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Association between nitrated lipoproteins and vascular function in type 2 diabetes

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1. ABSTRACT

Higher levels of nitrated lipoproteins (NT-HDL and NT-LDL) were found in blood and atherosclerotic plaques of patients with coronary artery disease. We aimed to examine the relationship between plasma NT-HDL and NT-LDL and diabetic vascular dysfunction. The study included 125 African-American patients with T2DM. NT-HDL and NT-LDL were quantified by ELISA. Microvascular function was assessed by vascular reactivity index (VRI). Large artery stiffness was assessed by carotid-femoral pulse wave velocity (PWV). Carotid intima-media thickness (CIMT) was assessed by B-

mode ultrasound imaging. In univariate analysis, NT-HDL was associated with VRI in total population and in patients with HbA1c ≤7.0% (β= -0.178, p= 0.034; β= -0.265, p= 0.042; respectively). In contrast, NT-LDL was associated with CIMT in total population and in patients with HbA1c >7.0% (β= -0.205, p= 0.022; β= -0.244, p= 0.042; respectively). Multivariable-adjusted regression analysis demonstrated that NT-HDL independently predicted VRI outcome in total population and in well-controlled patients (β= -0.282, p= 0.014; β= -0.400, p= 0.035, respectively). These results suggest that NT-HDL could be used as marker to identify diabetic patients at risk of developing early microvascular complications.

2. INTRODUCTION

The pathologic hallmark of type 2 diabetes mellitus (T2DM) involves the vasculature leading to both microvascular and macrovascular complications (1, 2). Hyperglycemia is associated with long-term damage and failure of various organs mainly affecting the eyes, nerves, kidneys, and heart (1). Patients with T2DM have a markedly increased risk of developing cardiovascular disease (CVD); and reducing levels of LDL-C using statin therapy only partially diminishes the increased propensity of these patients towards CVD events (3). African Americans suffer disproportionately from many chronic diseases including T2DM (4). Prevailing statistics suggest that African American adults are 50% to 100% more likely to have diabetes than are Whites (5). Therefore, the strategies in place to prevent disease development and progression warrant further examination.

The abnormal lipid profile that often accompanies T2DM is well-known to be associated with an increased risk of atherosclerotic vascular disease (6). This disorder is typically characterized by elevated serum triglycerides, low HDL-c concentrations and increased smaller, denser LDL-c particles (7). Small dense LDL (sdLDL) particles are believed to be particularly atherogenic due to increased susceptibility to oxidation, high endothelial permeability, decreased LDL receptor affinity, and an increased interaction with matrix components. sdLDL penetrates the vascular endothelium more easily and accelerates the atherosclerotic process (8). Also, there is evidence that some crucial functions of HDL, such as endothelial protection and repair, are adversely modified by diabetes, most likely through alteration of specific components of HDL particles (9). It has been shown that HDL from patients with T2D have a significant reduction in their antioxidative and endothelium-dependent vasorelaxant properties (7). HDL isolated from patients with T2DM have an impaired ability to stimulate nitric oxide synthase activity (10) and nitric oxide (NO) production from endothelial cells compared to HDL from healthy controls (11).

Circulating HDL and LDL particles are subjected to multiple enzymatic and non-enzymatic modifications including glycation, oxidation, nitration and carbamylation (12, 13). Plasma lipoproteins may represent a perfect biological sensor for oxidative stress in the arterial wall by their close interactions with endothelial cells in the vasculature and the susceptibility of their surface lipids to oxidative modification (14). ApoA-I and apoB have been shown to be specifically targeted for nitration by myeloperoxidase (MPO) leading to dysfunction of circulating HDL and LDL particles (15, 16). Moreover, MPO levels has been shown to predict accelerated progression of coronary atherosclerosis in diabetic patients (17). The pathways leading to the nitration of tyrosine residues in apoAI-HDL and apoB-LDL have been extensively studied (18, 19). High levels of nitrated tyrosine residues on apoA-I have been found in human plasma and atherosclerotic lesions from CAD patients, resulting in profound impairment of HDL particles and ABCA1-dependent cholesterol efflux (20, 21).

The endothelium is continuously exposed to various physiological molecules that may have a direct impact on NO actions. The damaging effects of oxidative stress on the cardiovascular system determine endothelial dysfunction through reduction in NO synthesis and bioavailability, inflammatory response, and lipid peroxidation (22). Interestingly, NO can play a dual role in atherosclerosis; and some of the pathological vascular conditions were linked with excessive rather than reduced NO production

(23). Altered NO levels in patients with T2D have been reported by different studies (24); however, results were controversial.

