Haptoglobin Phenotype Is Associated With High-Density Lipoprotein–Bound Hemoglobin Content and Coronary Endothelial Dysfunction in Patients With Mild Nonobstructive Coronary Artery Disease

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Objective—Coronary endothelial dysfunction (ED) is an early stage of atherosclerosis and is associated with impaired high-density lipoprotein (HDL) function. A functional polymorphism at the haptoglobin (Hp) gene locus (rs72294371) has been associated with marked differences in HDL structure and function. We sought to determine whether Hp phenotype was associated with coronary ED and whether the amount of hemoglobin (Hb) tethered to HDL via Hp was Hp-type dependent and associated with ED.

Approach and Results—Microvascular and epicardial coronary endothelial function was assessed in 338 individuals with nonobstructive coronary artery disease. Microvascular ED was defined as <50% change in coronary blood flow and epicardial ED as ≥20% decrease in coronary artery diameter after intracoronary acetylcholine infusion. The amount of Hb bound to HDL was measured by ELISA after HDL purification from plasma samples using immune-affinity chromatography. One hundred and seventy of the individuals in this study (50.3%) were diagnosed with microvascular ED, 143 (42.3%) with epicardial ED, and 67 (19.7%) had diabetes mellitus (DM). Hp phenotype was significantly associated with microvascular (P=0.01) and epicardial ED (P=0.04) among DM individuals. There was a significant and inverse correlation between the amount of HDL-bound Hb and change in coronary blood flow (r=–0.40; P<0.0001) and in coronary artery diameter (r=–0.44; P<0.0001) in response to acetylcholine infusion. Hb content of HDL was significantly increased in individuals with Hp 2-2 and DM. In a logistic regression model, Hp 2-2 phenotype was associated with microvascular ED (odds ratio, 1.9; P=0.03) and the amount of HDL-bound Hb was an independent predictor of both microvascular (odds ratio, 4.6 for each 1-SD increase; P<0.0001) and epicardial (odds ratio, 2.2; P<0.0001) ED.

Conclusions—Hp phenotype is significantly associated with coronary ED in DM individuals. This association is likely related to increased Hb tethering to HDL via Hp 2-2 in DM.

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Key Words: atherosclerosis ■ diabetes mellitus ■ haptoglobin ■ high-density lipoprotein ■ oxidative stress

Endothelial dysfunction (ED) represents an early stage in atherogenesis and is characterized by an imbalance between endothelium-dependent vasodilator and vasoconstrictor activity, as well as by altered anti-inflammatory and anticoagulant properties of the endothelium.[1–5] Among patients without obstructive coronary artery disease, the presence of coronary ED has been demonstrated as an independent risk factor for adverse cardiovascular events, supporting a role of coronary ED in the progression of coronary atherosclerosis.[6–8] Studies have shown that local coronary ED is associated with tissue characteristics suggestive of plaque vulnerability to rupture, including increased macrophage-rich necrotic plaques.[9] Moreover, there is a more pronounced local production of lipoprotein-associated phospholipase A2, an inflammatory mediator expressed by macrophages, and its products, lysophosphatidylcholine and oxidized fatty acids released from oxidized low-density lipoprotein (LDL), in patients with coronary ED, suggesting an involvement of oxidative stress and inflammation in the development of ED and progression of atherosclerosis.[3,10,11]

High-density lipoprotein (HDL) possesses antiatherogenic properties that are largely mediated by its ability to remove excess cholesterol from macrophages within the vessel wall, a process known as reverse cholesterol transport.[12] Cholesterol efflux capacity assessed in macrophages in vitro, reflecting HDL function, has been shown to be a strong predictor of

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Nonstandard Abbreviations and Acronyms	
CBF	coronary blood flow
DM	diabetes mellitus
ED	endothelial dysfunction
Hb	hemoglobin
HDL	high-density lipoprotein
Hp	haptoglobin

cardiovascular events independently of circulating HDL-cholesterol (HDL-C) levels.[13,14] HDL may become dysfunctional and proatherogenic under certain circumstances such as diabetes mellitus (DM) and high oxidative states.[15–17] The emerging importance of HDL functionality, rather than simply HDL levels, as a mediator of atherosclerotic cardiovascular events may explain the apparent failure of HDL-raising therapies to mitigate the risk of adverse cardiovascular events because increasing dysfunctional HDL may be harmful. In early stages of atherosclerosis, HDL function has been shown to be markedly impaired among subjects with coronary ED.[18] Moreover, subjects with early atherosclerosis and coronary ED have higher cholesterol content within the vessel wall than those with normal endothelial function.[19] These findings suggest that HDL dysfunction may contribute to the pathogenesis of ED and the progression of atherosclerotic lesions in humans.

We have previously demonstrated that HDL function is modulated by a genetic polymorphism in the haptoglobin (Hp) gene. Hp is a plasma free hemoglobin (Hb)–binding protein which has been shown to be a component of the HDL proteome and binds specifically to ApoA1 (apolipoprotein A1) as a complex with Hb.[20] A common 1.7-kb in-frame copy number variant polymorphism (rs72294371) has been described in the Hp gene, resulting in Hp allelic protein products that are associated with substantial differences in the structure and function of HDL in the setting of DM.[20–22] Specifically, in DM individuals homozygous for the Hp 2 allele (Hp 2-2 genotype), HDL has been shown to be more heavily oxidized, and its ability to promote cholesterol efflux from macrophages has been shown to be impaired.[20,21] Hp genotype–dependent differences in HDL function have been proposed as one reason for why Hp genotype appears to be predictive of incident cardiovascular disease in patients with DM in multiple longitudinal studies.[23–28] Although studies have shown that the extent of HDL dysfunction is determined by Hp type and that HDL dysfunction is implicated in coronary ED, the link between Hp genotype and coronary ED has not been studied.

We therefore proposed that Hp genotype is a determinant of coronary ED and that individuals with the Hp 2-2 genotype and DM have an increased prevalence of coronary ED that may be mediated by the presence of an increased amount of extracorpuscular Hb tethered to HDL via Hp, leading to HDL dysfunction.

Materials and Methods

A detailed data analysis section is available in the online-only Data Supplement. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Human Studies

The study protocol was approved by the Mayo Clinic Institutional Review Board. Informed consent was obtained from all subjects who participated in the study. Invasive coronary physiology study was performed to evaluate for ED in patients referred with a history of chest pain and normal luminal coronary arteries or mild nonobstructive coronary artery disease (<30% diameter stenosis), as determined by coronary angiography. Patients were defined as having normal or abnormal coronary endothelial function based on changes in coronary blood flow (CBF; microvascular ED) or change in epicardial artery diameter (epicardial ED) after intracoronary infusion of acetylcholine during cardiac catheterization.[6,9,11,29] Patients were excluded from the study if they had >30% diameter stenosis of any coronary artery, left ventricular ejection fraction <40%, acute coronary syndrome (unstable angina pectoris or acute myocardial infarction), cerebrovascular accident within 6 months, history of percutaneous coronary intervention, administration of radiographic contrast agents within 12 hours, severe renal or hepatic disorder, active inflammatory disease, or systemic infection within 4 weeks before catheterization. Vasoactive medications that can affect coronary vasoreactivity, such as nitrates and calcium channel blockers, were discontinued for at least 48 hours. All subjects were studied after fasting and discontinuation of tobacco use for at least 12 hours before the study. DM was defined based on the American Diabetes Association criteria for DM diagnosis, including patients who had HbA1c (hemoglobin A1c) value ≥6.5%, a fasting glucose level ≥126 mg/dL, or those on antihyperglycemic pharmacotherapy before participation in the study.

