lower PWV than JA among all the three PWV measures, while they had lower *cf*PWV and *fa*PWV than Whites. JA had higher PWVs than the other racial groups for all three measures.

Table 3 presents Spearman partial correlations of each subclass of lipoproteins with the three measures of PWVs, adjusted for age and race. *cf*PWV strongly correlated with both known atherogenic lipoprotein subclasses i.e. small LDL-P and total LDL-P (both $p<0.001$) even after adjusting for age and race, and also had a significant inverse correlation with large HDL-P ($p=0.0007$). In slight contrast, *ba*PWV significantly correlated with most lipoprotein subclasses, while having significant inverse correlations with large LDL-P ($p<0.0001$). Similarly, *fa*PWV had significant positive correlations with many lipoprotein subclasses, and a significant inverse correlation with large LDL-P ($p=0.0002$). Similar results were obtained while using Pearson's correlation coefficient for testing correlation between NMR lipoproteins and PWV (data not shown).

Table 4 presents multivariable-adjusted linear regression analysis of PWV measures with each of individual lipoprotein subclasses as main predictors. Standardized regression coefficients are shown which represent SD change in the PWV measure with every 1 SD change in the NMR lipoprotein subclass. When we adjusted for risk factors of CVD such as BMI, SBP, glucose, smoking and alcohol drinking status, NMR lipoprotein subclasses had significant associations with *ba*PWV and *fa*PWV. In contrast, the significant associations between atherogenic lipoproteins and *cf*PWV, as seen in the partial correlation analysis, became insignificant after multivariate adjustment. None of the lipoprotein subclasses was significantly associated with *cf*PWV after multivariate adjustment (all $p>0.01$). In contrast, baPWV had a significant positive association with medium HDL-P ($p=0.0068$), small HDL-P ($p<0.0001$), total HDL-P ($p<0.0001$), small LDL-P ($p<0.0001$), large VLDL-P ($p<0.0001$), medium VLDL-P ($p<0.0001$), total VLDL-P ($p<0.0001$), and VLDL size ($p<0.0001$). In addition, baPWV was significantly inversely associated with large LDL-P ($p<0.0001$) and LDL size ($p<0.0001$). Similar to *ba*PWV, *fa*PWV had significant associations with lipoprotein subclasses, except that it was not significantly associated with medium HDL-P.

We found no significant interaction between race and VLDL-P, or any of its subclasses in regression models for PWVs. Similarly, interaction terms with race were insignificant for

LDL-P, LDL-P subclasses, HDL-P, and HDL-P subclasses except interactions between race and large LDL-P in model for *fa*PWV (p=0.031), between race and HDL-P in model for *cf*PWV (p=0.049), and between race and HDL-P in the regression model for *ba*PWV (p=0.005). (See Supplementary Table 1)

Discussion

This is the first population-based study to examine the association between NMR lipoprotein subclasses and different measures of arterial stiffness. In the present study, *ba*PWV was significantly associated with lipoprotein subclasses independent of traditional cardiovascular risk factors. Similarly *fa*PWV, a measure of peripheral PWV, was significantly associated with many lipoprotein subclasses after adjusting for traditional risk factors. However, the significant correlation between *cf*PWV and lipoprotein subclasses became insignificant after adjusting for potential confounders in the regression analysis. The results might suggest that serum lipoproteins play a role in the pathogenesis of arterial stiffness in the peripheral muscular arteries, but not in the aorta among middle-aged men.

In our study, as well as in previous literature,[16, 17] *cf*PWV was not significantly associated with serum lipids, i.e. LDL-C, HDL-C, or triglycerides. Additionally, we did not find significant associations of *cf*PWV with any of the NMR lipoprotein subclasses. In a systematic-review on the association of *cf*PWV with CVD risk factors, most studies did not show any significant association between *cf*PWV and traditional measures of blood lipids i.e. LDL-C, HDL-C, or triglycerides.[9] On the other hand, in some previous studies *cf*PWV is associated with atherosclerotic plaque.[18, 19] One plausible explanation may be that calcification of the atherosclerotic plaque increases arterial stiffness. To support this hypothesis, we and others have previously reported significant association between *cf*PWV and aortic calcification, even as aortic calcification was not associated with traditional serum lipid measures i.e. LDL-C and HDL-C.[20–22] More research is needed to understand the pathogenesis of central arterial stiffness, and its association with serum lipids.