To our knowledge, there is no study reporting the direct link between plasma nitrated lipoproteins and endothelial dysfunction in diabetic microvascular and macrovascular complications. In this study, we aimed to investigate the relationship between circulating nitrated lipoproteins levels and subclinical vascular changes in African Americans diabetic patients.

3. MATERIALS AND METHODS

3.1. Patient blood sampling and nitrated lipoproteins quantification

A total of 125 patients with T2DM were recruited from the State University of New York Downstate Health Sciences University/Kings County Clinics between September 2016 and July 2017. The study protocol was approved by the Institutional Review Board of the State University of New York Downstate Health Sciences University (IRB protocol# 907067) and written informed consent was obtained from each participant. Patients were excluded from the study if, at baseline, patients were meeting one or more of the following criteria: patients are on chronic renal replacement therapy (hemodialysis, peritoneal dialysis, or transplantation), history of active malignancy (except those with basal cell carcinoma) within the last five years (prostatic cancer within the last two years), systemic lupus erythematosus and other autoimmune diseases that may affect kidney function, history of type 1 diabetes mellitus, acute infection or fever, pregnancy, chronic viral hepatitis or HIV infection, current unstable cardiac disease , history of hypercoagulable disorder, history of blood clots in arms, weak pulses in arms indicating low brachial artery flow, or history of vasculitis. Standard methods and definitions were adopted: Diabetes- Subjects with history of T2D on medication or HbA1c ≥ 6.5% or fasting glucose ≥126 mg/dL (≥7 mmol/L). Hypertension- Subjects with history of hypertension on medication. Dyslipidemia- Subjects with history of dyslipidemia on medication or fasting lipid profile with total cholesterol > 200 mg/dL or LDL > 70 mg/dL. CKD- Subjects with eGFR < 90 mL/min using modification of diet in renal disease (MDRD) equation or proteinuria (≥2+ on urine dipstick). Fasting blood samples were collected into EDTA-containing tubes from patients after at least 12-h fast in the morning and centrifuged 4°C at 3,000 rpm for 10 min to separate the serum for biochemical tests. All the samples for the measurement of nitrated lipoproteins levels were stored at -80°C until analysis. Plasma levels of NT-HDL and NT-LDL were measured in duplicate by using a well-established ELISA for NT-HDL (16, 19), and a commercially available ELISA kit for NT-LDL (Cell Biolabs, Inc., San Diego, CA, Catalog # STA-213). Total NO levels were assessed by ELISA kit (My BioSource, Inc., San Diego, CA, Catalog #MBS732723).

3.2. Quantification of vascular changes

Methods for noninvasive assessment of arterial stiffness and endothelial dysfunction has been well described by numerous studies (25). Vascular reactivity (VRI) is an index of blood vessel responsiveness to stimuli which measures microvascular function using differential distal digital thermal response following proximal peripheral vascular occlusion and release (Endothelix Vendys II, Palo Alto, CA). Studies were obtained from each subject under ambient room temperature after an overnight 12 hour fasting consisting of abstinence from food, alcohol, tobacco, caffeine, exercise and vasoactive medications. Briefly, subject was placed in supine position with cuff applied to both arms and baseline systolic blood pressure is obtained from non-test arm (left) after a rest interval of 30 minutes and recorded in the Vendys software. Temperature probes were placed on the palmar surface of index finger of both hands and the procedure was initiated and completed in 3 phases of 5 minutes duration generating a 15-minute curve; baseline stabilization, occlusion (cuff inflation of the test arm, right, to above 30mm Hg of the baseline systolic blood pressure) and deflation phases. Testing was fully automated and outcome results (area under the curve) are obtained from the Vendys software.

