High-density Lipoprotein Particle Concentration and Subclinical Atherosclerosis of the Carotid Arteries in Japanese Men

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Abstract

Objective—The association of high-density lipoprotein particle (HDL-P) with atherosclerosis may be stronger than that of HDL-cholesterol (HDL-C) and independent of conventional cardiovascular risk factors. Whether associations persist in populations at low risk of coronary heart disease (CHD) remains unclear. This study examines the associations of HDL-P and HDL-C with carotid intima-media thickness (cIMT) and plaque counts among Japanese men, who characteristically have higher HDL-C levels and a lower CHD burden than those in men of Western populations.

Methods—We cross-sectionally examined a community-based sample of 870 Japanese men aged 40-79 years, free of known clinical cardiovascular disease (CVD) and not on lipid-lowering medication. Participants were randomly selected among Japanese living in Kusatsu City in Shiga, Japan.

Results—Both HDL-P and HDL-C were inversely and independently associated with cIMT in models adjusted for conventional CHD risk factors, including low-density lipoprotein cholesterol (LDL-C) and diabetes. HDL-P maintained an association with cIMT after further adjustment for HDL-C (P<0.01), whereas the association of HDL-C with cIMT was noticeably absent after inclusion of HDL-P in the model. In plaque counts of the carotid arteries, HDL-P was significantly associated with a reduction in plaque count, whereas HDL-C was not.

Conclusion—HDL-P, in comparison to HDL-C, is more strongly associated with measures of carotid atherosclerosis in a cross-sectional study of Japanese men. Findings demonstrate that,

HDL-P is a strong correlate of subclinical atherosclerosis even in a population at low risk for CHD.

Keywords

High-density lipoprotein (HDL) particle; High-density lipoprotein (HDL) cholesterol; subclinical atherosclerosis; carotid intima-media thickness (cIMT); plaque count

Introduction

Many studies have reported an inverse association between high-density lipoprotein cholesterol (HDL-C) and coronary heart disease (CHD).[1-3] This has led to the notion that cardiovascular risk may drop significantly once HDL-C levels are increased. [4] However, recent trials involving pharmacological increases in HDL-C levels have reported no significant effects on the reduction of carotid intima-media thickness (cIMT)[5], the progression of coronary atherosclerosis,[6] or any other cardiovascular measurement.[7, 8] Also, a large mendelian randomization study has shown that some polymorphisms associated with genetically higher HDL-C levels do not lower risk of myocardial infarction[9]. Lack of improved cardiovascular outcomes with increased HDL-C has stressed the view that increasing HDL-C levels may not directly translate to decreases in cardiovascular risk[10] and, thus has led to a surge of interest in identifying other features of HDL that can be targeted for assessing cardiovascular risk.

Recently, total HDL particle (HDL-P) concentration has been shown to be a marker of reduced cardiovascular risk[11-13] and some evidence suggests that this is independent of HDL-C[12]. However, studies on HDL-P were largely limited to Western populations, which are known to have a higher risk of CHD and lower levels of HDL-C than less vulnerable regions of Asia, particularly Japan.[14-16] Whether associations persist in these regions at lower risk for CHD and with higher HDL-C levels remains unclear. Our objective is to evaluate the association of HDL-C and HDL-P with subclinical atherosclerosis in a population-based sample of Japanese men.

Methods

Study participants

The Shiga Epidemiological Study of Subclinical Atherosclerosis (SESSA) aims to examine various factors associated with subclinical atherosclerosis. The design of this study is described elsewhere.[17] In brief, from 2006 to 2008, 1,094 Japanese men aged 40 to 79 years were randomly selected from the general population in Kusatsu City, Shiga, Japan. After excluding those on lipid-lowering medications (n=168) and missing information on HDL-P, HDL-C or lipid-lowering medications (n=56), 870 remained for analysis in the current report. All participants provided written informed consent. The study complies with *the Declaration of Helsinki* and was approved by the Institutional Review Board of Shiga University of Medical Science, Otsu, Japan.

Factors collected through physical examinations include height, weight, blood pressure, and a variety of other measures. Body mass index (BMI) was calculated as weight (kg) divided

by height squared (m^2). Blood pressure was measured twice in a seated position after a 5 minute rest, using an automated sphygmomanometer (BP-8800; Omron Colin, Tokyo, Japan). The average of two measurements was used.