Diagnostic coronary angiography was performed with a 6F or 7F guiding catheter using standard clinical protocols. Nonionic contrast material was used, and no nitroglycerin was administered before the diagnostic procedure. Angiograms were carefully reviewed to exclude obstructive coronary disease (>30% stenosis) before the infusion of any pharmacological agents. Endothelial-dependent changes in CBF and coronary artery diameter were then performed right after the coronary angiography as previously described.[6,9,11,29] Briefly, after administration of unfractionated intravenous heparin for an activated clotting time of 200 to 250 s, endothelial function was assessed using a 0.014-inch Doppler tipped guidewire (FloWire: Volcano Corp, CA) that was positioned in the midportion of the left anterior descending coronary artery 2 to 3 mm distal to the tip of a coronary infusion catheter (Ultrafuse, Boston Scientific). Intracoronary acetylcholine was then infused in incremental concentrations up to a maximum tolerable dose (10^{-6}, 10^{-5}, 10^{-4} mol/L at 1 mL/min at 3-minute intervals). The infusion was terminated when the highest dose of acetylcholine (10^{-4} mol/L) was achieved. Baseline and maximal average peak velocities obtained after acetylcholine infusion were measured by Doppler echography and recorded. Doppler measurements and repeat coronary angiogram were obtained after each infusion. Coronary artery diameter was measured using a computer-based image analysis system performed in the segment 5 mm distal to the tip of the Doppler wire by an independent investigator blinded to the Doppler measurements. All coronary artery diameter measurements were made in the same angiographic projection and angulation and using the same level of magnification to confirm consistent coronary diameter measurements in all study participants. Endothelial-dependent CBF was then calculated from the Doppler-derived time velocity integral and vessel diameter, as previously described[29]: CBF = π(average peak velocity/2)(coronary artery diameter/2)^2. The maximal percentage change in CBF with acetylcholine infusion relative to baseline CBF was then extracted. Patients with <50% increase in CBF in response to acetylcholine infusion were determined as having microvascular ED. The maximal effect of acetylcholine was expressed as percent change in coronary artery diameter relative to baseline using quantitative coronary angiography, representing epicardial endothelial function. Impaired epicardial endothelial function was defined as ≥20% decrease in the coronary artery diameter in response to acetylcholine infusion. Using these cutoff percentages of change in CBF and coronary artery diameter, previous studies have demonstrated a strong association between coronary ED and adverse cardiovascular events.[6,29]

Hp Phenotyping

The Hp polymorphism assessed in this study is a copy number variant defined by the absence (Hp 1 allele) or presence (Hp 2 allele) of a 1.7-kb partial in-frame intragenic duplication of exons 3 and 4 of the Hp gene on chromosome 16.[30] The Hp monomeric protein product forms multimeric structures whose stoichiometry is dependent on the Hp type because of the presence of a free cysteine residue in exon 3 which mediates Hp multimerization. The Hp phenotype describes the polymers as detected by examining the Hp protein and directly corresponds with the Hp genotype describing the presence or absence of the copy number variant as detected by polymerase chain reaction. The protein product of the Hp 1 allele is monovalent, able to bind one Hp monomer, and accordingly in individuals with the Hp 1-1 phenotype, there are only dimers of Hp. The protein product of the Hp 2 allele is bivalent and is able to bind to 2 different Hp monomers, resulting in large cyclic polymers of Hp in individuals with the Hp 2-2 phenotype. In individuals with the Hp 2-1 phenotype, linear polymers of Hp are produced with both Hp 1 and Hp 2 monomers. We have recently described an ELISA which takes advantage of the polymeric structure of the different Hp types to rapidly and accurately perform Hp typing.[31]

Briefly, for the Hp typing ELISA, a microtiter plate is coated with a mAb to Hp. Then, 10 μL of plasma containing Hp, diluted 1:10 in sample diluent buffer (PBS with 1% BSA and 0.1% Tween-20), was added and incubated for 1 hour on a plate shaker to allow binding to the mAb. After washing the wells 5× with PBS containing 0.5% Tween-20, the same mAb conjugated to horseradish peroxidase was added to allow binding to the captured Hp with an additional hour of incubation. After extensive washing, Hp type was determined based on the level of intensity of the horseradish peroxidase signal measured by spectrophotometry after addition of a colorimetric substrate for horseradish peroxidase using known Hp type standards to establish a range for each Hp type in this assay. The use of this method for Hp typing was validated in over 8000 patient samples by comparing the results with those obtained by polyacrylamide gel electrophoresis and the TaqMan polymerase chain reaction method, demonstrating equivalent accuracy to gel electrophoresis and superiority to polymerase chain reaction.[31]

Quantitation of Hemoglobin Bound to HDL

Isolation of HDL From Serum by Immunoaffinity Chromatography

HDL was obtained from 200 μL of serum obtained from the study participants. One hundred microliter of polyclonal gout anti-human ApoA1 antibody conjugated to activated sepharose beads in PBS with 0.5 mol/L NaCl was added to serum in a final volume of 1 mL. The serum and anti-ApoA1 sepharose were mixed on rotary device for 2 hours at room temperature. The sepharose beads and solution were then transferred to a poly prep chromatography column (0.8×4 cm) (BioRad), and the solution was allowed to flow through. The beads were then washed with 10 mL of PBS with 0.5 mol/L NaCl and then with 10 mL of PBS. One hundred microliter of 0.1 mol/L Glycine pH 2.5 was added to the column and the eluate discarded (column void volume), and then the HDL was eluted with 3×100 μL of 0.1 mol/L Glycine pH 2.5 into tubes containing 30 μL 1 mol/L Tris pH 9 and 30 μL fetal calf serum.

Measurement of Hb Concentrations in Purified HDL by ELISA

A 96-well plate (Nunc immunoplate, ThermoScientific) was coated with monoclonal mouse anti-human Hb (Abcam) at 4.6 μg/mL in PBS and allowed to incubate overnight at 4°C. Plates were washed 5× with PBS containing 0.5% Tween-20 and then blocked with PBS containing 10% fetal calf serum for 1 hour while shaking at room temperature. One hundred microliter of immunoaffinity purified HDL (containing similar HDL concentrations) was then added to each well (all samples were examined in duplicate). In addition, a 1:10 dilution of the HDL sample was prepared in standard buffer (made by mixing one part 1 mol/L Tris pH 9, one part 1 mol/L glycine pH 2.5, one part fetal calf serum, and 7 parts water) and assayed as well. The Hb standards for the ELISA were prepared by creating a solution containing 10 ng of Hb in 100 μL of standard buffer. Ten serial dilutions of the standard were made in standard buffer. Standard buffer was also used as the blank in this assay. After aliquoting to the ELISA plates, standards and samples were incubated for 1 hour while shaking at room temperature, washed 5× with PBS containing 0.5% Tween-20, and to each well was added 100 μL horseradish peroxidase–conjugated goat anti-human Hb (ICL antibodies) at 2.5 μg/mL in PBS with 10% fetal calf serum. Plates were shaken for 1 hour at room temperature and then washed 5× with PBS containing 0.5% Tween-20. The plates were developed with 3′,3′,5,5′-tetramethylbenzidine substrate for 5 minutes, and reaction was then stopped with 100 μL of 1 mol/L H_2SO_4 and absorbance read at 450 nm. The intraday and interday precision (expressed as coefficient of variation) of the whole procedure (starting from immunoaffinity isolation of the HDL followed by the ELISA for Hb measurement) was <10% and <15%, respectively. Furthermore, with the use of a constant amount of anti-human ApoA1 antibody as the limiting factor, the amount of HDL in the HDL elute was similar in the different samples.

Statistical Analysis

All variables were tested for normal data distribution. Continuous clinical variables were reported as mean±SD for all normally distributed measurements and categorical variables as frequencies and percentages. Variables were compared between groups using χ^2 test for categorical variables and independent student *t* test or analysis of variance, as appropriate, for continuous variables with normal distribution. As Hb concentrations in HDL particles were not normally distributed, data were presented as median with the first and third quartiles (Q1, Q3), and the nonparametric Kruskal-Wallis test with the Steel-Dwass post hoc test for multiple comparisons were used to assess differences in variables with skewed distribution between the different groups. Correlations were examined using the Pearson method. A univariate logistic regression model was used to examine association of several variables, and those with significant association were included in a multivariate logistic regression model for examining the independent association of Hp phenotype and HDL-bound Hb concentrations with microvascular and epicardial ED. All significance tests were 2-tailed and conducted at the 5% significance level. Data were analyzed with the JMP 8.0 (SAS Institute, Inc, Cary, NC) and SPSS 23 (SPSS Inc, Chicago, IL) software.

