Journal of Clinical Lipidology (2019) 13, 664–672

Journal of Clinical Lipidology

Association of extremely high levels of high-density lipoprotein cholesterol with endothelial dysfunction in men

Yuji Takaeko, MD, Shogo Matsui, MD, PhD, Masato Kajikawa, MD, PhD, Tatsuya Maruhashi, MD, PhD, Shinji Kishimoto, MD, PhD, Haruki Hashimoto, MD, Yasuki Kihara, MD, PhD, Eisuke Hida, PhD, Kazuaki Chayama, MD, PhD, Chikara Goto, PhD, Yoshiki Aibara, MS, Farina Mohamad Yusoff, MD, Kensuke Noma, MD, PhD, Ayumu Nakashima, MD, PhD, Yukihito Higashi, MD, PhD, FAHA*

Department of Cardiovascular Medicine, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan (Drs Takaeko, Matsui, Maruhashi, Kishimoto, Hashimoto, and Kihara); Division of Regeneration and Medicine, Medical Center for Translational and Clinical Research, Hiroshima University Hospital, Hiroshima, Japan (Drs Kajikawa, Noma, and Higashi); Department of Biostatistics and Data Science, Osaka University Graduate School of Medicine, Osaka, Japan (Dr Hida); Department of Gastroenterology and Metabolism, Institute of Biomedical and Health Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan (Dr Chayama); Department of Physiol Therapy, Hiroshima International University, Hiroshima, Japan (Dr Goto); Department of Cardiovascular Regeneration and Medicine, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan (Drs Aibara, Yusoff, Noma, and Higashi); and Department of Stem Cell Biology and Medicine, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan (Dr Nakashima)

KEYWORDS:
High-density lipoprotein cholesterol;
Endothelial function;
Flow-mediated vasodilation;
Men;
Atherosclerosis

BACKGROUND: It is not clear whether a high level of high-density lipoprotein cholesterol (HDL-C) is associated with lower risk of atherosclerosis. It is likely that HDL-C is a double-edged sword for atherosclerosis.

OBJECTIVE: The purpose of this study was to evaluate the relationship between HDL-C levels and endothelial function in men.

METHODS: This was a cross-sectional study. We evaluated flow-mediated vasodilation (FMD) and serum levels of HDL-C in 5842 men aged 18 to 92 years who were not receiving lipid-lowering therapy. All participants were divided into four groups by HDL-C level: low HDL-C (<40 mg/dL),

Clinical Trial Registration Information: http://www.umin.ac.jp (University Hospital Medical Information Network Clinical Trials Registry; UMIN000012950).

Authors' contributions: Y.T. and Y.H. drafted the article and contributed to conception of this study; M.K., T.M., H.H., S.K., T.H., C.G., Y.A., A.N., F.M.Y., and K.N. acquired subjects and/or data; E.H., K.C., and Y.K. revised the article critically for important intellectual content. Y.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest: All authors have no conflicts of interests to report.

* Corresponding author. Department of Cardiovascular Regeneration and Medicine, Research Institute for Radiation Biology and Medicine (RIRBM), Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8551 Japan.

E-mail address: yhigashi@hiroshima-u.ac.jp

Submitted January 18, 2019. Accepted for publication June 6, 2019.

https://doi.org/10.1016/j.jacl.2019.06.004

moderate HDL-C (40–59 mg/dL), high HDL-C (60–79 md/dL), and extremely high HDL-C (≥80 mg/dL). We were not able to evaluate the amount of alcohol intake because there was limited information on the amount of alcohol drinking in our database.

RESULTS: FMD values were significantly smaller in the low group and the extremely high group than in the high group ($P = .001$ and $P = .016$, respectively). There was no significant difference in FMD between the low group and the extremely high group. Multiple logistic regression analysis revealed that extremely high HDL-C, but not low HDL-C, was independently associated with the lowest quartile of FMD (odds ratio: 1.39, 95% confidence interval: 1.09–1.77; $P = .009$).

CONCLUSIONS: An extremely high level of HDL-C in men (8.1% of this population) was associated with a significant reduction in FMD.

Introduction

Several lines of evidence have shown an inverse relationship between high-density lipoprotein cholesterol (HDL-C) levels and cardiovascular disease.[1–3] It is well known that HDL has an atheroprotective effect through transportation of excess cholesterol from macrophages in the liver and bile, namely, cholesterol reverse transport.[4] In addition, HDL activates endothelial nitric oxide synthase (eNOS), has antioxidant and anti-inflammatory effects and an antithrombotic effect, and prevents apoptosis of endothelial cells induced by tumor necrosis factor alpha.[5–8]

However, cohort studies have shown that an extremely high HDL-C group had high mortality and morbidity rates of cardiovascular disease and that there was a U-shaped curve between HDL-C levels and mortality and morbidity rates of cardiovascular disease.[9–11] In addition, recent large clinical trials have shown that an increase in HDL-C levels with pharmacologic interventions does not reduce the risk of cardiovascular events. Cholesteryl ester transfer protein (CETP) modulates the transfer of cholesteryl esters from HDL to apolipoprotein B–containing lipoproteins.[12] Pharmacologic inhibition of CETP raises HDL-C levels and decreases low-density lipoprotein cholesterol (LDL-C) levels.[13–16] Randomized clinical trials using CETP inhibitors such as torcetrapib, dalcetrapib, and evacetrapib have shown an increase in mortality or a lack of efficacy, whereas CETP inhibitors increased HDL-C levels.[13–15] On the other hand, the CETP inhibitor anacetrapib reduced the risk of major coronary events during a 4-year treatment period.[16] The mechanism of this reduction seems to be largely explained by lowering of non–HDL-C rather than increase in HDL-C.[16] Results of recent human genetic studies have shown that genetic conditions leading to increased HDL-C levels may not always be associated with lower risk of atherosclerosis.[17] Moreover, a loss-of-function coding variant in *SCARB1* leads to increased risk of coronary heart disease despite elevation in HDL-C levels.[18] These findings suggest that extremely high HDL-C levels are associated with an increase in the risk of mortality and morbidity of cardiovascular disease. HDL-C may be a double-edged sword for atherosclerosis.