Hypertension was defined as systolic blood pressure (SBP) 140mm Hg, diastolic blood pressure (DBP) 90mm Hg, or as the use of antihypertensive medications. Diabetes mellitus (DM) was defined as a hemoglobin A1c (HbA1c) 6.1% (Japan Diabetes Society criteria; equivalent to HbA1c 6.5% in National Glycohemoglobin Standardization Program)[18] a fasting glucose 6.99 mmol/l (126 mg/dL), or the use of antidiabetic medications.

A self-administered questionnaire was used to collect data on medical history, medication use, smoking, alcohol intake, and other lifestyle behaviours with confirmation by trained technicians.

Laboratory Measurements

Blood samples were drawn from participants after a 12-hour fast and centrifuged soon after coagulation. Standard lipids, including total cholesterol and triglycerides (TG), were measured using enzymatic techniques. HDL-C was measured after heparin-calcium precipitation. Measurements were standardized according to guidelines from the Center for Disease Control and Prevention/Cholesterol Reference Method Laboratory Network (CDC/CRMLN).[19] Friedewald's formula was used to estimate low-density lipoprotein cholesterol (LDL-C) levels in men with TG <4.52mmol/l (400 mg/dl). For higher TG levels, LDL-C was treated as missing.

HDL-P concentration was determined by nuclear magnetic resonance (NMR) spectroscopy using serum samples stored at −80°C,[20] and shipped on dry ice to LipoScience Inc (Raleigh, North Carolina, US). Concentrations were obtained from amplitudes of distinct spectroscopic NMR signals of the lipid methyl group, characteristic of each subclass. Reproducibility of NMR-measured HDL-P has been examined and measurements have a coefficient of variation <2%. [21]

Intima-media thickness and plaque counts of carotid arteries

Ultrasound measurements of the carotid arteries were performed by sonographers following an established protocol of the Ultrasound Research Laboratory at the University of Pittsburgh.[17, 22] A Toshiba XarioSSA-660A scanner (Toshiba Medical Systems, Japan), equipped with a 7.5MHz linear-array imaging probe, was used for high-resolution B-mode ultrasound of the carotid arteries. Sonographers scanned both right and left carotid arteries.

In both arteries, the IMT of the common carotid artery (CCA), carotid bulb, and internal carotid artery were measured. For the CCA segment, both near and far walls were examined 1 cm proximal to the bulb. For the bulb and internal carotid artery segments, only far walls were examined. cIMT was defined as the mean of the eight IMT values measured in both arteries.

Plaque was defined as focal thickening lesion (>10% protrusion compared to adjacent areas) with an IMT of 1mm. The total number of plaques in CCA, bulb, and internal carotid artery of both left and right carotid arteries were counted.

Statistical Analyses

Participant demographics were described according to quartile of HDL-P and HDL-C. P-values for trend across the quartiles were determined either using linear regression when a response variable is continuous (such as age), or using logistic regression when it is categorical (such as current smoker or not).

A dose-response relationship between HDL measures and subclinical atherosclerosis was investigated by obtaining adjusted means of cIMT and plaque counts across quartiles of HDL-P and HDL-C using linear regression. We then calculated a difference in cIMT per 1 standard deviation (SD) increase in HDL-P or HDL-C, treating them as continuous variables.

For carotid plaque, we modeled plaque count as an overdispersed integer response following a negative binomial distribution. Regression coefficients have been transformed to percentages, indicating the percent reduction (or excess) in plaque counts per 1 SD increase in HDL-P or HDL-C.

In regression models, we chose the following adjusting covariates as they are established cardiovascular risk factors: age (years), SBP (mmHg), hypertension medication (yes/no), current smoker (yes/no), current alcohol intake (g/day), DM (yes/no), LDL-C (mmol/l) [this set was defined as "base covariates"] and HDL-P or HDL-C (mmol/l).

Analyses were performed using SAS version 9.3 (SAS Institute, Cary, North Carolina) and two-tailed *P*-values of <0.05 were considered significant.

Results

Study Participants and characteristic trends with HDL-P and HDL-C

Characteristics of study participants according to quartiles of HDL-P and HDL-C are displayed in Table 1A and 1B. Mean (SD) characteristics of all participants included 63.3(10) years for age, 834 (184)μm for cIMT and 2.4(2.4) for plaque count (75.4% of all participants had presence of plaque 1). Men with higher HDL-P tended to be younger, leaner, have less prevalence of DM, and consumed more alcohol. The same was also true for HDL-C with the exception of age. Additionally, men with higher HDL-C tended to have less prevalence of hypertension and were less likely to be current smokers. Among lipids, HDL-P and HDL-C were positively related to each other. LDL-C was negatively associated with both HDL-P and HDL-C.