We found that *ba*PWV was significantly associated with atherogenic serum lipoproteins, i.e. small LDL-P, independent of other risk factors. Takahashi et al. have also reported a significant association between very small LDL-C and *ba*PWV among men with impaired glucose tolerance, even as lipoprotein subclasses were measured using high performance liquid chromatography in their study, a technique which provides lipoprotein subclass measurement in serum but does not provide lipoprotein particle numbers.[23] A possible reason for the association between atherogenic serum lipoproteins and *ba*PWV might be that *ba*PWV, in addition to central arterial stiffness, is also influenced by peripheral arterial atherosclerosis and its related pathophysiological conditions. Consistent with the opposite direction of association for small LDL and large LDL, we found that increasing LDL size was inversely associated with baPWV. Our result is consistent with findings of Paynter et al as they also found lower incidence of hypertension among women with larger LDL size.[24] We also found a significant association between small LDL-P and *fa*PWV which further indicates that PWV within a peripheral muscular arterial tree might be a product of arterial stiffness as well as atherosclerosis.

We found that the associations were stronger for lipoprotein subclasses with baPWV than with faPWV, in spite of baPWV encompassing carotid-femoral region where the associations with lipoprotein subclasses are statistically insignificant. One possible reason for this finding might be the inclusion of another peripheral muscular region in the measurement of baPWV i.e. the carotid-brachial segment. We do not have separate heart-brachial PWV data in this study to allow us measure its association with lipoprotein subclasses.

We did not find any significant associations between arterial stiffness and atherogenic serum lipoproteins in the central arterial tree in our middle-aged male population. In contrast, this association was present in the more muscular peripheral arteries. van Popele et al. found a significant association between arterial stiffness and carotid atherosclerosis in an older population.[18] In aging arteries, vascular changes occur via an inflammatory milieu leading to increased arterial stiffness.[25] It is possible that the arterial stiffening process exposes the arterial wall intima to the deleterious effects of atherogenic LDL particles, leading to stimulation of the localized atherosclerosis, a process that may appear earlier in the peripheral arteries than in central arteries.

We found that total HDL-P as well as small HDL-P was significantly associated with *ba*PWV and with *fa*PWV. In contrast, large HDL-P had an inverse association with *ba*PWV and with *fa*PWV. The reasoning behind our finding of the positive association of total and small HDL-P with PWV is not known, but might be hidden in yet un-deciphered complex pathobiology of HDL synthesis and mode of action. HDL particles are small and disc-shaped to start with, and gain functionality after becoming spherical through a series of poorly understood processes.[26] It is possible that large HDL-P represents these functional spherical HDL particles that are likely atheroprotective. Many studies have reported findings similar to ours. From the Women's Health Study (WHS), Paynter et al have reported independent positive association of total HDL-P (OR = 1.43 (CI: 1.26, 1.61) quintile 5 vs. quintile 1), medium HDL-P (OR = 1.26 (CI: 1.12, 1.41) quintile 5 vs. quintile 1), small HDL-P (OR = 1.23 (CI: 1.30, 1.50) quintile 5 vs. quintile 1) with incident hypertension. In contrast, yet similar to our results, the association between large HDL-P and incident hypertension was protective (OR = 0.81 (CI: 0.72, 0.91) quintile 5 vs. quintile 1).[24] Our findings were different from Otvos et al, as they found small HDL-P to be protective of a CHD event in a trial of gemfibrozil among patients with low HDL-C.[27] More research is needed to understand the different types of HDL particles and their relative physiological mechanisms.

We found stronger association for baPWV with total HDL-p than any of its individual components i.e. large HDL-p, medium HDL-p, and small HDL-p. This may be due to differences in the relative distribution of HDL-p components among individuals. In our data, there was a significant inverse correlation of large HDL-p with small HDL-p ($\rho = -0.22$, $p<0.0001$). As these are associated with baPWV in opposite directions (Table 4), their sum as in total HDL-p has stronger association with baPWV than either of them.