Endothelial dysfunction is the initial step of atherosclerosis and plays an important role in the development of atherosclerosis.[19,20] Flow-mediated vasodilation (FMD) of the brachial artery has been used for noninvasive assessment of endothelial function, and FMD has been shown to be significantly associated with cardiovascular risk factors including HDL-C and to be an independent predictor of cardiovascular events.[21–25]

However, the role of marked elevation of HDL-C in endothelial function remains unclear.[26–28] Therefore, we evaluated the relationship between HDL-C levels and endothelial function assessed by FMD in men not receiving lipid-lowering therapy.

Materials and methods

Subjects

A total of 10,247 Japanese adults (7385 subjects from the FMD-J study and 2862 subjects who underwent a health checkup at Hiroshima University Hospital between August 2007 and August 2016) were enrolled in this study. From this registry, 7682 men aged 18 to 92 years were recruited. Subjects with unclear images of the brachial artery interfaces and subjects without information on HDL-C level were excluded. To eliminate the influence of pharmacologic therapy, subjects who were receiving lipid-lowering medicine (eg, statins, ezetimibe, proprotein convertase subtilisin/kexin type 9 inhibitors, bile acid sequestrants, fibrates, eicosapentaenoic acid, and niacin) were also excluded. Finally, 5842 subjects were enrolled in this study. Hypertension was defined as treatment with oral antihypertensive agents or systolic blood pressure of more than 140 mm Hg or diastolic blood pressure of more than 90 mm Hg measured in a sitting position on at least three different occasions. Diabetes mellitus was defined according to the American Diabetes Association recommendation.[29] Dyslipidemia was defined according to the third report of the National Cholesterol Education Program.[30] Smokers were defined as those who were current smokers. Coronary heart disease included angina pectoris, myocardial infarction, and unstable angina. Cerebrovascular disease included ischemic stroke, hemorrhagic stroke, and transient ischemic attack. Cardiovascular disease was defined as coronary

heart disease and cerebrovascular disease. Framingham risk score was calculated by points of risk factors: age, total cholesterol level, HDL-C level, systolic blood pressure, diabetes mellitus, and smoking status.[31]

All participants were divided into 4 groups according to the definitions used in a previous study in Japan: low HDL-C (<40 mg/dL), moderate HDL-C (40–59 mg/dL), high HDL-C (60–79 mg/dL), and extremely high HDL-C (≥80 mg/dL).[9,32]

The ethical committees of the participating institutions approved the study protocol. The study was executed in accordance with the Good Clinical Practice guidelines. Informed consent for participation in the study was obtained from all subjects. The protocol was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000012950).

Study protocol

Subjects fasted overnight for at least 12 hours and abstained from caffeine, alcohol, smoking, and antioxidant vitamins on the day of the FMD examination. The subjects were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature of 22°C–25°C) throughout the study. A 23-gauge polyethylene catheter was inserted into the deep antecubital vein to obtain blood samples. At 30 minutes of maintaining a supine position, FMD is measured. The observers were blind to the form of examination.

Measurement of FMD

We evaluated the vascular response to reactive hyperemia in the brachial artery for assessment of endothelium-dependent FMD. A high-resolution linear artery transducer was coupled to computer-assisted analysis software (UNEXEF18G; UNEX Co, Nagoya, Japan) that used an automated edge detection system for measurement of brachial artery diameter. A blood pressure cuff was placed around the forearm. The brachial artery was scanned longitudinally 5 to 10 cm above the elbow. When the clearest B-mode image of the anterior and posterior intimal interfaces between the lumen and vessel wall was obtained, the transducer was held at the same point throughout the scan by a special probe holder (UNEX Co) to ensure consistency of the image. Depth and gain setting were set to optimize the images of the arterial lumen wall interface. When the tracking gate was placed on the intima, the artery diameter was automatically tracked, and the waveform of diameter changes over the cardiac cycle was displayed in real time using the FMD mode of the tracking system. This allowed the ultrasound images to be optimized at the start of the scan and the transducer position to be adjusted immediately for optimal tracking performance throughout the scan. Pulsed Doppler flow was assessed at baseline and during peak hyperemic flow, which was confirmed to occur within 15 seconds after cuff deflation. Blood flow velocity was calculated from the color Doppler data and was displayed as a waveform in real time. The baseline longitudinal image of the artery was acquired for 30 seconds, and then the blood pressure cuff was inflated to 50 mm Hg above systolic pressure for 5 minutes. The longitudinal image of the artery was recorded continuously until 5 minutes after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 seconds at baseline and for 10 seconds immediately after cuff deflation. Changes in brachial artery diameter were immediately expressed as percentage change relative to the vessel diameter before cuff inflation. FMD was automatically calculated as the percentage change in peak vessel diameter from the baseline value. Percentage of FMD ([peak diameter − baseline diameter]/baseline diameter) was used for analysis. Blood flow volume was calculated by multiplying the Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area ($-r^2$). Reactive hyperemia was calculated as the maximum percentage increase in flow after cuff deflation compared with baseline flow. The coefficient of variation for FMD was 10.1% in our laboratory. The correlation coefficient between FMD analyzed at the core laboratory and participant institutions was 0.84 ($P < .001$).