Results

Study Participants

A total of 338 patients with mild nonobstructive coronary artery disease who met our inclusion criteria underwent assessment of coronary endothelial function at the Mayo Clinic. Of these, 170 patients had microvascular ED (defined as <50% change from baseline in CBF in response to intracoronary acetylcholine infusion) and 168 patients had normal microvascular endothelial function. Subjects with microvascular ED were older than those with normal microvascular endothelial function (51.1±12.2 versus 48.3±11.2; *P*=0.03; Table 1). Approximately two thirds of participants were women and had family history of ischemic heart disease, 40% had hypertension, and 15% were active smokers, without significant differences between the 2 groups. Out of the 338 participants, 67 patients (19.7%) had DM: 27 patients (16.1%) in the normal microvascular endothelial function group compared with 40 patients (23.5%) in the microvascular ED group (*P*=0.09). All DM patients included in the study were diagnosed with type 2 DM. Among those with DM, HbA1c levels and rates of insulin therapy did not differ significantly between the

Table 1. Baseline Characteristics of the Study Population

	Patients Without ED (n=168)	Patients With ED (n=170)	*P* Value
Age, y	48.3±11.2	51.1±12.2	0.03
Female, n (%)	105 (62.5)	105 (61.8)	0.89
Race (white), n (%)	151 (89.9)	158 (92.9)	0.32
BMI, kg/m^2	29.2±6.1	29.6±6.1	0.60
Hypertension, n (%)	71 (42.3)	71 (41.8)	0.93
Diabetes mellitus, n (%)	27 (16.1)	40 (23.5)	0.09
HbA1c, %	7.0±2.1	7.4±2.0	0.43
Insulin therapy, n (%)	6 (22.2)	10 (25.0)	0.78
Hyperlipidemia	88 (52.4)	87 (51.2)	0.83
Smoking (current), n (%)	30 (17.9)	23 (13.5)	0.27
Smoking (previous), n (%)	60 (35.7)	55 (32.4)	0.34
Peripheral vascular disease, n (%)	11 (6.6)	17 (10.0)	0.25
Family history of IHD	99 (58.9)	108 (63.5)	0.37
Total cholesterol, mg/dL	189.3±42.6	184.1±41.0	0.27
Triglycerides, mg/dL	139.6±97.3	140.9±88.5	0.90
HDL cholesterol, mg/dL	54.0±16.8	50.6±17.2	0.70
LDL cholesterol, mg/dL	109.7±34.8	104.3±33.6	0.17
Hs-CRP, mg/dL	2.4±3.6	1.7±2.6	0.13
Homocysteine, μmol/L	7.7±2.7	8.2±2.8	0.12
BNP, pg/mL	42.9±60.2	36.5±42.4	0.45
Lipoprotein(a), mg/dL	27.8±31.8	23.7±31.0	0.37
Hemoglobin, g/dL	13.7±1.2	13.7±1.4	0.79
Creatinine, mg/dL	0.97±0.21	0.98±0.19	0.68
BUN, mg/dL	14.5±5.3	16.0±5.5	0.06
Uric acid, mg/dL	4.8±1.6	5.3±1.6	0.02
Aspirin, n (%)	79 (47.0)	96 (56.5)	0.08
Statins, n (%)	50 (29.8)	72 (42.4)	0.02
Anticoagulation, n (%)	20 (12.0)	23 (13.5)	0.68
Nitrates, n (%)	56 (33.3)	56 (32.9)	0.94
Diuretics, n (%)	26 (15.5)	27 (15.9)	0.92
CCB, n (%)	76 (45.2)	58 (34.1)	0.04
BB, n (%)	37 (22.0)	51 (30.0)	0.10
ACE inhibitor, n (%)	28 (16.7)	26 (15.3)	0.73
Antiarrhythmic drugs, n (%)	7 (4.2)	7 (4.1)	0.98
LVEF, %	60.6±6.7	62.1±9.3	0.33

Data expressed as mean (±SD) or n (%) as appropriate.

ACE indicates angiotensin-converting enzyme; BB, beta blockers; BMI, body mass index; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; CCB, calcium channel blockers; ED, endothelial dysfunction; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; Hs-CRP, high resolution C-reactive protein; IHD, ischemic heart disease; LDL, low-density lipoprotein; and LVEF, left ventricular ejection fraction.

normal microvascular endothelial function and microvascular ED groups. HDL levels were similar in patients with and without microvascular ED (50.6±17.2 versus 53.0±16.8 mg/dL, respectively; *P*=0.70), as were levels of LDL (*P*=0.17), lipoprotein (a) (*P*=0.37), triglycerides (*P*=0.90), and levels of inflammatory markers (Hs-CRP; *P*=0.13). The use of statins was higher (42% versus 30%; *P*=0.02) and the use of calcium channel blockers was lower (34% versus 45%; *P*=0.04) in patients with microvascular ED, while rates of aspirin, anticoagulation, and nitrate therapy were similar in the 2 groups.

Hp Phenotype Is Associated With Microvascular ED

Hp type was examined in all study participants by monoclonal anti-human Hp antibody-based ELISA using plasma EDTA samples.[31] In the overall cohort, there were 44 patients (13%) with Hp 1-1, 162 (48%) with Hp 2-1, and 132 (39%) with the Hp 2-2 type (Figure 1), a distribution similar to that previously reported in the United States. Among patients with microvascular ED, Hp 2-2 type was found in 45.3%, Hp 2-1 in 44.1%, and Hp 1-1 in 10.6% as compared to 32.7%, 51.8%, and 15.5% in patients with normal microvascular endothelial function, respectively (*P*=0.048). The higher prevalence of Hp 2-2 type in the microvascular ED group was particularly pronounced among patients with DM, with 55% of patients with DM having Hp 2-2, 37.5% having Hp 2-1, and 7.5% having Hp 1-1 compared with 18.5%, 55.6%, and 25.9% in the normal microvascular endothelial function group (*P*=0.01). In patients without DM, there was no significant association between the Hp phenotype and the presence of microvascular ED, with 42.3% having Hp 2-2, 46.2% having Hp 2-1, and 11.5% having Hp 1-1 compared with 35.4%, 51.1%, and 13.5% in patients with and without microvascular ED, respectively (*P*=0.51).

HDL-Bound Hb Content Is Significantly Associated With Microvascular ED

Hb content of HDL was measured as described in Methods in all samples obtained from subjects at the time of their coronary endothelial function study. The median (Q1, Q3) Hb concentrations in all samples was 7.6 (2.8, 13.1) ng/mL of HDL. As shown in Figure 2A, the median (Q1, Q3) amount of Hb bound to HDL in subjects with microvascular ED was significantly higher than in subjects with normal microvascular endothelial function (10.5 [4.7, 10.5] versus 3.9 [1.9, 9.0]; *P*<0.0001; for the microvascular ED and non-ED groups, respectively). To validate this relationship, the percentage of change in CBF from baseline after intracoronary acetylcholine infusion was used to examine the correlation between HDL-bound Hb content and coronary endothelial function both as continuous variables. We found a significant inverse correlation between HDL-bound Hb concentrations and change in CBF (r=–0.40; *P*<0.0001; Figure 2B). Moreover, when patients were stratified according to quartiles of HDL-bound Hb content, 77% of patients with the upper quartile of Hb levels (>13.1 ng/mL) had microvascular ED as compared to 32% of patients with the lower quartile (*P*<0.0001; Figure 3A). The dose-response pattern observed by Hb quartile analysis for association with microvascular ED was more pronounced among DM individuals, with 88% of patients with the upper quartile of Hb content of HDL having microvascular ED as compared with 15% of those with the lower quartile (*P*<0.000; *P*=0.04 for interaction; Figure 3B).

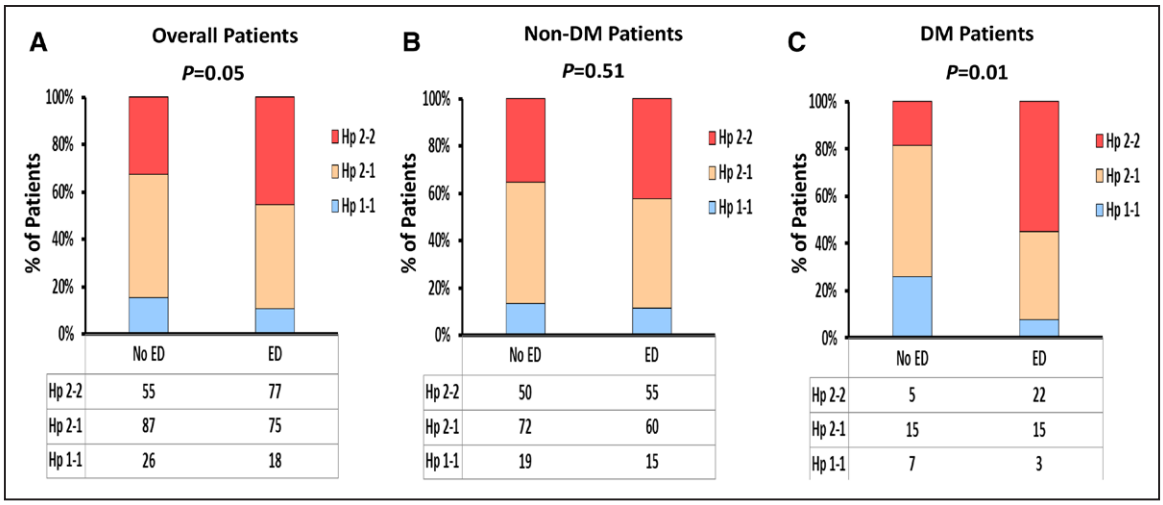

Figure 1. Distribution of haptoglobin (Hp) phenotypes in patients with and without microvascular endothelial dysfunction (ED). Data are presented as percentage of patients with Hp 1-1, Hp 2-1 and Hp 2-2 in patients with and without microvascular ED, and the absolute number of patients in each group are presented in the table under each histogram. In overall patients (**A**), there was more patients with Hp 2-2 in the microvascular ED group (*P*=0.05) but not in patients without diabetes mellitus (DM; **B**). In patients with DM, there were significantly more patients with the Hp 2-2 and less with the Hp 1-1 in the microvascular ED group compared with the non-ED group (*P*=0.01; **C**).