Pulse wave velocity (PWV) is a measurement of arterial stiffness between two major arteries and or measurement sites, in this case:

carotid-femoral sites (26). Assessment involves measurement of two parameters: transit time of arterial pulse along the analyzed arterial segments and distance on the skin between both recording sites (26). To obtain this measurement, subject (fasting and abstaining from coffee and tobacco 12 hours prior) was placed in a supine position for about 5 minutes to assume physiologic baseline condition under ambient room temperature (22-24˚C) after which an average of 2 reading of blood pressure was obtained using automated blood pressure measurement device. The distance between carotid artery and sternal notch was subtracted from that between femoral artery and sternal notch and recorded (measurement done transcutaneously using a tape measure), this is done to correct for overestimation (26). Actual measurement of carotid-femoral PWV (cf-PWV) was done using SphygmoCor system (ArtCor, Sydney, Australia): a cutaneous probe is placed in succession over the carotid and femoral arteries both signals synchronized with R wave on EKG made possible by placing connected (connected to Sphygmocor system) EKG rhythm leads on participant's torso prior to procedure. It is noteworthy that PWV can be obtained between several peripheral arterial segments but has been observed that the cf-PWV is the most widely used, highly predictive and most reproducible index of arterial stiffness (27).

Carotid intima-media thickness (CIMT) was assessed by high-resolution B-mode ultrasound image analysis as described elsewhere (28). An ultrasound machine (Philips Sonos 7500 Cardiac Ultrasound) with 8–15 MHz variable frequency linear probe was used for the study. The examination was carried out with each subject lying supine, neck slightly extended and turned contralaterally to the carotid along artery being examined. Continuous scans were done in the longitudinal and transverse planes after the application of ultrasound gel. Measurement of CIMT was established on the opposite wall of the right distal common carotid artery (CCA), as an average thickness of locating the segment of 10 mm proximal to the bifurcation. Edges and distances were detected and calculated using semi-automatic software. All measurements of the intima-media thickness were made in the longitudinal plane at the point of maximum thickness of the far wall of the CCA a 1 cm section of the artery proximal to the carotid bulb. Measurements were repeated for 3 times and the maximal thickness of the intima-media width at both right and left distal CCAs were measured to give an average of six readings as mean value of intima-media thickness of both sides.

3.3. Statistical Analysis

Statistical analyses were performed using SPSS software version 24 (IBM Corp., Armonk, NY-USA). Continuous variables were presented as means ± SD and medians (interquartile ranges), and comparisons between groups were performed by using Wilcoxon rank-sum test. Categorical variables were presented as frequencies and percentages, and comparisons between groups were performed by using Pearson's chi-square or Fisher's exact test. Associations between nitrated lipoproteins and other variables were assessed using non-parametric Spearman's correlation test. Univariate and multiregression analysis were conducted to determine possible associations between plasma nitrated lipoproteins and other variables. Multiple linear regression analysis was performed to evaluate the association between plasma nitrated lipoproteins levels and vascular outcome, using based models for covariates assessment including factors such as sex, age, weight, hypertension, stroke, smoking, creatinine, total cholesterol, LDLc, HDLc, triglycerides, HbA1c, duration of diabetes and total NO. All estimated β-coefficients were accompanied by approximate 95% confidence limits. Two-sided tests with p-values < 0.05 were considered statistically significant.

4. RESULTS

4.1. Clinical and biochemical characteristics of study population and plasma nitrated lipoproteins

The baseline characteristics of study subjects were shown in Table 1. Patients population was divided in two groups based on HbA1c median value in the entire population (HbA1c ≤7%, N=54 vs. HbA1c >7%, N=71). Mean patient age was 59.7±7.8 years (female 63%). Eighty percent (78%) had hypertension, 76% had dyslipidemia and 12% had chronic kidney disease. Mean HbA1c levels were 8.06%±2.02% (median HbA1c=7.3%). Among all the parameters, systolic blood pressure, HbA1c levels,

Table 1. Baseline characteristics of the total study population and stratified groups based on HbA1c levels