Statistical analysis

Results are presented as means ± SD or median (interquartile range) for continuous variables. All reported probability values were 2 sided, and a probability value of <.05 was considered statistically significant. An association between FMD and HDL-C was explored visually using a locally weighted regression smoothing (Locally Weighted Scatterplot Smoothing) plot. The relation between FMD and HDL-C was determined by Pearson's correlation analysis. Comparison of variables among four groups by difference of the HDL-C level was performed using one-way analysis of variance or the Kruskal–Wallis test depending on normality of the data. Normality was assessed by visual inspection of histograms. Tukey's post-hoc test was performed to compare the differences in FMD between groups. Multiple logistic regression analysis was performed to identify independent variables associated with lower quartile of FMD (<3.9%). Age, body mass index, systolic blood pressure, LDL-C, glucose, and presences of current smoking were entered into the multiple logistic regression analysis. As a sensitivity analysis, propensity score analysis was used to generate a set of matched cases (subjects with extremely high HDL-C levels) and controls (subjects with high HDL-C levels). A logistic regression model was used to estimate the propensity of extremely high HDL-C levels based on variables associated with HDL-C, including age, body mass index, systolic blood pressure, total cholesterol, triglycerides, glucose, use of anti-hypertensive drugs (yes or no), current smoking (yes or no), and history of cardiovascular disease (yes or no). With these propensity scores using a caliper width of 0.2 standard deviations of the logit of the propensity score, two well-matched groups based on clinical characteristics were created for comparison of the prevalence of

endothelial dysfunction defined as FMD of <3.9%, the division point for the lowest quartile of FMD in all participants. All analyses were conducted using JMP version 13.0 software (SAS Institute, Cary, NC) and Stata version 15 (Stata Corporation, College Station, TX).

Results

Baseline characteristics

Baseline characteristics of all subjects are summarized in Table 1. The age range was 18 to 92 years. Of the 5842 subjects, 2473 (42.3%) had hypertension, 2560 (43.8%) had dyslipidemia, 486 (8.3%) had diabetes mellitus, 259 (4.4%) had previous cardiovascular disease, and 2156 (37.0%) were current smokers. There were significant differences among the four groups in age, body mass index, systolic blood pressure, diastolic blood pressure, heart rate, total cholesterol, triglycerides, HDL-C, LDL-C, glucose, use of antihypertensive drugs and antidiabetic drugs, prevalence of hypertension, dyslipidemia, and diabetes mellitus, and percentage of smokers.

Relationship between HDL-C and endothelial function

The scatter plot between FMD and HDL-C with Locally Weighted Scatterplot Smoothing smoothed curve is shown in Figure S1 in the online-only Data Supplement. FMD gradually increased to about 70 to 80 mg/dL of HDL-C levels and decreased in relation to the increase in HDL-C levels after about the 70 to 80 mg/dL of HDL-C. Using a linear regression analysis, there was no significant relationship between FMD and HDL-C ($r = 0.03$, $P = .052$). FMD values were 5.6 ± 3.1% in the low HDL-C group, 6.0 ± 3.0% in the moderate HDL-C group, 6.2 ± 3.2% in the high HDL-C group, and 5.7 ± 3.0% in the extremely high HDL-C group. FMD values were significantly smaller in the low HDL-C group and extremely high HDL-C group than in the high HDL-C group ($P = .001$ and $P = .016$, respectively; Fig. 1). There was no significant difference in FMD between the low HDL-C group and the extremely high HDL-C group. Multiple logistic regression analysis revealed that extremely high HDL-C was independently associated with the lowest quartile of FMD (odds ratio: 1.39, 95% confidence interval: 1.09–1.77; $P = .009$) and that low HDL-C and moderate HDL-C were not associated with FMD (Table 2).

We next evaluated the association between extremely high HDL-C and FMD in the propensity score–matched population. There were significant differences in triglycerides, HDL-C, LDL-C, Framingham risk score, and prevalence of dyslipidemia (Table S1 in the online-only Data Supplement). There were no significant differences in other baseline variables between the two groups. After matching for confounding factors, FMD was significantly lower in subjects with extremely high HDL-C than in subjects

Table 1 Clinical characteristics of the subjects on the basis of HDL-C

Variables	Total (n = 5842)	Low <40 mg/dL (n = 466)	Moderate 40–59 mg/dL (n = 3115)	High 60–79 mg/dL (n = 1787)	Extremely high ≥80 mg/dL (n = 474)	*P* value for trend
Age, y	50.2 ± 11	50.9 ± 12	49.8 ± 11	50.1 ± 12	51.9 ± 11	.002
Body mass index, kg/m^2	23.6 ± 3.2	25.2 ± 3.5	24.1 ± 3.2	22.7 ± 2.9	22.0 ± 2.7	<.001
Systolic blood pressure, mm Hg	128 ± 16	128 ± 15	129 ± 16	127 ± 16	129 ± 18	.001
Diastolic blood pressure, mm Hg	81 ± 12	80 ± 11	81 ± 12	79 ± 12	80 ± 12	<.001
Heart rate, bpm	64 ± 11	65 ± 11	65 ± 11	63 ± 11	63 ± 12	<.001
Total cholesterol, mg/dL	202 ± 33	193 ± 37	200 ± 33	203 ± 32	213.7 ± 31	<.001
Triglycerides, mg/dL	112 (79, 161)	185 (128, 269)	125 (90, 175)	90 (67, 123)	77 (58, 109)	<.001
HDL-C, mg/dL	58 ± 15	36 ± 3	50 ± 6	68 ± 5	91 ± 11	<.001
LDL-C, mg/dL	119 ± 30	117 ± 32	123 ± 29	116 ± 28	106 ± 29	<.001
Glucose, mg/dL	101 ± 22	105 ± 26	102 ± 24	100 ± 19	100 ± 18	<.001
Medications, n (%)						
Antihypertensive therapy	1353 (23.2)	156 (33.5)	746 (24.0)	340 (19.0)	111 (23.1)	<.001
Antihyperglycemic therapy	303 (5.2)	49 (10.5)	162 (5.2)	70 (3.9)	22 (4.6)	<.001
Framingham risk score, %	9.2 ± 7.8	12.4 ± 10.0	10.3 ± 8.3	7.0 ± 5.9	7.4 ± 5.8	<.001
Medical history, n (%)						
Hypertension	2473 (42.3)	219 (47.0)	1371 (44.0)	668 (37.4)	215 (45.4)	<.001
Dyslipidemia	2560 (43.8)	421 (90.3)	1523 (48.9)	522 (29.2)	94 (19.8)	<.001
Diabetes mellitus	486 (8.3)	70 (15.0)	279 (9.0)	108 (6.0)	29 (6.1)	<.001
Current smokers	2156 (37.0)	209 (45.0)	1257 (40.5)	557 (31.2)	133 (28.1)	<.001
Previous cardiovascular disease	259 (4.4)	30 (6.4)	134 (4.3)	75 (4.2)	20 (4.2)	.231

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
Data are presented as mean ± standard deviation or median (interquartile range).