Hp Phenotype Is Associated With Epicardial ED

Epicardial ED was defined as ≥20% decrease in coronary artery diameter in response to acetylcholine infusion. We found that 143 patients (42.3%) developed epicardial ED. Of those with epicardial ED, 55 (38.5%) had normal microvascular endothelial function and 88 (61.5%) had microvascular ED. Hp phenotype was significantly associated with epicardial ED in the overall cohort, with 43.4%, 48.8%, and 8.4% versus 35.9%, 47.7%, and 16.4% having Hp 2-2, Hp 2-1, and Hp 1-1 in patients with and without epicardial ED, respectively (*P*=0.025). Similar to microvascular ED, the association between epicardial ED and Hp type was specific to the DM population. Among patients with DM and epicardial ED, Hp 2-2 type was found in 48.5%, Hp 2-1 in 45.4%, and Hp 1-1 in 6.1% compared with 40.7%, 44.1%, and 23.5% in patients with normal epicardial endothelial function, respectively (*P*=0.038). Among non-DM patients with epicardial ED, 41.8% had Hp 2-2, 49.1% had Hp 2-1, and 9.1% had Hp 1-1 compared with 36.6%, 48.5%, and 14.9% in the normal endothelial function group, respectively (*P*=0.14).

Further analysis showed that there were 88 patients (26% of the overall cohort) who had both microvascular and epicardial ED (62 patients without DM [22.9% of all non-DM] and 26 patients with DM [38.8% of all DM]). The association between Hp type and the presence of combined microvascular and epicardial ED was remarkable in the DM group (57.7%, 38.5%, and 3.9% had Hp 2-2, Hp 2-1, and Hp 1-1, respectively; *P*=0.026), while no significant association was found in the non-DM group (43.5%, 45.2%, and 11.3% with Hp 2-2, Hp 2-1, and Hp 1-1; *P*=0.66) or in the overall group (47.7%, 43.2%, and 9.1% with Hp 2-2, Hp 2-1, and Hp 1-1; *P*=0.14).

HDL-Bound Hb Content Is Significantly Associated With Epicardial ED

We found a significant association between HDL-bound Hb concentration and change in epicardial artery diameter in response to acetylcholine (r=–0.44; *P*<0.0001; Figure 2D). Patients with epicardial ED had markedly higher HDL-bound Hb content compared with those with normal epicardial endothelial function (median 10.79 [4.21, 21.4] versus 4.51 [1.95, 9.82] for patients with and without epicardial ED, respectively; *P*<0.0001; Figure 2C). Further analysis demonstrated that patients with both microvascular and epicardial ED had the highest amount of HDL-bound Hb while those with both normal microvascular and epicardial endothelial function had the lowest amount of HDL-bound Hb (median 3.7 [1.65, 7.63] versus 13.0 [7.17, 26.95]; *P*<0.0001). Patients with either microvascular or epicardial ED had intermediate HDL-bound Hb content. In addition, these differences in the amount of HDL-bound Hb were exaggerated among patients with DM (median 2.98 [1.56, 5.84] versus 27.7 [18.93, 42.80] in patients with both normal microvascular and epicardial endothelial function versus those with combined ED, respectively; *P*<0.0001; Figure 4).

Hb Content of HDL Is Significantly Increased in Patients With Hp 2-2 and DM

To test whether the association between Hp type and ED is correlated with the amount of Hb tethered to HDL, we examined differences in HDL-bound Hb concentrations in both DM and non-DM individuals according to their Hp type. We found significant Hp type–dependent differences in the amount of Hb bound to HDL particles (*P*=0.003; Figure 5A). Hp 2-2 participants had ≈2.5-fold increased HDL-bound Hb content compared with the Hp 1-1 participants (8.9 [3.4, 20.0] versus 3.9 [1.5, 11.3] ng/mL, respectively; *P*=0.007), while Hp 2-1 participants had intermediate amount of Hb bound to HDL (6.2 [3.3, 12.0]; *P*=0.07 as compared to Hp 2-2 participants). Among non-DM patients, Hp 2-2 was not associated with significant differences in the amount of HDL-bound Hb content compared with Hp 1-1 (7.7 [3.1–13.2] versus 3.6 [1.0–10.4]; *P*=0.08) and Hp 2-1 (7.7 [3.1–13.2] versus 5.3 [3.3–10.5]; *P*=0.23; Figure 5B). Among DM participants, Hp 2-2 was associated with ≈5-fold (23.2 [8.1, 36.8] versus 5.0 [1.7, 13.3]