HDL-P and HDL-C associations with cIMT and carotid plaque

Results of quartile analyses are depicted in Figure 1. With adjustment for base covariates, higher quartiles of HDL-P and HDL-C were both associated with smaller cIMT (panels A & B, dashed blue lines). The overall inverse relationship of HDL-P was maintained with

further adjustments for HDL-C (panel A, solid red line). In contrast, the observed inverse association of HDL-C was noticeably absent after adjustments for HDL-P (panel B, solid red line). Higher quartiles of HDL-P were associated with lower mean plaque count in both models (panel C), with and without adjustments for HDL-C. Across quartiles of HDL-C, an association with plaque counts was absent (panel D).

In Table 2, a 1 SD increase in HDL-P and HDL-C was associated with 47.2μm and 22.1μm lower cIMT, respectively, (unadjusted models). In models adjusted for base covariates, 22.1 μm and 11.1 μm lower cIMT was estimated per 1 SD increase in HDL-P and HDL-C, respectively. After adjustment for HDL-C, the estimated cIMT differences in relation to HDL-P remained significant. In contrast, differences in cIMT with HDL-C were absent when adjusted for HDL-P.

Table 3 depicts the estimated reduction or excess in total number of carotid artery plaque counts per 1 SD increase in HDL-P and HDL-C. In unadjusted models, a 1 SD increase in HDL-P and HDL-C was associated with 20.4% and 8.8% reduction in total plaques, respectively. HDL-P was associated with significant reductions in plaque counts even after adjustment for base covariates and HDL-C. Here, a 1 SD increase in HDL-P was associated with 10.4% reduction in number of plaques in the final model adjusted for HDL-C. In contrast, HDL-C had no significant associations with carotid artery plaque in any of the adjusted models.

Discussion

HDL and carotid atherosclerosis

In this cross-sectional study of Japanese men, free of clinical CVD and not on lipid-lowering medication, the inverse association of HDL-P with cIMT was independent of conventional cardiovascular risk factors, including HDL-C. In contrast, the association of HDL-C with cIMT was attenuated with adjustments for these factors and was absent after adjustment for HDL-P. Furthermore, higher HDL-P, but not higher HDL-C, was inversely and independently associated with lower number of carotid artery plaque after adjustment for cardiovascular risk factors. We demonstrated stronger associations of HDL-P, compared to HDL-C, with two different measures of carotid atherosclerosis (i.e. cIMT and carotid plaque) among a community-based sample of Japanese men. Whether effects of HDL-P are more noticeable in the higher ranges of HDL-C, normally thought to be atheroprotective, warrants consideration.

Our findings are consistent with those of other studies.[11-13] The Multi-Ethnic Study of Atherosclerosis (MESA),[12] in the United States, for example, reported a significant inverse association of cIMT with HDL-P, but not with HDL-C after adjustments for each other and known risk factors. The Woman's Health Study (WHS), however, did not find a significant inverse association of HDL-P with CHD [23] and instead, only confirmed the inverse association between HDL-C and cardiovascular risk. Possible explanations for the difference in findings may not only be due to the sample population, but also to the randomized clinical trial study design of WHS, involving low-dose aspirin and vitamin E in primary prevention of CVD and cancer.

It is noteworthy that Japanese populations have higher HDL-C levels[16] and lower risk of CHD compared to populations of Western countries.[24] In addition, we previously reported significantly lower measurements of cIMT and higher levels of HDL-P among Japanese men compared to Caucasian men in the US.[15] Despite having a different cardiovascular risk profile, we found that in Japanese men, HDL-P, but not HDL-C, was significantly inversely associated with two measures of carotid atherosclerosis. Hence, our finding, together with results of other studies, suggests that HDL-P may be a novel marker for, and may possibly play a biological role against, the pathogenesis of atherosclerosis.

We have also analyzed HDL size subclass: small (7.3-8.2 nm), medium (8.2-8.8 nm), and large (8.8-13 nm) and their associations with cIMT and carotid plaque counts. However, we found no significant associations of any size with either measure of subclinical atherosclerosis in models adjusted for HDL-C. An atheroprotective effect of subclass size is also controversial [25] and is in need of focused attention.