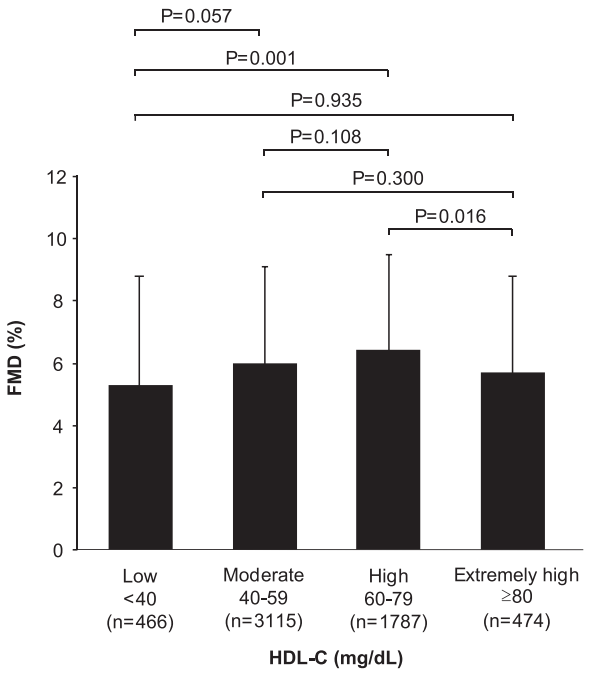

Figure 1 Bar graphs show flow-mediated vasodilation (FMD) in the low levels of high-density lipoprotein cholesterol (HDL-C), moderate levels of HDL-C, high levels of HDL-C, and extremely high levels of HDL-C groups. The error bars indicate the standard deviation.

with high HDL-C ($P < .001$; Figure S2 in the online-only Data Supplement).

Discussion

In the present study, we demonstrated for the first time that endothelial function was impaired not only in subjects with low HDL-C levels but also in subjects with extremely high HDL-C levels. After adjustment for traditional cardiovascular risk factors, extremely high levels of HDL-C were significantly associated with endothelial dysfunction.

It is well known that treatment with drugs such as fibrates, CETP inhibitors, and statins increases HDL-C levels, and lipid-lowering therapy, including HDL-C-elevating therapy, per se improves endothelial function. Therefore, in the present study, we carefully excluded subjects receiving lipid-lowering therapy to eliminate the influence of lipid-lowering medication on HDL-C levels.

Previous studies have shown that there is an inverse association between HDL-C levels and incidence of events of coronary heart disease.[1–3] HDL plays an important role in atherosclerosis through cholesterol reverse transport, activation of eNOS, antioxidation, anti-inflammation, and prevention of endothelial cell apoptosis.[4–8] However, some studies showed that high levels of HDL-C are paradoxically associated with high mortality and vascular events.[9–11] In clinical trials, elevating HDL-C levels by inhibition of CETP failed to decrease cardiovascular disease.[13–15] The first CETP inhibitor, torcetrapib, increased the risk of mortality and morbidity of cardiovascular disease rather than reducing the risk of cardiovascular events, while torcetrapib increased HDL-C levels.[13] In addition, torcetrapib increased systolic blood pressure and impaired endothelial function because of unexpected off-target effects.[33] Dalcetrapib, another CETP inhibitor, increased HDL-C levels without affecting blood pressure or biomarkers of inflammation and oxidative stress but did not reduce the risk of cardiovascular events and did not improve endothelial function after 12 and 36 weeks in a high-risk population.[14,34] The CETP inhibitor evacetrapib also increased HDL-C levels, but the trial of this CETP inhibitor was terminated early because of a lack of efficacy.[15] The Randomized Evaluation of the Effects of Anacetrapib through Lipid Modification trial showed a significant reduction of cardiovascular events during a 4-year follow-up period.[16] The reasons for the discrepant results of trials using CETP inhibitors are unclear. Although anacetrapib reduced cardiovascular events in the Randomized Evaluation of the Effects of Anacetrapib through Lipid Modification trial, it has been proposed that a reduction of non–HDL-C rather than an increase in HDL-C is the main

Table 2 Multivariate analysis of the relationship between FMD and HDL-C

	Model 1		Model 2		Model 3		Model 4	
Variables	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
HDL-C, mg/dL								
<40	1.42 (1.13–1.79)	.002	1.39 (1.10–1.77)	.007	1.09 (0.84–1.41)	.523	1.21 (0.94–1.57)	.135
40–59	1.10 (0.96–1.26)	.185	1.13 (0.98–1.54)	.072	0.97 (0.84–1.41)	.355	1.02 (0.87–1.18)	.837
60–79	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
≥80	1.39 (1.11–1.75)	.005	1.30 (1.02–1.65)	.029	1.36 (1.07–1.73)	.014	1.39 (1.09–1.77)	.009

BMI, body mass index; CI, confidence interval; FMD, flow-mediated vasodilation; HDL-C, high-density lipoprotein cholesterol; OR, odds ratio for lowest quartile of FMD (<3.9%).

Model 1: unadjusted model.

Model 2: adjusted for age.

Model 3: adjusted for age, BMI, the presence of hypertension, dyslipidemia, diabetes, and current smoking.