Several recent studies have shown that the inverse association of HDL-C with CHD events is greatly attenuated by adjusting for correlated levels of triglycerides and atherogenic

lipoprotein particles measured by apolipoprotein B100 (apoB) or LDL-P,[28, 29] but that inverse associations of concentrations of total HDL-P and medium HDL-P with CHD are relatively independent of triglycerides, LDL-P or apoB.[28, 30] When we further adjusted for LDL-P in our analysis for association between HDL-P subclasses and PWVs, the results were essentially unchanged thus indicating that the association between HDL-P and its subclasses and PWVs is independent of traditional risk factors and also LDL-P. (See Supplementary Table 2)

We found strong associations for each of large and medium VLDL-p with baPWV as well as faPWV, but not with cfPWV. VLDL particles are secreted by the liver as large, triglyceride-rich particles, deliver fatty acids to peripheral tissues, and become progressively smaller in the circulation as they are depleted of triglycerides. Their direct role in pathogenesis of atherosclerosis or arterial stiffness is not known.[31] It is likely that individuals with large number of VLDL-p also have higher LDL-p, thus explaining association between VLDL-p components and, baPWV and faPWV. In our study, the unadjusted Pearson's correlations of small LDL-p and small HDL-p were significant with large VLDL-p (ρ=0.47 and ρ=0.34 respectively, both $p<0.0001$), medium VLDL-p (ρ=0.55 and ρ=0.39 respectively, both $p<0.0001$), and with total VLDL-p (ρ=0.52 and ρ=0.40 respectively, both $p<0.0001$) (data not shown). Further research is needed to understand the role played by VLDL particles in pathogenesis of arterial stiffness.

We found no significant association between total LDL-P and any of the measures of PWV, independent of traditional risk factors. One likely reason for this unexpected finding might be that the major components of total LDL-P i.e. large LDL-P and small LDL-P are significantly associated with *ba*PWV and *fa*PWV, but in opposite directions. Combining the two lipoprotein subclasses probably obliterated these two associations. In the WHS study, incident hypertension was also inversely associated with large LDL-P and positively associated with small LDL-P, but also with total LDL-P.[24] In a supplementary analysis where we additionally adjusted for LDL-C in the regression analysis, we found significant positive association of total LDL-P with *ba*PWV (p=0.004), and with *fa*PWV (p= 0.003), but only weak association with *cf*PWV (p=0.018) (data not shown). LDL-C is more strongly correlated with large LDL-P (Spearman's ρ=0.50, $p<0.0001$) than with small LDL-P (Spearman's ρ=0.28, $p<0.0001$) in our study (data not shown) as would be expected as large LDL particles are likely to have larger amount of cholesterol per particle than small LDL particles, and so have stronger correlation with LDL-C. Thus, adjusting for LDL-C possibly adjusted more for the effect of large LDL-P than effect of small LDL-P, resulting in a significant positive association of total LDL-P with each of *ba*PWV and *fa*PWV.

We found lower cfPWV and baPWV among Koreans than any other racial group. Also, Japanese Americans had higher baPWV and faPWV than the other two groups. However, we combined the population groups for the analysis as we did not find significant interaction by race for most of the lipoprotein subclasses (Supplementary Table 1).

Our study had several limitations. As this is a cross-sectional study, we cannot establish temporality of the association between lipoprotein particles and PWV. We had only cross-sectional data for analysis, thus we can't comment on causality of increased PWV. Our

study population comprised of men within a relatively narrow age range, so our results cannot be extrapolated to women or other age groups. However, the narrow age range likely provided us with more precision in detecting the association between the two sets of CVD markers. We excluded individuals with diabetes from this analysis. The results, however, remained unchanged after including them in a supplementary analysis (data not shown). By keeping the cut-off p-value at 0.01, we might have missed potentially important associations between the two sets of biomarkers. However, keeping α at 0.01 likely prevented us from detecting false associations that occur by chance.