Potential Mechanisms

The failure of recent randomized controlled trials on HDL-C-increasing drugs for CVD prevention resulted in questioning a causal protective role of HDL-C, which may only be an indicator of cardioprotective mechanisms at work. Nevertheless, the cardioprotective association of HDL is far from being ruled out. It has been suggested that increased particle concentrations of HDL may be indicative of higher reverse cholesterol transport activity.[12] The reverse cholesterol transport pathway mediates the efflux of cholesterol from peripheral cells to the liver [26] which is believed to be a key process in preventing plaque formation and progression[27] and, thus, many CVDs. Indeed, macrophage-specific cholesterol efflux was found to have a strong inverse association with cIMT and CHD. [28] Furthermore, recent studies have found that HDL-P, and not HDL-C, concentrations are positively associated with cholesterol efflux in patients with type 2 diabetes[29] and patients undergoing coronary angiography. [30] These findings parallel our results, with total HDL-P having inverse associations with cIMT. It may be that serum HDL-P concentration is more closely related to the performance rate of cholesterol efflux in the reverse cholesterol transport pathway than HDL-C, with more particles being analogous to increased pathway activity. How HDL affects the cholesterol transport and protects against CVD may depend on its structure and composition, leading to a variety of biological activities, such as anti-inflammation, antioxidation, and vasodilation,[25] all of which cannot be assessed by HDL-C alone.[26, 31]

Limitations and strengths

As our study is cross-sectional and observational, causality cannot be proven in the associations of HDL-P with cIMT and carotid plaque counts. Other limits of our study include the study population being restricted to men of a single ethnic group. However, this is not without its advantage, as homogeneity in a population minimizes confounding from genetic variation. The size of carotid plaques was also not taken into account. Total plaque count may not define the grade or vulnerability of plaques, nevertheless it has been reported that the presence of plaque, alone, in the carotid arteries is positively associated with increased risk of cardiovascular events.[34] Thus, carotid plaque count can be used as an

alternate indicator of subclinical atherosclerosis.[35] The main protein component of HDL particle, apolipoprotein A-1, is a strong predictor of CHD and a key player in reverse cholesterol transport.[32, 33] Unfortunately, apolipoprotein A-1 levels were not measured in our serum samples and thus we were unable to look at possible confirmatory associations of HDL-P with cIMT and plaque.

This is the first study, of which we are aware of, to report a significant and inverse association of HDL-P, and not HDL-C, with plaque count in the carotid arteries, even after adjustments for conventional risk factors. This finding, as well as the confirmatory finding of cIMT associations, is in agreement with most literature published on HDL-P and both clinical CVD and subclinical atherosclerosis.[11, 12, 36] The fact that we could identify a relationship of HDL-P with cIMT and plaque in a population at low risk of CHD, indicates that HDL-P may be an important predictor of subclinical atherosclerosis and perhaps even more so in populations at high risk. Presently, there are few population-based studies on HDL-P, let alone any on a Japanese population, that characteristically has higher serum HDL-C levels compared to those of Western populations. Thus, our findings provide additional information to the current modest body of knowledge in this area.

Conclusion

In a community-based sample of Japanese men, free of clinical CVD, HDL-P was associated with measures of carotid atherosclerosis (cIMT and plaque count) independent of lipids or lipoproteins and other traditional CVD risk factors. In contrast, associations with HDL-C were absent after accounting for HDL-P. There is need for more scrutiny towards the properties of HDL in general, in order to better understand its involvement in CVD risk processes.

Acknowledgments

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Highlights

- We examined a cross-sectional sample of Japanese men aged 40-79 years.

- HDL-P was associated with subclinical atherosclerosis (cIMT and carotid plaque).

- The association persisted after adjustment for other factors including HDL-C.

- In contrast, HDL-C was unrelated to either measure after HDL-P adjustment.