Model 4: adjusted for age, BMI, systolic blood pressure, low-density lipoprotein cholesterol, glucose, and current smoking.

mechanism of cardiovascular risk reduction.[16] These findings suggest that a large CETP inhibition–induced increase in HDL-C levels does not directly contribute to the reduction in cardiovascular events. Several studies have suggested that the endothelial effects of HDL are highly heterogeneous, and that atheroprotective effects of HDL are impaired in patients with type II diabetes mellitus, metabolic syndrome, coronary artery disease and chronic heart failure.[35–38] The present study showed that FMD was significantly smaller in subjects with extremely high HDL-C as well as subjects with low HDL-C than in subjects with high HDL-C. After adjustment for cardiovascular risk factors, extremely high HDL-C, but not low HDL-C, was significantly associated with endothelial dysfunction.

Although environmental factors such as obesity, smoking, alcohol, diet, and physical activity have been shown to influence HDL-C levels, it has been estimated that heritability of HDL-C levels is about 40% to 60%.[39] There are several rare genetic variant effects on HDL metabolism, and they are inherited in a Mendelian fashion.[17] Loss-of-function mutations in the genes *LIPG*, *SCARB1*, and *CETP* cause conditions of extremely high HDL-C.[18,40–43] Moreover, recent genome-wide association studies have revealed novel loci associated with HDL-C levels.[17] The *LIPG* gene encodes endothelial lipase, which has been shown to mediate HDL catabolism.[40] A Mendelian randomization study has shown that a loss-of-function variant in the LIPG gene is not associated with risk of myocardial infarction.[40] The *SRARB1* gene encodes scavenger receptor class B type 1.[18] Scavenger receptor class B type 1 is an HDL receptor and promotes the uptake of HDL cholesteryl esters into cells, especially hepatocytes.[18] It has been shown that a loss-of-function variant in *SCARB1*, P376L, is associated with increased coronary heart disease (odds ratio = 1.79, $P = .0018$).[18] CETP deficiency has been reported to be one of the major causes of increased HDL-C levels in Japan.[41–43] It is thought that the presence of CETP deficiency contributes to a longevity condition.[32,44] On the other hand, some studies showed that elevation of HDL-C levels caused by a *CETP* gene mutation is a risk factor for coronary heart disease, and CETP deficiency may not always be a longevity condition.[45–48] It has been shown that subjects with CETP deficiency have large and cholesteryl ester–rich HDL-C and polydisperse LDL-C.[49] Triglyceride-rich LDL decreases affinity for the LDL receptor.[50] It remains controversial whether cholesteryl ester–rich HDL-C reduces or enhances the ability to promote cholesterol efflux from macrophages.[51,52] Gomaraschi et al.[53] showed that HDL-C isolated from carriers of CETP deficiency impaired the activation of eNOS. In addition, inhibition of CETP may lead to increases in prooxidant and proinflammatory properties by an increase in dysfunctional HDL-C production. Measurements of markers of oxidative stress and inflammation would enable more specific conclusions concerning the role of HDL-C in endothelial function to be drawn. It is likely that an increase in dysfunctional HDL attenuates the eNOS/NO pathway. When considering the effects of HDL on atherosclerosis including vascular function, attention should be paid to HDL function as well as circulating levels of HDL-C.

In the present study, FMD was significantly smaller in the low HDL-C group (<40 mg/dL) than in the high HDL-C group (60–79 md/dL). However, after adjustment for cardiovascular risk factors, there was no significant difference in FMD between the low HDL-C group and the high HDL-C group. In the low HDL-C group, the prevalence of hypertension, prevalence of diabetes mellitus, and percentage of current smokers were higher than those in the other groups, suggesting that these confounding factors reflect endothelial function in subjects with low HDL-C. In clinical trials, isolated low levels of HDL-C were not associated with coronary heart disease and mortality in Japan.[11,54]

Study limitations

There are a number of limitations in the present study. First, this study was a cross-sectional study. We were able to evaluate association but not causality. Second, we were not able to evaluate the amount of alcohol drinking because there was limited information on the amount of alcohol drinking in our database. It has been shown that there is a positive gradient of HDL-C levels with alcohol consumption.[55] We previously reported that endothelial function is impaired in relation to alcohol intake in men.[56] The amount of alcohol drinking might have an impact on the relationship between HDL-C and endothelial function. Third, we did not assess *CETP* mutations. Finally, HDL-C functions other than endothelial function, including anti-inflammation and proinflammation, antioxidation and pro-oxidation, antithrombosis and prothrombosis, eNOS/NO pathway, and cholesterol efflux capacity, were not evaluated. Future studies are needed to confirm the effects of HDL functions, especially cholesterol efflux capacity, on endothelial function.

Conclusions

In the present study, an extremely high level of HDL-C in men (8.1% of this population) was associated with a significant reduction in FMD. Subjects with extremely high HDL-C may have dysfunctional HDL that has harmful effects on vascular function.

Acknowledgments

The authors would like to thank all patients who participated in this study. In addition, they thank Miki Kumiji, Megumi Wakisaka, Ki-ichiro Kawano, and Satoko Michiyama for their excellent secretarial assistance; FMD-J investigators of Takayuki Hidaka, MD, PhD; Shuji Nakamura, MD, PhD; Junko Soga, MD, PhD; Yuichi Fujii, MD, PhD; Naomi Idei, MD; Noritaka Fujimura, MD, PhD; Shinsuke Mikami, MD, PhD; Yumiko Iwamoto, MD;