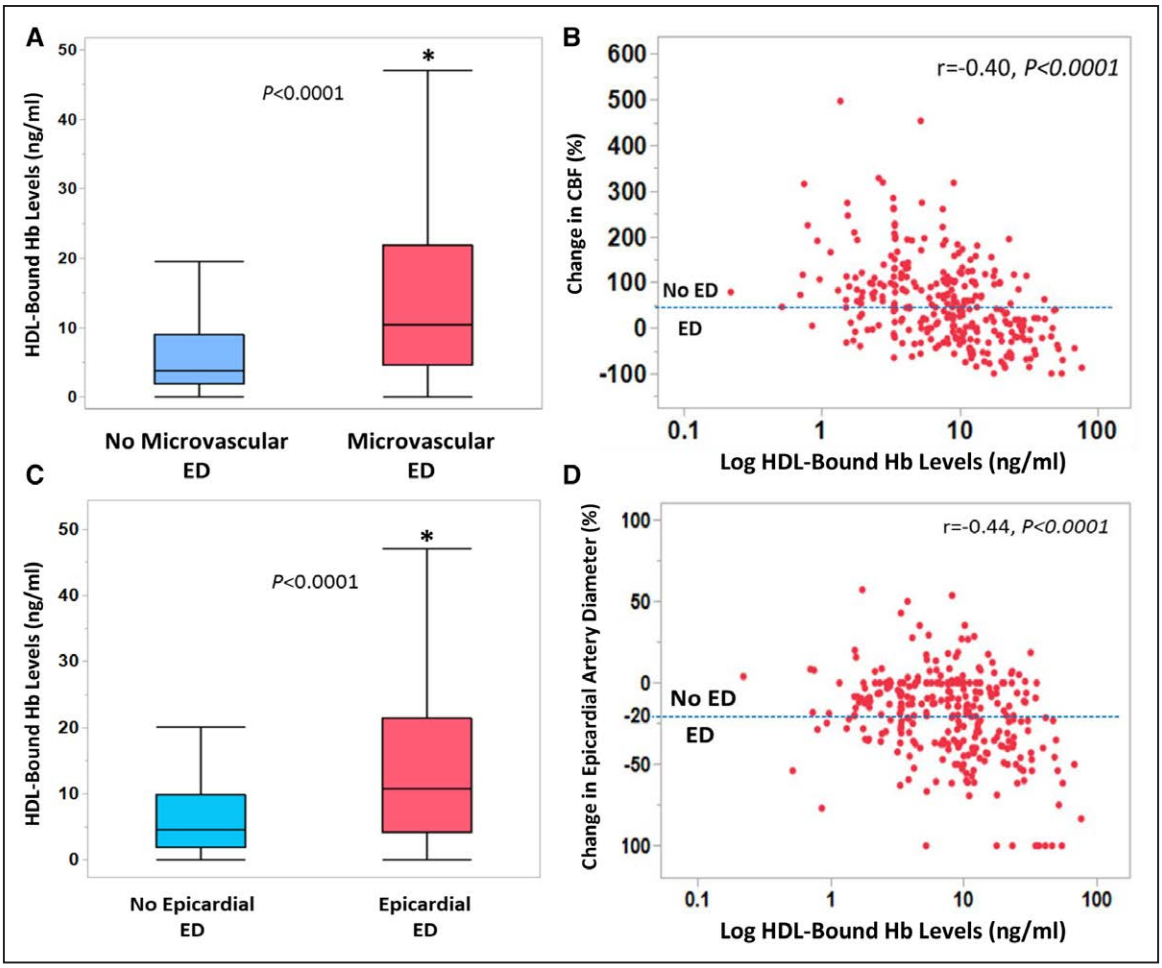

Figure 2. High-density lipoprotein (HDL)–bound hemoglobin (Hb) concentrations in patients with and without endothelial dysfunction (ED). Hb was assessed in all patients using ELISA performed in HDL purified by immuneaffinity chromatography as described in Methods. Change in coronary blood flow (CBF) was measured after intracoronary acetylcholine infusion with <50% increase in CBF relative to baseline defined as microvascular ED. Change in epicardial artery diameter was measured after intracoronary acetylcholine infusion, with ≥20% decrease in diameter relative to baseline defined as epicardial ED. **A** and **C**, The amount of Hb bound to HDL in subjects with microvascular ED (**A**) and epicardial ED (**C**) was remarkably higher than in subjects with normal endothelial function (P<0.0001). Data are presented as box-and-whisker plots (25%–75% percentiles) with the median value and range presented as lines. **B** and **D**, Pearson correlation analysis of the covariance showing a significant and inverse correlation between HDL-bound Hb concentrations and percentage of change in CBF (**B**) and epicardial artery diameter (**D**) in response to acetylcholine compared with baseline.

ng/mL; P=0.01) and 2-fold (23.2 [8.1, 36.8] versus 11.1 [3.1, 21.7] ng/mL; P=0.06) increased HDL-bound Hb concentration compared with Hp 1-1 and Hp 2-1, respectively (Figure 5C). Moreover, there was a highly significant interaction between the Hp 2-2 type and DM on HDL-bound Hb content (P<0.001 for interaction). In a multivariate logistic regression model that included Hp 2-2 phenotype, DM, age, sex, and plasma HDL-C, CRP, and Hb levels, both Hp 2-2 phenotype (odds ratio [OR], 2.3; 95% confidence interval [CI], 1.30–4.13; P=0.004) and DM (OR, 2.2; 95% CI, 1.11–4.15; P=0.023) were independently associated with elevated (greater than or equal to the third quartile) HDL-bound Hb levels.

Hp Phenotype and Hb Content of HDL Are Significantly Associated With Microvascular ED

A logistic regression model was performed to identify significant indicators for the presence of microvascular ED. Plasma HDL-C levels were not significantly associated with microvascular ED (OR, 1.02; 95% CI, 0.82–1.27, per 1-SD change; P=0.86). Similarly, the presence of DM was not a significant predictor for ED, despite a trend toward an increased odds of ED observed in DM individuals (OR, 1.61; 95% CI, 0.93–2.77; P=0.09; Figure 6A). The association between Hp type and ED was examined by stratifying patients 2 two groups based on having the Hp 2-2 phenotype risk marker (Hp 2-2 versus non-Hp 2-2 groups). We found that Hp 2-2 was associated with significantly higher risk of microvascular ED (unadjusted OR, 1.70; 95% CI, 1.09–2.65; P=0.018) compared with non-Hp 2-2 (Figure 6A). Specifically, this relationship was more remarkable among those subjects with DM (unadjusted OR, 5.38; 95% CI, 1.70–17.05; 0.004) but not in those without DM (unadjusted OR, 1.33; 95% CI, 0.82–2.18; P=0.248; P=0.023 for interaction between the Hp 2-2 type and DM for predicting the presence of microvascular ED; Table 2). In addition, there was a highly significant association between the amount of HDL-bound Hb and microvascular ED, with >3-fold increase in odds ratio of ED for each 1 SD increase in the Hb content of purified HDL (OR, 3.18; 95% CI, 2.19–4.62; P<0.0001;

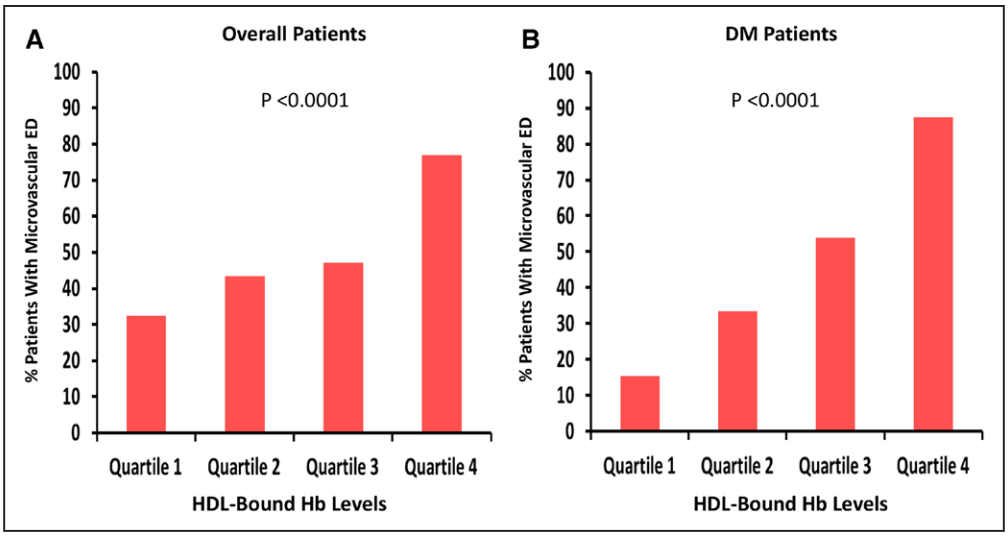

Figure 3. Probability of microvascular endothelial dysfunction (ED) according to quartiles of high-density lipoprotein (HDL)–bound hemoglobin (Hb) concentrations. The proportion of (**A**) overall patients and (**B**) patients with diabetes mellitus (DM) with microvascular ED is presented in each one of the Hb quartiles. Hb concentrations were <2.8, 2.8–7.5, 7.6–13.1, >13.1 ng/mL HDL for quartiles 1, 2, 3, and 4, respectively. Of all patients, 77% with the upper quartile of Hb levels had ED vs 32% with the lower quartile (P<0.0001). Of DM patients, 88% vs 15% with the upper and lower quartiles of Hb had microvascular ED, respectively (P<0.0001).

Figure 6A). The association between HDL-bound Hb content and microvascular ED was more robust in patients with DM (unadjusted OR, 6.9; P<0.0001 versus OR, 2.60; P<0.0001, per 1 SD increase, in the DM and non-DM groups; P=0.048 for interaction; Table 2).

In a univariate logistic regression model that tested multiple variables for association with microvascular ED, we found that, in addition to Hp 2-2 and HDL-bound Hb, age, uric acid levels, use of calcium channel blockers, and statins were significantly associated with ED, while DM was only marginally associated with ED (Table 3). In a multivariate logistic regression model that included all important variables observed in the univariate model as well as plasma HDL levels (another important variable to be adjusted for when assessing the effect of HDL-bound Hb content on ED), we found that Hp 2-2 remained a significant indicator of microvascular ED (adjusted OR, 1.90; 95% CI, 1.06–3.39; P=0.030). Elevated HDL-bound Hb content was also independently associated with significantly increased prevalence of microvascular ED after adjustment for the same variables (adjusted OR, 4.64; 95% CI, 2.59–8.30, per 1 SD increase; P<0.0001; Table 3).