Conclusion

In summary, our results demonstrate that among apparently healthy middle-aged men, NMR spectroscopy-derived lipoprotein subclasses are associated with *ba*PWV and *fa*PWV, but not with *cf*PWV. While small LDL-P and small HDL-P have significant positive associations with *ba*PWV and *fa*PWV, the association of each of large LDL-P and large HDL-P with each of *ba*PWV and *fa*PWV are in inverse direction. In contrast, traditionally measured VLDL-C, LDL-C and HDL-C do not have significant associations with any measure of arterial stiffness i.e. *cf*PWV, *ba*PWV or *fa*PWV. More research is needed to understand the differences in the pathogenesis of central vs. peripheral arterial stiffness.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Summary Table

What is known about this topic

- Serum cholesterol is a risk factor for high blood pressure
- However, serum LDL-cholesterol is not significantly associated with central measure of arterial stiffness i.e. *cf*PWV

What this study adds

- Serum lipoprotein particle numbers but not serum cholesterol measures, are associated with measures of arterial stiffness
- The association is statistically significant for *ba*PWV and *fa*PWV but not with *cf*PWV

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Table 1

Descriptive characteristics of the study population[1]

	All subjects (n=701)	Whites (n=248)	Japanese Americans (n=191)	Koreans (n=262)	Difference between racial groups[2]
Age	45.1 (2.9)	44.8 (2.8)	46.0 (2.9)	44.7 (2.8)	¶¥
BMI (kg/m^2)	26.2 (3.8)	27.5 (4.3)	26.7 (3.6)	24.5 (2.6)	‡¥
Systolic blood pressure (mmHg)	122.2 (12.0)	122.2 (11.3)	125.8 (12.1)	119.5 (12)	¶‡¥
Diastolic blood pressure (mmHg)	74.4 (9.2)	72.6 (8.7)	76.5 (8.9)	74.6 (9.7)	¶‡
Hypertension (%)	9.4	7.7	15.2	6.9	¶¥
Heart rate	65.4 (9.0)	64.1 (9.3)	66.4 (8.5)	65.8 (9.1)	¶
Glucose (mg/dL)	101.5 (11.7)	99 (7.8)	105.5 (7.8)	100.9 (15.7)	¶¥
Smoking (pack-years)	7.5 (12.0)	3.7 (8.5)	4.0 (8.1)	13.9 (14.4)	‡¥
Alcohol drinker[3]	42.1	45.6	37.5	42.2	none
Total cholesterol (mg/dL)	205.4 (36.7)	215.3 (37.4)	211.9 (35.6)	191.6 (32.6)	‡¥
LDL cholesterol (mg/dL)	127.0 (33.6)	137.6 (33.2)	129.1 (31.9)	115.4 (31.5)	¶‡¥
HDL cholesterol (mg/dL)	48.3 (12.6)	48.7 (13.1)	51.0 (12.5)	45.9 (11.9)	‡¥
Triglycerides (mg/dL)	153.5 (96.5)	145 (80.7)	163.6 (111.9)	154.1 (97.6)	none
NMR Lipoproteins					
Total VLDL-P (nmol/L)	81.2 (46.1)	82.8 (43.7)	95.4 (51.6)	69.4 (40.9)	¶‡¥
Large VLDL-P (nmol/L)	5.1 (7.4)	5.5 (7.4)	6.3 (8.7)	3.9 (6.0)	¥
Medium VLDL-P (nmol/L)	35.1 (30.8)	36.9 (29.3)	42.1 (34.9)	28.3 (27.6)	‡¥
Small VLDL-P (nmol/L)	41.1 (25.3)	40.5 (24.1)	47.1 (26.8)	37.3 (24.5)	¶¥
Total LDL-P (nmol/L)	1309.4 (347.0)	1474.4 (338.39)	1290.8 (334.8)	1166.9 (294.0)	¶‡¥
Intermediate DL-P (nmol/L)	143.5 (96.5)	131.6 (99.7)	139.0 (100.0)	158.0 (89.2)	‡
Large LDL-P (nmol/L)	546.8 (279.9)	665.0 (308.6)	455.7 (245.5)	501.3 (233.8)	¶‡
Small LDL-P (nmol/L)	619.1 (339.4)	677.7 (350.9)	696.2 (338.0)	507.5 (298.5)	‡¥
Total HDL-P (μmol/L)	31.8 (6.4)	31.5 (5.8)	36.4 (5.7)	28.7 (5.4)	¶‡¥
Large HDL-P (μmol/L)	3.8 (3.0)	3.3 (2.7)	4.6 (3.1)	3.8 (2.9)	¶¥
Medium HDL-P (μmol/L)	7.8 (4.2)	7.7 (4.0)	10.2 (4.5)	6.0 (3.1)	¶‡#
Small HDL-P (μmol/L)	20.2 (4.5)	20.6 (4.4)	21.5 (4.2)	18.9 (4.3)	‡¥