Akimichi Iwamoto, MD, PhD; Takeshi Matsumoto, MD, PhD; Nozomu Oda, MD, PhD (Department of Cardiovascular Medicine, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan); Kana Kanai, PhD; Hraruka Morimoto, PhD (Department of Cardiovascular Regeneration and Medicine, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan); Tomohisa Sakashita, MD, PhD; Yoshiki Kudo, MD, PhD (Department of Obstetrics and Gynecology, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan); Taijiro Sueda, MD, PhD (Department of Surgery, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan); Hirofumi Tomiyama, MD, PhD, FAHA; Akira Yamashina, MD, PhD (Department of Cardiology, Tokyo Medical University, Tokyo, Japan); Bonpei Takase, MD, PhD, FAHA (Division of Biomedical Engineering, National Defense Medical College Research Institute, Tokorozawa, Japan); Takahide Kohro, MD, PhD (Department of Cardiology, Tokyo Medical University, Tokyo, Japan); Toru Suzuki, MD, PhD (Cardiovascular Medicine, University of Leicester, Leicester, UK); Tomoko Ishizu, MD, PhD (Cardiovascular Division, Institute of Clinical Medicine, University of Tsukuba, Ibaraki, Japan); Shinichiro Ueda, MD, PhD (Department of Clinical Pharmacology and Therapeutics, University of the Ryukyu School of Medicine, Okinawa, Japan); Tsutomu Yamazaki, MD, PhD (Clinical Research Support Center, Faculty of Medicine, The University of Tokyo, Tokyo, Japan); Tomoo Furumoto, MD, PhD (Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Hokkaido, Japan); Kazuomi Kario, MD, PhD (Division of Cardiovascular Medicine, Jichi Medical University School of Medicine, Tochigi, Japan); Teruo Inoue, MD, PhD (Department of Cardiovascular Medicine, Dokkyo Medical University, Mibu, Tochigi, Japan); Shinji Koba, MD, PhD (Department of Medicine, Division of Cardiology, Showa University School of Medicine, Tokyo, Japan); Kentaro Watanabe, MD, PhD (Department of Neurology, Hematology, Metabolism, Endocrinology and Diabetology (DNHMED), Yamagata University School of Medicine, Yamagata, Japan); Yasuhiko Takemoto, MD, PhD (Department of Internal Medicine and Cardiology, Osaka City University Graduate School of Medicine, Osaka, Japan); Takuzo Hano, MD, PhD (Department of Medical Education and Population-based Medicine, Postgraduate School of Medicine, Wakayama Medical University, Wakayama, Japan); Masataka Sata, MD, PhD (Department of Cardiovascular Medicine, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan); Yutaka Ishibashi, MD, PhD (Department of General Medicine, Shimane University Faculty of Medicine, Izumo, Japan); Koichi Node, MD, PhD (Department of Cardiovascular and Renal Medicine, Saga University, Saga, Japan); Koji Maemura, MD, PhD (Department of Cardiovascular Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan); Yusuke Ohya, MD, PhD (The Third Department of Internal Medicine, University of the Ryukyus, Okinawa, Japan); Taiji Furukawa, MD, PhD (Department of Internal Medicine, Teikyo University School of Medicine, Tokyo, Japan); Hiroshi Ito, MD, PhD (Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan); Hisao Ikeda, MD, PhD (Faculty of Fukuoka Medical Technology, Teikyo University, Omuta, Japan).

Financial disclosure

Grant-in-Aid for Scientific Research from the Ministry of Education,Science and Culture of Japan (18590815 and 21590898 to Y.H.) and a Grant in Aid of Japanese Arteriosclerosis Prevention Fund (to Y.H.).

References

1. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am J Med*. 1977;62:707–714.
2. Assmann G, Schulte H. Relation of high-density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease (the PROCAM experience). Prospective Cardiovascular Munster study. *Am J Cardiol*. 1992;70:733–737.
3. Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation*. 1989;79:8–15.
4. Rader DJ, Alexander ET, Weibel GL, Billheimer J, Rothblat GH. The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis. *J Lipid Res*. 2009;50(Suppl):S189–S194.
5. Yuhanna IS, Zhu Y, Cox BE, et al. High-density lipoprotein binding to scavenger receptor-BI activates endothelial nitric oxide synthase. *Nat Med*. 2001;7:853–857.
6. Navab M, Hama SY, Cooke CJ, et al. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: step 1. *J Lipid Res*. 2000;41:1481–1494.
7. Cockerill GW, Rye KA, Gamble JR, Vadas MA, Barter PJ. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. *Arterioscler Thromb Vasc Biol*. 1995;15:1987–1994.
8. Sugano M, Tsuchida K, Makino N. High-density lipoproteins protect endothelial cells from tumor necrosis factor-alpha-induced apoptosis. *Biochem Biophys Res Commun*. 2000;272:872–876.
9. Hirata A, Okamura T, Sugiyama D, et al. The relationship between very high levels of serum high-density lipoprotein cholesterol and cause-specific mortality in a 20-year follow-up study of Japanese general population. *J Atheroscler Thromb*. 2016;23:800–809.
10. Madsen CM, Varbo A, Nordestgaard BG. Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies. *Eur Heart J*. 2017;38:2478–2486.
11. Hirata A, Sugiyama D, Watanabe M, et al. Association of extremely high levels of high-density lipoprotein cholesterol with cardiovascular mortality in a pooled analysis of 9 cohort studies including 43,407 individuals: The EPOCH-JAPAN study. *J Clin Lipidol*. 2018;12:674–684.e5.
12. Tall AR. Plasma cholesteryl ester transfer protein. *J Lipid Res*. 1993;34:1255–1274.
13. Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med*. 2007;357:2109–2122.