Hb Content of HDL and Microvascular ED Are Significantly Associated With Epicardial ED

A univariate logistic regression analysis demonstrated that similar to microvascular ED, the amount of HDL-bound Hb was a significant indicator of epicardial ED (OR, 2.40;

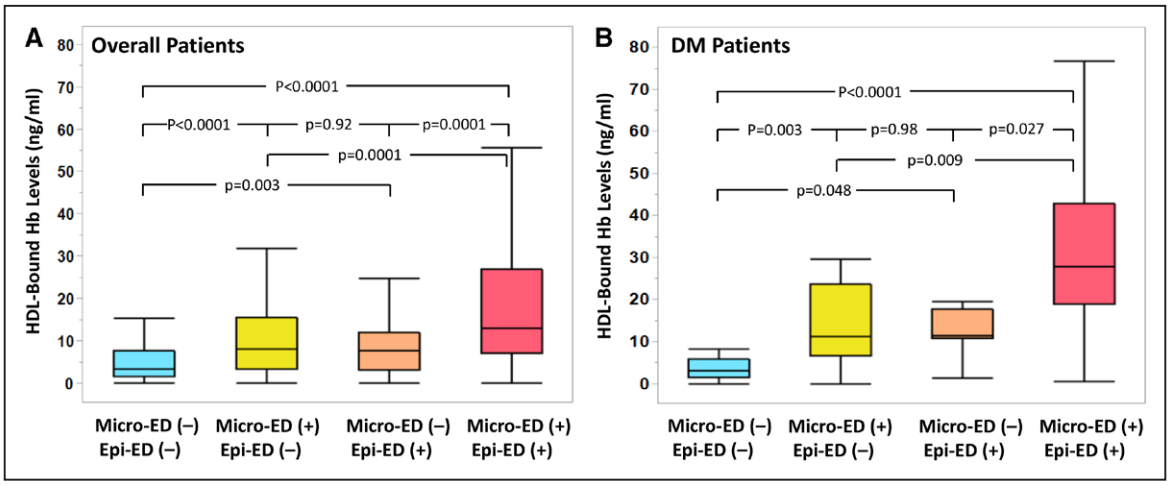

Figure 4. Differences in the amount of high-density lipoprotein (HDL)–bound hemoglobin (Hb) stratified by microvascular and epicardial endothelial function. Data are presented as box-and-whisker plots of hemoglobin concentration (ng/ml HDL) in (**A**) overall patients and (**B**) patients with diabetes mellitus (DM). Patients with both microvascular and epicardial endothelial dysfunction had significantly higher amount of Hb attached to HDL than those with either epicardial or microvascular endothelial dysfunction (ED) or those with both normal microvascular and epicardial endothelial function, and these differences were more accentuated in DM. P<0.0001 by Kruskal-Wallis test in overall and in DM patient groups. Differences between the different subgroups were tested for significance by Steel-Dwass post hoc analysis.

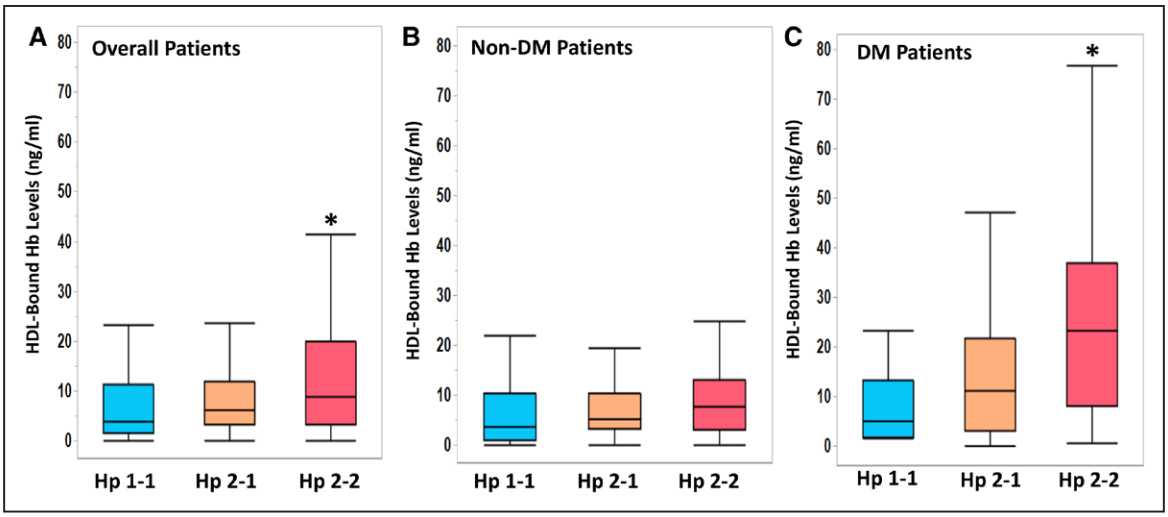

Figure 5. Differences in high-density lipoprotein (HDL)–bound hemoglobin (Hb) concentrations by haptoglobin (Hp) phenotype. Data are presented as box-and-whisker plots of hemoglobin concentration (ng/ml HDL) in (**A**) overall patients, (**B**) patients without diabetes mellitus (DM), and (**C**) patients with DM. Patients with the Hp 2-2 type had significantly more Hb attached to HDL, and these differences were more accentuated in DM. *P*<0.0001 by Kruskal-Wallis test in overall and in DM patient groups. **P*<0.01 for differences between the Hp 2-2 and Hp 1-1 subgroups tested by Steel-Dwass post hoc analysis.

95% CI, 1.77–3.25, per 1 SD increase; *P*<0.0001). In addition, the presence of microvascular ED was significantly associated with epicardial ED (OR, 2.20; 95% CI, 1.42–3.43; *P*=0.0004). Despite the significant dose effect of Hp 2 allele on epicardial ED, using a logistic regression model by stratifying patients into 2 groups (Hp 2-2 versus non-Hp 2-2), we found that Hp 2-2 was nonsignificant predictor of epicardial ED (OR, 1.37; 95% CI, 0.88–2.13; *P*=0.17; Figure 6B). This might be explained by the relatively higher rates of epicardial ED in the Hp 2-1 group. Indeed, Hp 1-1 was still found to be significantly associated with normal epicardial endothelial function (OR, 0.47; 95% CI, 0.23–0.94; *P*=0.03). Examining other variables in the univariate model also revealed plasma HDL-C levels as inversely associated with epicardial ED (OR, 0.78; 95% CI, 0.62–0.98, per 1 SD increase; *P*=0.04), as well as nitrate therapy (OR, 1.60; 95% CI, 1.01–2.52; *P*=0.044). The presence of DM was not significantly correlated with the presence of epicardial ED, though a trend toward an increased odds of ED was observed in DM similar to that seen in association with microvascular ED (OR, 1.42; 95% CI, 0.83–2.43; *P*=0.09; Table 4).

In a multivariate logistic regression that included the important indicators observed in the univariate model, we found HDL-bound Hb content (adjusted OR, 2.17; 95% CI, 1.42–3.33, per 1 SD increase; *P*<0.0001), plasma HDL levels (adjusted OR, 0.59; 95% CI, 0.41–0.85, per 1 SD increase; *P*=0.003), and microvascular ED (adjusted OR, 2.33; 95% CI, 1.20–4.52; *P*=0.013) but not Hp 2-2 (adjusted OR, 1.17; 95% CI, 0.64–2.11; *P*=0.611) as independently associated with the presence of epicardial ED (Table 4).

Discussion

The present study provides strong evidence that Hp 2-2 phenotype is significantly associated with microvascular ED and with increased HDL-bound Hb, particularly in individuals with DM. Moreover, increased Hb content of HDL was associated with increased rates of microvascular and epicardial ED, thus providing a suggestive mechanistic link between impaired HDL function and coronary ED. The genetic heterogeneity in the amount of Hb tethered to HDL, as determined by the Hp type and its implication in HDL oxidative modifications and ED, suggests a new potential target for preventing HDL dysfunction and reducing cardiovascular disease.

We have previously demonstrated the ability of the Hp type to regulate reverse cholesterol transport, specifically in DM, with Hp 2-2 associated with markedly impaired HDL function. The Hp type dependence of HDL function is likely related to differences in the trafficking of extracellular Hb by the different Hp proteins.[20] Extracorpuscular Hb is rapidly bound to Hp to form a Hp-Hb complex that is cleared by the monocyte/macrophage CD163 receptor.[32] We have shown that the Hp 2-2-Hb complex is cleared more slowly than the Hp 1-1-Hb particularly in the setting of DM.[20] This decreased clearance of circulating Hp 2-2-Hb complex results in its binding to ApoA1.[33] We have previously hypothesized[20] that the HDL from Hp 2-2 DM individuals might contain more Hb. Using a novel method developed for quantification of HDL-bound Hb, we now demonstrate an ≈5-fold increase in the amount of Hb bound to HDL from Hp 2-2 DM individuals and that this high Hb content is associated with the presence of both microvascular and epicardial ED.