Values are expressed as mean (SD)

[1] Participants taking antihypertensive (n=140), lipid-lowering (n=132) and hypoglycemic medications (n=33) were removed from the analysis (Total excluded = 305)

[2] ¶ denotes significant difference between whites and JA, ‡ denotes significant difference between whites and Koreans, and ¥ denotes significant difference between JA and Koreans

[3] Alcohol drinker was defined as alcohol intake ≥ twice/week

BMI = body-mass index, LDL = low-density lipoprotein, HDL = high-density lipoprotein, VLDL = very low-density lipoprotein, NMR = nuclear magnetic resonance

Table 2

Mean pulse wave velocity values in the study populations

	***cf*PWV (SD), cm/s**	***ba*PWV (SD), cm/s**	***fa*PWV (SD), cm/s**
Whites	889.6 (268.3)	1310.7 (150.4)	987.5 (160.4)
Japanese Americans	882.7 (189.6)	1413.1 (179.0)	1062.5 (111.7)
Koreans	802.9 (128.8)	1286.4 (137.2)	1026.1 (98.0)
Total	854.4 (205.2)	1329.8 (162.6)	1023.1 (129.0)
Difference between racial groups[1]	‡¥	¶¥	¶‡¥

[1] ¶ denotes significant difference between whites and JA, ‡ denotes significant difference between whites and Koreans, and ¥ denotes significant difference between JA and Koreans

*cf*PWV = carotid-femoral pulse wave velocity, *ba*PWV = brachial-ankle pulse wave velocity, *fa*PWV = femoral-ankle pulse wave velocity

Table 3

Spearman Rank Correlations[1] between measures of serum lipoproteins and pulse wave velocity in the study population

	*cf*PWV		*ba*PWV		*fa*PWV	
	Spearman ρ	**p-value**	**Spearman ρ**	**p-value**	**Spearman ρ**	**p-value**
Total VLDL-P	0.1133	0.0034	0.2218	<0.0001	0.1840	<0.0001
Large VLDL-P	0.2351	<0.0001	0.3326	<0.0001	0.2898	<0.0001
Medium VLDL-P	0.1517	<0.0001	0.2555	<0.0001	0.2128	<0.0001
Small VLDL-P	−0.0318	0.4120	0.0127	0.7425	0.0019	0.9602
Triglycerides	0.2176	<0.0001	0.2763	<0.0001	0.2325	<0.0001
VLDL Size	0.2038	<0.0001	0.2674	<0.0001	0.2428	<0.0001
Intermediate DL-P	0.0928	0.0164	0.0978	0.0112	0.0991	0.0105
Total LDL-P	0.1827	<0.0001	0.1651	<0.0001	0.1102	0.0044
Large LDL-P	−0.0569	0.1415	−0.1836	<0.0001	−0.1444	0.0002
Small LDL-P	0.1961	<0.0001	0.2981	<0.0001	0.1963	<0.0001
LDL-C	0.0576	0.1370	0.0314	0.4170	−0.0016	0.9665
LDL Size	−0.1271	0.0010	−0.2794	<0.001	−0.1951	<0.0001
Total HDL-P	0.0701	0.0702	0.2552	<0.0001	0.2186	<0.0001
Large HDL-P	−0.1302	0.0007	−0.0746	0.0536	−0.0187	0.6305
Medium HDL-P	0.0407	0.2938	0.1556	<0.0001	0.0920	0.0176
Small HDL-P	0.1387	0.0003	0.2416	<0.0001	0.2162	<0.0001
HDL-C	−0.0614	0.1125	0.0373	0.3345	0.0387	0.3178
HDL Size	−0.1089	0.0048	−0.0513	0.1843	0.0096	0.8038