Baseline Characteristics	Total Population (N=125)	Patients with HbA1c ≤ 7.0 % (N=54)	Patients with HbA1c > 7.0 % (N=71)	p-value
Age (years)				
Mean (SD) 60.2 (54.8, 65.6)	59.7 (7.8)	59.2 (8.1) 59.0 (55.2, 66.0)	60.0 (7.6) 60.3 (53.9, 65.2)	0.899
Weight (kg)				
• Mean (SD)	85.9 (20.5)	85.3 (17.5)	86.4 (22.6)	0.433
Height (cm)				
• Mean (SD)	165.5 (17.6)	165.6 (10.1)	165.4 (21.8)	0.188
Waist Circumference (cm)				
• Mean (SD)	95.6 (24.8)	93.5 (27.6)	97.2 (20.5)	0.243
Systolic BP (mmHg)				
• Mean (SD)	134.3 (19.3)	127.3 (15.1)	139.6 (20.5)	**0.0001**
Diastolic BP (mmHg)				
• Mean (SD)	75.7 (10.7)	75.5 (10.0)	75.8 (11.3)	0.310
HbA1c (%)				
• Mean (SD)	8.1 (2.0) 7.3 (6.7, 9.3)	6.4 (0.4) 6.5 (6.2, 6.9)	9.3 (1.9) 8.8 (7.8, 10.3)	**0.0001**
Diabetes duration (year)				
• Mean (SD)	10.5 (7.6)	7.8 (5.4)	12.7 (8.5)	**0.002**
Total Cholesterol (mg/dL)				
• Mean (SD)	172.6 (41.3)	173.7 (34.9)	171.8 (45.7)	0.413
LDL-c (mg/dL)				
• Mean (SD)	95.8 (36.4)	98.8 (37.1)	92.7 (35.8)	0.656
HDL-c (mg/dL)				
• Mean (SD)	55.5 (17.8)	56.9 (18.4)	54.5 (17.5)	0.053
Triglycerides (mg/dL)				
• Mean (SD)	110.3 (53.7)	106.5(58.1)	113.2 (50.1)	0.110
Pooled Cohort Score (ASCVD Score)				
• Mean (SD) • Median (IQR)	21.6 (13.5) 19.2 (11.2, 29.1)	19.1 (13.3) 16.6 (8.8, 28.3)	23.5 (13.3) 21.5 (13.4, 29.7)	**0.041**
Pulse Waive Velocity (m/s)				
• Mean (SD) • Median (IQR)	8.5 (2.8) 8.1 (6.5, 10.2)	8.2 (2.5) 8.1 (6.3, 10.2)	8.8 (3.1) 8.2 (6.8, 10.4)	0.675
Vascular Reactivity Index				
• Mean (SD) • Median (IQR)	1.2 (0.5) 1.1 (0.9, 1.4)	1.0 (0.5) 1.0 (0.8, 1.2)	1.3 (0.5) 1.3 (1.0, 1.6)	**0.005**
Nitric Oxide (µmol/L)				
• Mean (SD) • Median (IQR)	27.7 (36.6) 20.1 (16.3, 30.7)	21.7 (8.4) 19.3 (16.2, 25.3)	32.3 (47.7) 21.0 (16.6, 34.7)	0.509
Nitrated LDL (ng/mL)				
• Mean (SD) • Median (IQR)	2.3 (0.7) 2.2 (1.8, 2.7)	2.5 (0.7) 2.4 (2.0, 3.0)	2.1 (0.7) 2.0 (1.6, 2.4)	**0.016**

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Table 1. Contd...

Baseline Characteristics	Total Population (N=125)	Patients with HbA1c ≤ 7.0 % (N=54)	Patients with HbA1c > 7.0 % (N=71)	P-value
Nitrated HDL (ng/mL)				
• Mean (SD) • Median (IQR)	217.2 (199.2) 156.7 (113.7, 231.3)	255.4 (227.4) 175.1 (124.6, 299.3)	188.1 (170.8) 152.0 (109.1, 183.7)	0.242
Medication				
Insulin, n (%)	35 (28.0)	13 (24.1)	22 (31.0)	0.449
Sulfonylurea, n (%)	25 (20.0)	6 (11.1)	19 (26.8)	0.158
Metformin, n(%)	91 (72.8)	42 (77.8)	49 (69.0)	0.999
DPP-4 inhibitors, n (%)	41 (32.8)	15 (27.8)	26 (36.6)	0.296
GLP-1 agonists, n (%)	1 (0.8)	1 (1.9)	10 (14.1)	N/D
SGLT2 inhibitors, n (%)	16 (12.8)	6 (11.1)	10 (14.1)	N/D
Thiazolidinediones, n (%)	16 (12.8)	6 (11.1)	10 (14.1)	N/D
Meglitinide, n (%)	3 (2.4)	1 (1.9)	2 (1.5)	N.D
Calcium channel blockers, n (%)	38 (30.4)	19 (35.2)	19 (26.8)	0.730
ACE inhibitors, n (%)	43 (34.4)	16 (29.6)	27 (38.0)	0.914
Beta blockers, n (%)	27 (21.6)	7 (13.0)	20 (28.2)	0.357
Diuretics, n (%)	30 (24.0)	15 (27.8)	15 (21.1)	0.423
Nitrates, n (%)	6 (4.8)	6 (11.1)	6 (8.5)	N/D
Anti-platelets, n (%)	37 (29.6)	16 (29.6)	21 (29.6)	0.532
Statins, n (%)	71 (56.8)	28 (51.9)	43 (60.6)	0.132