- HDL-P is a stronger correlate of subclinical atherosclerosis than HDL-C.
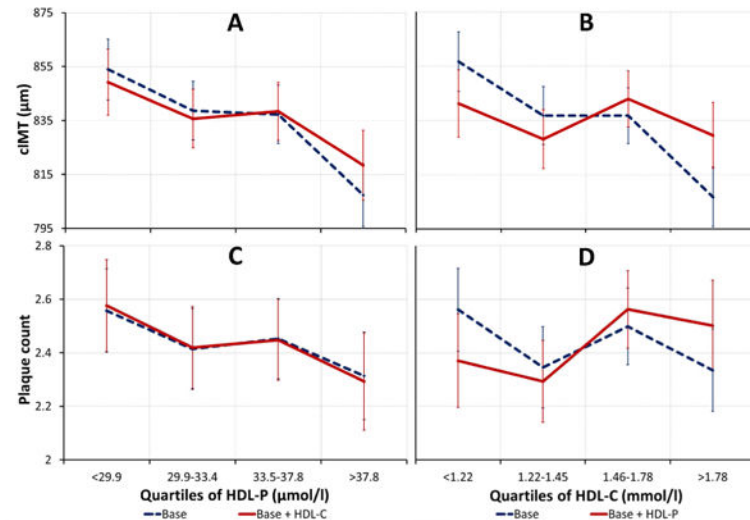
Figure 1.
Adjusted mean cIMT (n=870) and plaque count across quartiles of HDL-P and HDL-C. Means were adjusted for base covariates [Base]: age (years), systolic blood pressure (mmHg), hypertension medication (yes/no), smoking status (yes/no), alcohol intake (g/day), diabetes (yes/no) and LDL-C (mg/dl), or adjusted for base covariates and HDL-C (mmol/l) or HDL-P (μmol/L) [Base + HDL-C or HDL-P]. P-values are for linear trend. All linear trends are significant p<0.05, except for HDL-C models: Base + HDL-P (p=0.886) for cIMT and Base (p=0.763) and Base + HDL-P (p=0.187) for plaque counts. Error bars represent standard error of mean.

Table 1A

Characteristics of participants (n=870), aged 40-79 years, across quartiles of HDL particle concentration, 2006 - 2008, Kusatsu, Shiga, Japan

Characteristic	Quartile of HDL-P				P trend
	1	2	3	4	
Age, years	68.2 ± 8.3	64.4 ± 9.7	60.7 ± 10.2	60.0 ± 9.6	<0.001
Body mass index, kg/m^2	23.9 ± 3.2	23.1 ± 2.8	23.3 ± 3.0	23.1 ± 2.8	0.016
SBP, mmHg	138 ± 16	133 ± 19	133 ± 19	140 ± 22	0.089
Hypertension, %[†]	57.7	47.0	46.1	53.2	0.803
Diabetes, % (Type 2)	21.8	17.7	16.6	17.0	0.033
Current smoker, %	34.6	36.3	27.2	35.8	0.512
Alcohol intake (g/day)	12.3 ± 18.4	17.3 ± 20.8	23.4 ± 25.2	43.4 ± 34.1	<0.001
Triglycerides, mmol/l	1.36 ± 0.70	1.31 ± 0.74	1.38 ± 1.00	1.53 ± 1.11	0.010
LDL-C, mmol/l[‡]	3.31 ± 0.87	3.33 ± 0.80	3.33 ± 0.71	3.05 ± 0.83	<0.001
HDL-C, mmol/l	1.20 ± 0.31	1.44 ± 0.34	1.62 ± 0.36	1.88 ± 0.47	<0.001

Values are mean ± SD, or % (as indicated).

[†] Hypertension is defined as SBP 140mmHg or DBP 90mmHg or use of anti- hypertensive medication. Diabetes is defined as glycated hemoglobin 6.5% (NGSP) or fasting glucose 6.99 mmol/l or use of anti-diabetic medication.

[‡] LDL-C was calculated by Friedewald equation. [LDL-C (mg/dl) = total cholesterol (mg/dl) - HDL cholesterol (mg/dl) -triglyceride(mg/dl)/5] SBP, systolic blood pressure; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HDL-P, HDL particle; LDL, low-density lipoprotein cholesterol.

Quartiles of HDL-P are as follows (1): 13.9 to 29.8μmol/l; n=220, (2): 29.9 to 33.4 μmol/l; n=215, (3): 33.5 to 37.8 μmol/l; n=217, and (4): 37.9 to 68.9 μmol/l; n=218. P-values for trend were obtained using linear regression (for continuous variables) or logisitc regression (for categorical variables) as per 1 unit increase in HDL-P.