[1] Partial correlation after adjusting for age and race

Spearman ρ = Spearman rank correlation coefficient. Cut-off p-value of p<0.01 was used for determining significance.

Unit for VLDL-P and LDL-P was nmol/L, unit for HDL-P was μmol/L. Unit for pulse wave velocity was cm/s

*cf*PWV = carotid-femoral pulse wave velocity, *ba*PWV = brachial-ankle pulse wave velocity, *fa*PWV = femoral-ankle pulse wave velocity

VLDL-P = very low-density lipoprotein particle number, VLDL-C = very low-density lipoprotein cholesterol, LDL-P = low-density lipoprotein particle number, LDL-C = low-density lipoprotein cholesterol, HDL-P = high-density lipoprotein particle number, HDL-C = high-density lipoprotein cholesterol

Table 4

Association between NMR subclasses and carotid-femoral, brachial-ankle and femoral-ankle pulse wave velocities in the study population

	Standardized coefficients (β)					
	***cf*PWV[1]**	**p-value**	***ba*PWV[1]**	**p-value**	***fa*PWV[1]**	**p-value**
Large VLDL-P	0.0608	0.1113	0.1825	<0.0001	0.1857	<0.0001
Medium VLDL-P	0.0645	0.0850	0.1914	<0.0001	0.1561	<0.0001
Small VLDL-P	−0.0339	0.3420	0.0152	0.6463	0.0310	0.3904
Total VLDL-P	0.0308	0.4069	0.1600	<0.0001	0.1465	<0.0001
Triglycerides[2]	0.0687	0.0768	0.1650	<0.0001	0.1541	<0.0001
VLDL Size	0.0440	0.2543	0.1465	<0.0001	0.1608	<0.0001
Intermediate DL-P	−0.0068	0.8523	0.0479	0.1573	0.0622	0.0910
Large LDL-P	0.0104	0.7826	−0.1428	<0.0001	−0.0871	0.0218
Small LDL-P	0.0625	0.1125	0.1867	<0.0001	0.1296	0.0011
Total LDL-P	0.0700	0.0798	0.0736	0.0473	0.0715	0.0771
LDL-C	0.0152	0.6824	0.0177	0.6091	−0.0014	0.9711
LDL Size	−0.0132	0.7297	−0.1911	<0.0001	−0.1426	0.0002
Large HDL-P	−0.0210	0.5966	−0.0357	0.3339	−0.0387	0.3364
Medium HDL-P	−0.0028	0.9420	0.0954	0.0068	0.0402	0.2946
Small HDL-P	0.0545	0.1481	0.1584	<0.0001	0.1742	<0.0001
Total HDL-P	0.0313	0.4316	0.1759	<0.0001	0.1477	0.0002
HDL-C	−0.0185	0.6390	0.0106	0.7720	−0.0133	0.7378
HDL Size	−0.0175	0.6598	−0.0213	0.5661	−0.0234	0.5615

[1]Linear regression derived beta (β) values

[2]Triglyceride values were log-transformed for analysis

Table after adjusting for age, race, body-mass index, systolic blood pressure, heart rate, glucose, smoking and alcohol Intake. Cut-off p-value of p<0.01 was used for determining significance.

Unit for VLDL-P and LDL-P was nmol/L, unit for HDL-P was μmol/L

*cf*PWV = carotid-femoral pulse wave velocity, *ba*PWV = brachial-ankle pulse wave velocity, *fa*PWV = femoral-ankle pulse wave velocity

LDL-P = low-density lipoprotein particle number, HDL-P = high-density lipoprotein particle number, VLDL-P = very low-density lipoprotein particle number