Increased Hb attachment to HDL may contribute to ED through its ability to interfere with HDL function. First, Hb binding to HDL results in oxidative modification of HDL-associated lipids and proteins because of its iron-driven oxidative injury by the Fenton reaction, leading to inactivation of antioxidant enzymes, such as glutathione peroxidase, and paraoxonase.[15] Second, Hb can oxidize ApoA1, resulting in significant impairment of its ability to promote cholesterol efflux from macrophages.[34,35] Hp exerts antioxidant properties by its binding to free Hb and inhibiting Hb-induced oxidation. However, the ability of the Hp 1-1 and Hp 2-2 proteins to neutralize the oxidative effects of Hb

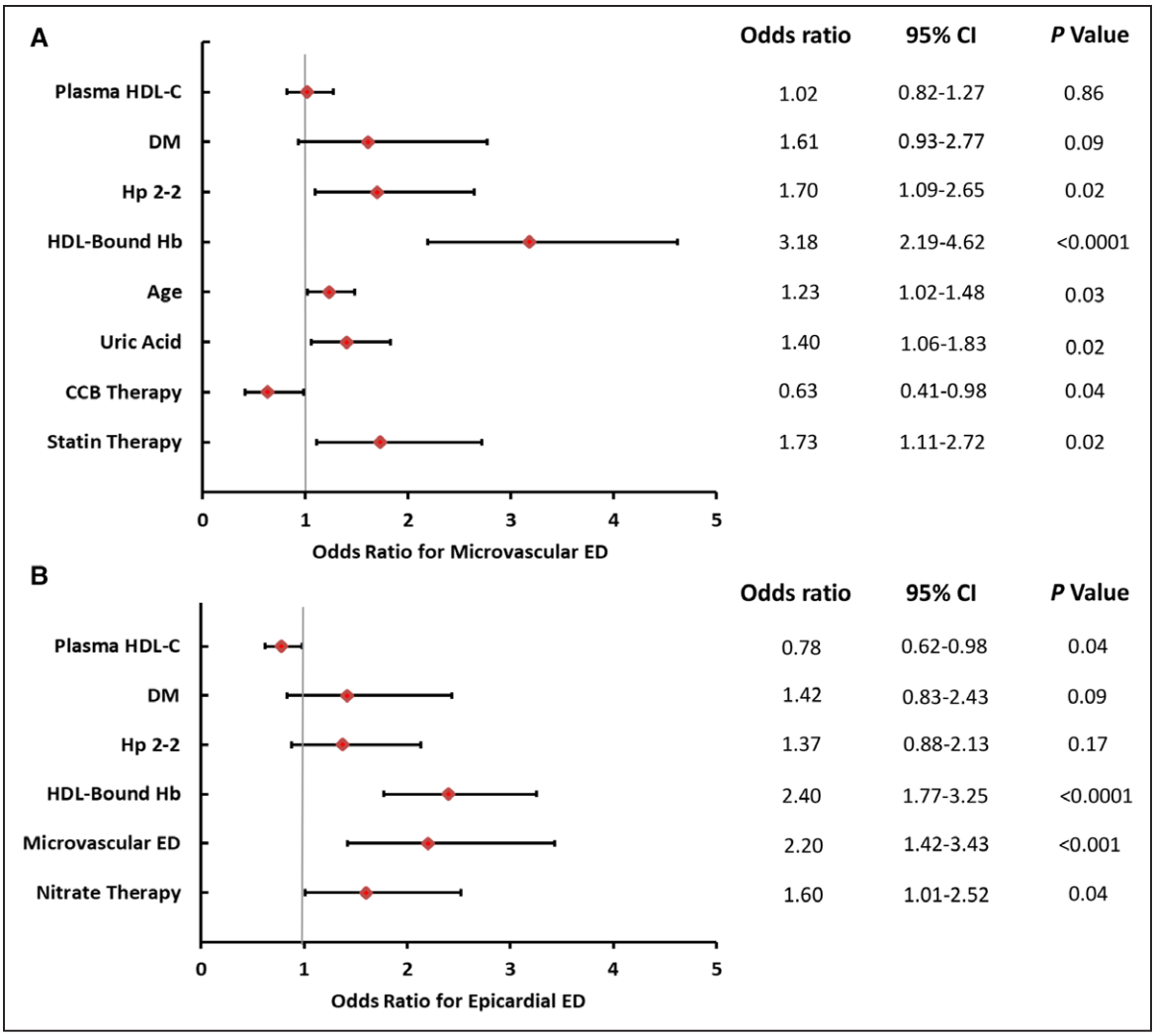

Figure 6. Summary of univariate predictors of microvascular (**A**) and epicardial (**B**) endothelial dysfunction (ED). Unadjusted ORs for ED were calculated using a logistic regression model. Bars represent 95% confidence interval (CI) for each OR. For plasma HDL-cholesterol (HDL-C), HDL-bound Hb, and uric acid levels, ORs are presented per 1 SD increase. For age, ORs are presented per 1 decade increase. CCB indicates calcium channel blockers; DM, diabetes mellitus; Hb, hemoglobin; and HDL, high-density lipoprotein.

has been shown to be different, with Hp 2-2 demonstrated as a poor antioxidant,[36] particularly against glycosylated Hb.[37] Accordingly, Hp 2-2-Hb bound to HDL would be expected to display more pro-oxidative activity and result in more HDL dysfunction than an equivalent mass of Hb bound to Hp 1-1 protein.

ED is one of the early hallmarks in the pathogenesis of atherosclerosis. HDL dysfunction has been implicated in the development of atherosclerotic cardiovascular disease at different stages, including its involvement in ED. Among patients with early-stage coronary atherosclerotic disease, we have previously shown that impaired HDL-C efflux capacity is a significant predictor of coronary ED even after adjusting for HDL-C levels.[18] The mechanisms by which HDL affects endothelial function are not fully understood but may involve regulation of endothelial cell nitric oxide (NO) bioavailability by HDL and the ability of HDL to regulate vascular repair via cholesterol efflux–related pathways. Studies have shown that cholesterol efflux by HDL is required for lipoprotein-mediated signaling in endothelial cells, which underlies the capacity of HDL to activate endothelial NO synthase and promote endothelial repair as part of its atheroprotective effects.[38,39]

Hb association with HDL has been shown to result in increasing scavenging of NO and thereby impaired vascular protective effects of HDL.[20,40] This may have clinical significance that is of greater importance than the effect of Hb on the function of HDL in reverse cholesterol transport.[41] In the current study, microvascular coronary endothelial function was assessed based on maximal vasorelaxation in response to intracoronary acetylcholine infusion, which is largely reflected by the activity of NO available for vasomotor regulation. Therefore, increased HDL-bound Hb content resulting in more sequestering of NO may provide a mechanistic explanation for the increased likelihood of microvascular ED in Hp 2-2 DM. Importantly, coronary ED in humans is associated with increased local oxidative stress and increased NO consumption without a significant decrease in basal NO release.[42] In support of these findings, we have recently presented evidence for decreased bioavailability of NO in Hp 2-2 DM individuals,[43] which may reflect the increased Hb association

Table 2. Summary of Unadjusted Odds Ratios (95% Confidence Intervals) Associated With Hp 2-2 Phenotype and HDL-Bound Hb Content For Endothelial Dysfunction

	Non-Hp 2-2	Hp 2-2	*P* Value	HDL-Bound Hb (per 1 SD increase)	*P* Value
All group (n=338)	n=206	n=132			
Microvascular ED	1.0	1.70 (1.09–2.65)	0.018	3.18 (2.19–4.62)	<0.0001
Epicardial ED	1.0	1.37 (0.88–2.13)	0.165	2.40 (1.77–3.25)	<0.0001
Non-diabetic mellitus group (n=271)	n=166	n=105			
Microvascular ED	1.0	1.33 (0.82–2.18)	0.248	2.57 (1.71–3.94)	<0.0001
Epicardial ED	1.0	1.24 (0.76–2.04)	0.391	2.00 (1.41-2.86)	0.0001
Diabetic group (n=67)	n=40	n=27			
Microvascular ED	1.0	5.38 (1.70–17.05)	0.004	6.90 (2.60–18.30)	<0.0001
Epicardial ED	1.0	1.97 (0.73–5.30)	0.181	4.55 (2.14–9.68)	<0.0001
P Value for interaction for microvascular ED			0.023		0.047
P Value for interaction for epicardial ED			0.414		0.037

ED indicates endothelial dysfunction; Hb, hemoglobin; HDL, high-density lipoprotein; and Hp, haptoglobin.

with HDL in Hp 2-2 DM individuals and its association with coronary ED observed in this study. Taken together, our findings demonstrating higher prevalence of microvascular ED in patients with Hp 2-2 DM may be explained by the presence of increased amount of Hb content attached to HDL thus contributing to the decreased NO bioavailability and HDL dysfunction in this group.