14. Schwartz GG, Olsson AG, Abt M, et al. Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N Engl J Med*. 2012; 367:2089–2099.
15. Lincoff AM, Nicholls SJ, Riesmeyer JS, et al. Evacetrapib and cardiovascular outcomes in high-risk vascular disease. *N Engl J Med*. 2017; 376:1933–1942.
16. Group HTRC, Bowman L, Hopewell JC, et al. Effects of anacetrapib in patients with atherosclerotic vascular disease. *N Engl J Med*. 2017; 377:1217–1227.
17. Vitali C, Khetarpal SA, Rader DJ. HDL cholesterol metabolism and the risk of CHD: new insights from human genetics. *Curr Cardiol Rep*. 2017;19:132.
18. Zanoni P, Khetarpal SA, Larach DB, et al. Rare variant in scavenger receptor BI raises HDL cholesterol and increases risk of coronary heart disease. *Science*. 2016;351:1166–1171.
19. Ross R. Atherosclerosis–an inflammatory disease. *N Engl J Med*. 1999;340:115–126.
20. Higashi Y, Noma K, Yoshizumi M, Kihara Y. Endothelial function and oxidative stress in cardiovascular diseases. *Circ J*. 2009;73: 411–418.
21. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002; 39:257–265.
22. Benjamin EJ, Larson MG, Keyes MJ, et al. Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation*. 2004;109:613–619.
23. Donald AE, Halcox JP, Charakida M, et al. Methodological approaches to optimize reproducibility and power in clinical studies of flow-mediated dilation. *J Am Coll Cardiol*. 2008;51:1959–1964.
24. Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *J Am Coll Cardiol*. 2002;40:505–510.
25. Yeboah J, Folsom AR, Burke GL, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. *Circulation*. 2009;120:502–509.
26. Maruhashi T, Soga J, Fujimura N, et al. Relationship between flow-mediated vasodilation and cardiovascular risk factors in a large community-based study. *Heart*. 2013;99:1837–1842.
27. Kajikawa M, Maruhashi T, Matsumoto T, et al. Relationship between serum triglyceride levels and endothelial function in a large community-based study. *Atherosclerosis*. 2016;249:70–75.
28. Gokce N, Keaney JF Jr., Hunter LM, Watkins MT, Menzoian JO, Vita JA. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation*. 2002;105:1567–1572.
29. American Diabetes Association: clinical practice recommendations 1999. *Diabetes Care*. 1999;22(Suppl 1):S1–S114.
30. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA*. 2001;285:2486–2497.
31. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation*. 1998;97:1837–1847.
32. Moriyama Y, Okamura T, Inazu A, et al. A low prevalence of coronary heart disease among subjects with increased high-density lipoprotein cholesterol levels, including those with plasma cholesteryl ester transfer protein deficiency. *Prev Med*. 1998;27:659–667.
33. Simic B, Hermann M, Shaw SG, et al. Torcetrapib impairs endothelial function in hypertension. *Eur Heart J*. 2012;33:1615–1624.
34. Luscher TF, Taddei S, Kaski JC, et al. Vascular effects and safety of dalcetrapib in patients with or at risk of coronary heart disease: the dal-VESSEL randomized clinical trial. *Eur Heart J*. 2012;33: 857–865.
35. Besler C, Heinrich K, Rohrer L, et al. Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. *J Clin Invest*. 2011;121:2693–2708.
36. Carvalho LS, Panzoldo N, Santos SN, et al. HDL levels and oxidizability during myocardial infarction are associated with reduced endothelial-mediated vasodilation and nitric oxide bioavailability. *Atherosclerosis*. 2014;237:840–846.
37. Adams V, Besler C, Fischer T, et al. Exercise training in patients with chronic heart failure promotes restoration of high-density lipoprotein functional properties. *Circ Res*. 2013;113:1345–1355.
38. Sorrentino SA, Besler C, Rohrer L, et al. Endothelial-vasoprotective effects of high-density lipoprotein are impaired in patients with type 2 diabetes mellitus but are improved after extended-release niacin therapy. *Circulation*. 2010;121:110–122.
39. Weissglas-Volkov D, Pajukanta P. Genetic causes of high and low serum HDL-cholesterol. *J Lipid Res*. 2010;51:2032–2057.
40. Voight BF, Peloso GM, Orho-Melander M, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet*. 2012;380:572–580.
41. Inazu A, Brown ML, Hesler CB, et al. Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. *N Engl J Med*. 1990;323:1234–1238.
42. Koizumi J, Mabuchi H, Yoshimura A, et al. Deficiency of serum cholesteryl-ester transfer activity in patients with familial hyperalphalipoproteinaemia. *Atherosclerosis*. 1985;58:175–186.
43. Maruyama T, Sakai N, Ishigami M, et al. Prevalence and phenotypic spectrum of cholesteryl ester transfer protein gene mutations in Japanese hyperalphalipoproteinemia. *Atherosclerosis*. 2003;166:177–185.
44. Saito F. A pedigree of homozygous familial hyperalphalipoproteinemia. *Metabolism*. 1984;33:629–633.
45. Hirano K-I, Nagasaka H, Kobayashi K, et al. Disease-associated marked hyperalphalipoproteinemia. *Mol Genet Metab Rep*. 2014;1: 264–268.
46. Zhong S, Sharp DS, Grove JS, et al. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. *J Clin Invest*. 1996;97:2917–2923.
47. Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Jensen G, Tybjaerg-Hansen A. Elevated HDL cholesterol is a risk factor for ischemic heart disease in white women when caused by a common mutation in the cholesteryl ester transfer protein gene. *Circulation*. 2000;101:1907–1912.
48. Hirano K, Yamashita S, Nakajima N, et al. Genetic cholesteryl ester transfer protein deficiency is extremely frequent in the Omagari area of Japan. Marked hyperalphalipoproteinemia caused by CETP gene mutation is not associated with longevity. *Arterioscler Thromb Vasc Biol*. 1997;17:1053–1059.
49. Yamashita S, Sprecher DL, Sakai N, Matsuzawa Y, Tarui S, Hui DY. Accumulation of apolipoprotein E-rich high density lipoproteins in hyperalphalipoproteinemic human subjects with plasma cholesteryl ester transfer protein deficiency. *J Clin Invest*. 1990; 86:688–695.
50. Sakai N, Yamashita S, Hirano K, et al. Decreased affinity of low density lipoprotein (LDL) particles for LDL receptors in patients with cholesteryl ester transfer protein deficiency. *Eur J Clin Invest*. 1995; 25:332–339.
51. Ishigami M, Yamashita S, Sakai N, et al. Large and cholesteryl ester-rich high-density lipoproteins in cholesteryl ester transfer protein (CETP) deficiency can not protect macrophages from cholesterol accumulation induced by acetylated low-density lipoproteins. *J Biochem*. 1994;116:257–262.
52. Matsuura F, Wang N, Chen W, Jiang XC, Tall AR. HDL from CETP-deficient subjects shows enhanced ability to promote cholesterol efflux from macrophages in an apoE- and ABCG1-dependent pathway. *J Clin Invest*. 2006;116:1435–1442.
53. Gomaraschi M, Ossoli A, Pozzi S, et al. eNOS activation by HDL is impaired in genetic CETP deficiency. *PLoS One*. 2014;9:e95925.