Table 1B

Characteristics of participants (n=870), aged 40-79 years, across quartiles of HDL cholesterol concentration, 2006 - 2008, Kusatsu, Shiga, Japan

Characteristic	Quartile of HDL-C				P trend
	1	2	3	4	
Age, years	64.1 ± 9.5	63.7 ± 9.5	62.2 ± 10.6	63.4 ± 10.3	0.129
Body mass index, kg/m^2	24.6 ± 3.0	23.9 ± 2.8	23.0 ± 2.9	21.9 ± 2.6	<0.001
SBP, mmHg	137 ± 17	136 ± 19	136 ± 18	135 ± 23	0.168
Hypertension, %[†]	55.2	50.5	56.4	41.4	0.008
Diabetes, % (Type 2)	20.8	22.0	20.5	9.5	0.001
Current smoker, %	42.5	32.7	35.0	23.3	<0.001
Alcohol intake (g/day)	18.4 ± 23.8	19.0 ± 24.2	27.9 ± 30.0	30.9 ± 30.9	<0.001
Triglycerides, mmol/l	1.91 ± 1.01	1.49 ± 1.11	1.23 ± 0.63	0.96 ± 0.43	<0.001
LDL-C, mmol/l[‡]	3.36 ± 0.85	3.47 ± 0.82	3.18 ± 0.72	3.02 ± 0.79	<0.001
HDL-P, μmol/l	28.9 ± 4.6	32.5 ± 4.2	36.4 ± 5.7	39.0 ± 7.1	<0.001

Values are mean ± SD, or % (as indicated).

[†] Hypertension is defined as SBP 140mmHg or DBP 90mmHg or use of anti- hypertensive medication. Diabetes is defined as glycated hemoglobin 6.5% (NGSP) or fasting glucose 6.99 mmol/l or use of anti-diabetic medication.

[‡] LDL-C was calculated by Friedewald equation. [LDL-C (mg/dl) = total cholesterol (mg/dl) - HDL cholesterol (mg/dl) -triglyceride(mg/dl)/5]

Quartiles of HDL-C are as follows (1): 0.67 to 1.19 mmol/l; n=212, (2): 1.22 to 1.45 mmol/l; n=214, (3): 1.46 to 1.78 mmol/l; n=234, and (4): 1.81 to 3.88 mmol/l; n=210. P-values for trend were obtained using linear regression (for continuous variables) or logisitc regression (for categorical variables) as per 1 unit increase in HDL-C.

Table 2

Estimated reduction (-) or excess (+) in cIMT per 1 standard deviation increase in HDL-P or HDL-C (n=870), 2006 - 2008

Parameter	Model	cIMT (μm)	95% CI	*P* value
	Unadjusted	-47.2	-59.0 , -35.3	<0.001
HDL-P	Base covariates	-22.1	-34.4 , -9.9	<0.001
	Base covariates + **HDL-C**	-22.8	-37.9 , -7.7	0.003
	Unadjusted	-22.1	-34.3 , -10.0	<0.001
HDL-C	Base covariates	-11.1	-22.1 , 0.0	0.050
	Base covariates + **HDL-P**	+1.0	-12.6 , +14.6	0.886

Base covariates include: age, SBP, hypertension medication (yes/no), and smoking status (yes/no), alcohol intake, LDL-C, diabetes (yes/no) and HDL-P or HDL-C. 1 standard deviation of HDL-P = 6.7 μmol/l and of HDL-C = 0.45 mmol/l.

cIMT, carotid intima-media thickness; CI, confidence interval.

Table 3

Estimated percent reduction (-) or excess (+) in total number of carotid artery plaque count per 1 standard deviation increase in HDL-P or HDL-C (n=870), 2006-2008

Parameter	Model	Estimate (%)	95% CI	P value
	Unadjusted	-20.4	-27.4 , -13.5	<0.001
HDL-P	Base covariates	-7.8	-15.1 , -0.5	0.037
	Base covariates + HDL-C	-10.4	-19.7 , -1.1	0.029
	Unadjusted	-8.8	-15.7 , -1.9	0.012
HDL-C	Base covariates	-2.0	-8.5 , +4.5	0.552
	Base covariates + HDL-P	+3.7	-4.5 , +11.9	0.380

Base covariates include: age, SBP, hypertension medication (yes/no), and smoking status (yes/no), alcohol intake, LDL-C, diabetes (yes/no) and HDL-P or HDL-C. 1 standard deviation of HDL-P = 6.7 μmol/l and of HDL-C = 0.45 mmol/l.