Our findings are supportive of more deleterious effects of Hp 2-2 on the microvascular than on the epicardial endothelial function in DM individuals. As control of CBF to the myocardium is mainly at the level of the microcirculation, we believe that the change in CBF in response to acetylcholine (microvascular ED) more than the change in epicardial coronary artery diameter (epicardial ED) is the predominant factor determining endothelial function and myocardial perfusion in patients presented with angina and nonsignificant coronary artery stenosis. Moreover, we found that microvascular ED is strongly correlated with epicardial ED, which is consistent with our previous study.[44] These findings suggest that coronary atherosclerotic disease is a continuous process that starts with the development of microvascular ED in response to oxidative stress and inflammation and progresses from microvascular to

Table 3. Summary of Univariate and Multivariate Models for Association With Microvascular Endothelial Dysfunction

	Univariate Model		Multivariate Model	
Variable	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Age (per decade)	1.23 (1.02–1.48)	0.027	1.14 (0.86–1.52)	0.367
Gender (male)	1.03 (0.66–1.60)	0.889	—	—
Smoking status	0.71 (0.40–1.29)	0.265	—	—
Diabetes mellitus	1.61 (0.93–2.77)	0.087	0.67 (0.30–1.48)	0.319
Plasma HDL levels (per 1 SD increase)	1.02 (0.82–1.27)	0.855	1.27 (0.89–1.82)	0.182
Uric acid (per 1 SD increase)	1.40 (1.06–1.83)	0.015	1.43 (1.04–1.97)	0.025
Nitrate therapy	0.98 (0.62–1.55)	0.939	—	—
CCB therapy	0.63 (0.41–0.98)	0.041	0.41 (0.21–0.80)	0.009
Statin therapy	1.73 (1.11–2.72)	0.016	1.57 (0.80–3.08)	0.194
HDL-bound Hb (per 1 SD increase) (Hp 2-2 not included in the model)	3.18 (2.19–4.62)	<0.0001	4.64 (2.59–8.30)	<0.0001
HDL-bound Hb (per 1 SD increase) (Hp 2-2 included in the model)	—	—	4.57 (2.54–8.24)	<0.0001
Hp 2-2 phenotype (HDL-bound Hb not included in the model)	1.70 (1.09–2.65)	0.018	1.90 (1.06–3.39)	0.030
Hp 2-2 phenotype (HDL-bound Hb included in the model)	—	—	1.55 (0.82–2.95)	0.180

CCB indicates calcium channel blocker; CI, confidence interval; Hb, hemoglobin; HDL, high-density lipoprotein; Hp, haptoglobin; and OR, odds ratio.

Table 4. Summary of Univariate and Multivariate Models for Association With Epicardial Endothelial Dysfunction

	Univariate Model		Multivariate Model	
Variable	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Age (per decade)	1.09 (0.90–1.31)	0.376	—	—
Gender (male)	1.35 (0.87–2.10)	0.185	—	—
Smoking status	1.05 (0.58–1.89)	0.877	—	—
Diabetes mellitus	1.42 (0.83–2.43)	0.090	0.60 (0.28–1.27)	0.180
Plasma HDL levels (per 1 SD increase)	0.78 (0.62–0.98)	0.040	0.59 (0.41–0.85)	0.003
Uric acid (per 1 SD increase)	1.12 (0.86–1.47)	0.398	—	—
Nitrate therapy	1.60 (1.01–2.52)	0.044	1.58 (0.82–3.03)	0.141
CCB therapy	1.03 (0.66–1.60)	0.904	—	—
Statin therapy	1.08 (0.69–1.68)	0.751	—	—
Microvascular ED	2.20 (1.42–3.43)	0.0004	2.33 (1.20–4.52)	0.013
HDL-bound Hb (per 1 SD increase) (Hp 2-2 not included in the model)	2.40 (1.77–3.25)	<0.0001	2.17 (1.42–3.33)	<0.0001
HDL-bound Hb (per 1 SD increase) (Hp 2-2 included in the model)	—	—	2.17 (1.42–3.33)	<0.0001
Hp 2-2 phenotype (HDL-bound Hb not included in the model)	1.37 (0.88–2.13)	0.165	1.17 (0.64–2.11)	0.611
Hp 2-2 phenotype (HDL-bound Hb included in the model)	—	—	1.01 (0.54–1.88)	0.980

CCB indicates calcium channel blocker; CI, confidence interval; ED, endothelial dysfunction; Hb, hemoglobin; HDL, high-density lipoprotein; Hp, haptoglobin; and OR, odds ratio.

epicardial ED manifestation over time. Based on these findings, we propose that Hp 2-2 in DM patients may be involved early in the pathogenesis of coronary microvascular ED that promotes subsequent epicardial ED and atherosclerosis progression. This process might be mediated by increased Hb tethering to HDL by the Hp 2-2 protein, leading to HDL dysfunction and decreased NO bioavailability.

We have previously demonstrated that HDL-bound lipid peroxidation and HDL dysfunction can be reversed by administration of vitamin E to individuals with Hp 2-2 and DM.[20] The present study further extends our previous studies as well as the recent study by Monette et al,[18] which reported an association between impaired HDL-C efflux capacity and ED. It may be speculated that a Hp-based pharmocogemonic approach may be helpful in identifying those individuals at high ED risk who may derive a clinical benefit from early vitamin E therapy or from potential therapies targeting the association of Hb to HDL.

This study is limited by insufficient power to evaluate for an association with clinical cardiovascular events. As the study population comprised patients with early coronary atherosclerotic disease and normal LV function, the findings cannot be generalized to patients with significant coronary artery disease or with depressed LV function. The low number of participants with DM is an additional limitation of this study. Though previous studies have suggested that decreased NO bioavailability and increased HDL oxidative modifications can contribute to the higher rates of ED in patients with Hp 2-2 and DM, our study did not include functional studies to provide this mechanistic link and future studies are still needed to further elucidate this association. The clinical characteristics of our study population are limited to patients with chest pain and mild nonobstructive coronary artery disease. Therefore, our findings may not be generalized to patients with more advanced atherosclerosis. Further, the presence of minimal luminal obstruction may yet represent significant eccentric plaque burden with possible complex plaque morphology that has not been investigated in the current study. However, we have previously shown, in patients with similar clinical characteristics, that ED is associated with more features of plaque vulnerability and more accelerated plaque progression as assessed by serial coronary intravascular ultrasound volumetric measurements.[45] Finally, the study was a cross-sectional design, and all participants had only a one-time coronary endothelial function assessment. Despite these limitations, our study includes a large sample size of patients with similar baseline characteristics of patients with and without microvascular and epicardial ED who underwent a validated study for accurate assessment of coronary endothelial function and with a novel and reliable assay developed here for measurement of Hb content of HDL.

Conclusions

The current study demonstrates that the Hp type is independently associated with the presence of coronary ED and is a major determinant of the amount of HDL-bound Hb, particularly in the setting of DM. These findings suggest a genetic susceptibility for ED determined by the Hp polymorphism and may provide a mechanistic explanation of the association between impaired HDL function and coronary ED. Our

observations suggest that Hp typing and measurement of Hb content of purified HDL may be helpful in selecting patients with ED for early intervention with antioxidant therapy to improve endothelial function and mitigate the progression of atherosclerotic cardiovascular disease.

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Disclosures

A.P. Levy is the author of a patent owned by his university regarding use of haptoglobin (Hp) genotype to predict susceptibility to cardiovascular disease in individuals with diabetes mellitus and the ability of the Hp type to predict benefit from vitamin E. The other authors report no conflicts.

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Highlights

- The present study demonstrates that haptoglobin phenotype is significantly associated with the presence of coronary endothelial dysfunction and a determinant of HDL (high density lipoprotein)-bound hemoglobin content, specifically in diabetes mellitus.
- These findings support a genetic susceptibility for endothelial dysfunction determined by the haptoglobin polymorphism and may provide a mechanistic explanation of the association between impaired HDL function and endothelial dysfunction.
- Our observations suggest that haptoglobin typing and measurement of hemoglobin content of purified HDL may be helpful in selecting patients at high endothelial dysfunction risk who may benefit from early intervention to improve endothelial function and mitigate the progression of coronary atherosclerotic disease.