54. Hirata T, Sugiyama D, Nagasawa SY, et al. A pooled analysis of the association of isolated low levels of high-density lipoprotein cholesterol with cardiovascular mortality in Japan. *Eur J Epidemiol*. 2017; 32:547–557.
55. Ernst N, Fisher M, Smith W, et al. The association of plasma high-density lipoprotein cholesterol with dietary intake and alcohol consumption. The Lipid Research Clinics Prevalence Study. *Circulation*. 1980;62:Iv41–Iv52.
56. Oda N, Kajikawa M, Maruhashi T, et al. Endothelial function is impaired in relation to alcohol intake even in the case of light alcohol consumption in Asian men; Flow-mediated Dilation Japan (FMD-J) Study. *Int J Cardiol*. 2017;230:523–528.

Table S1 Clinical characteristics in propensity score matched subjects

Variables	High 60–79 mg/dL (n = 451)	Extremely high ≥80 mg/dL (n = 451)	*P* value
Age, y	51.4 ± 11	51.5 ± 11	.892
Body mass index, kg/m^2	22.1 ± 2.6	22.0 ± 2.6	.623
Systolic blood pressure, mmHg	129 ± 17	129 ± 17	.695
Diastolic blood pressure, mmHg	81 ± 12	80 ± 12	.239
Heart rate, bpm	63 ± 11	63 ± 12	.956
Total cholesterol, mg/dL	216 ± 32	213 ± 30	.138
Triglycerides, mg/dL	84 (64, 109)	77 (58, 110)	.021
HDL-C, mg/dL	68 ± 6	91 ± 11	<.001
LDL-C, mg/dL	129 ± 29	105 ± 28	<.001
Glucose, mg/dL	101 ± 20	100 ± 17	.500
Medications, n (%)			
Anti-hypertensive therapy	98 (21.7)	104 (23.1)	.632
Anti-hyperglycemic therapy	19 (4.2)	18 (4.0)	.867
Framingham risk score, %	4.0 ± 3.5	3.1 ± 3.4	<.001
Medical history, n (%)			
Hypertension	200 (44.4)	203 (45.0)	.841
Dyslipidemia	165 (36.6)	86 (19.1)	<.001
Diabetes mellitus	29 (6.4)	24 (5.3)	.479
Current smokers	127 (28.2)	131 (29.1)	.768
Previous cardiovascular disease	12 (2.7)	16 (3.6)	.442

HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
Data are presented as mean ± SD or median (interquartile range).

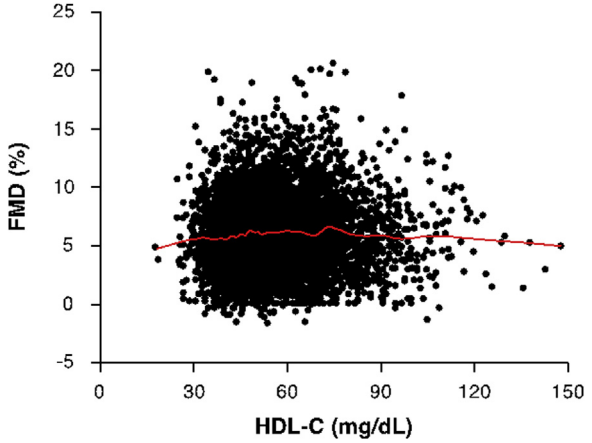

Figure S1 Scatter plot shows the relationship between flow-mediated vasodilation (FMD) and high-density lipoprotein cholesterol (HDL-C). Red line represents the estimated Lowest smoothed curve.

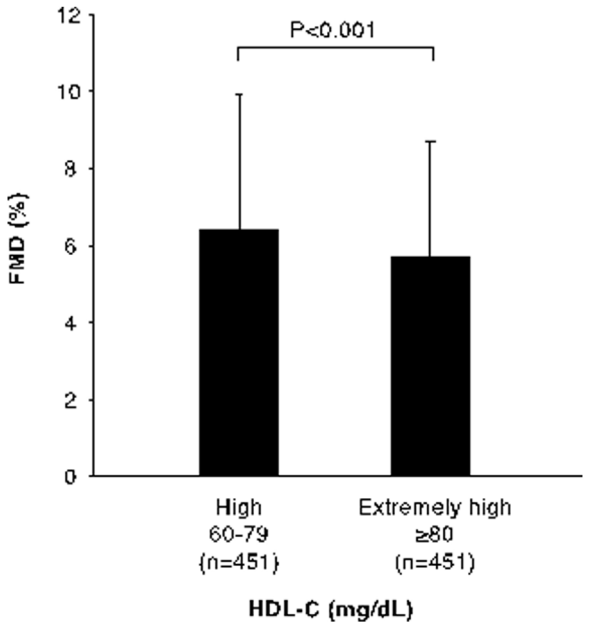

Figure S2 Bar graphs show flow-mediated vasodilation (FMD) in high levels of high-density lipoprotein cholesterol (HDL-C) and in extreme high levels of HDL-C in propensity score matched subjects. The error bars indicate the standard deviation.