Data are presented as frequencies (percentages) for categorical variables and as mean (standard deviation, SD) or median (25%-75% interquartile ranges, IQR) for continuous variables. ND: not determined due to low frequency or proportion of variable is constant. Statistical significance: p-values < 0.05 were considered statistically significant.

duration of diabetes, and triglyceride levels were significantly elevated in poorly controlled group of participants. NT-LDL levels were significantly reduced in patients with HbA1c ≤7%. Median VRI was significantly elevated in elevated in poorly controlled group of participants. There was no significant change in total NO levels between the groups.

4.2. Correlations between circulating nitrated lipoproteins and clinical variables

In total population, NT-HDL levels positively correlated with NT-LDL levels (r= 0.259, p= 0.002; Table 2) and negatively correlated with HbA1c and total NO levels (r= -0.242, p= 0.003 and r= -0.186, p= 0.025, respectively; Table 2). In patients with HbA1c ≤ 7%, NT-HDL correlated negatively with HDLc (r= -0.326, p= 0.012; Table 2) and NT-LDL levels (r= 0.250, p= 0.052; Table 2). There was no correlation between NT-HDL and NT-LDL in the group of patients with HbA1c > 7% (Tables 2 & 3). NT-HDL negatively correlated with total NO levels in total population and in patients with HbA1c >7% (r= -0.186, p= 0.025 and r= -0.364, p= 0.001; respectively, Table 2). In contrast, NT-LDL levels negatively correlated with HbA1c levels, duration of diabetes and CIMT outcome in the total population (r= -0.347, p= 0.0001, r= -0.316, p= 0.0001, r= -0.198, p= 0.027, respectively; Table 3). In patients with HbA1c > 7%, NT-LDL negatively correlated with HbA1c, total NO levels and CIMT outcome (r= -0.234, p= 0.031, r= -0.265, p= 0.014, r= -0.252, p= 0.034, respectively; Table 3).

4.3. Linear regression analysis between circulating NT-HDL and NT-LDL

Linear regression analysis revealed that NT-HDL was significantly associated with NT-LDL in

Table 2. Spearman correlation between NT-HDL, lipid parameters and vascular outcome in African-Americans diabetic patients

Variables	Total population		HbA1c ≤ 7.0%		HbA1c > 7.0%	
	R	p-value	R	p-value	R	p-value
Gender	0.024	0.776	0.063	0.628	0.049	0.653
Age	0.042	0.612	-0.021	0.872	0.075	0.496
Weight	-0.034	0.687	-0.010	0.939	-0.030	0.784
PWV	-0.070	0.413	-0.090	0.500	-0.005	0.965
VRI	-0.076	0.367	-0.081	0.544	-0.002	0.986
CIMT	0.072	0.427	0.062	0.655	0.095	0.432
HbA1c	**-0.242**	**0.003**	-0.012	0.930	-0.187	0.087
Total cholesterol	-0.029	0.729	-0.117	0.377	-0.028	0.728
LDL-c	0.012	0.888	-0.026	0.844	0.048	0.664
HDL-c	-0.127	0.131	**-0.326**	**0.012**	-0.091	0.411
Triglycerides	-0.001	0.988	-0.138	0.293	-0.199	0.073
Diabetes duration	-0.140	0.128	-0.066	0.640	-0.142	0.248
Creatinine	-0.031	0.727	-0.021	0.881	0.021	0.857
Nitric oxide	**-0.186**	**0.025**	0.165	0.203	**-0.364**	**0.001**
Platelets count	-0.117	0.234	0.029	0.856	0.164	0.196
NT-LDL	**0.259**	**0.002**	**0.250**	**0.052**	0.166	0.128

R: Rho coefficient. Statistical significance: p-values < 0.05 were considered statistically significant.

total population and in the group with HbA1c > 7% after adjustment to other independent variables (β= 0.337, p= 0.003 and β= 0.571, p=0.001, respectively; Table 4). Among the list of variables that were included in the model, the age and weight were found to be the main confounding factors in the association between NT-HDL and LDL in the poorly-controlled patients (Table 4).

4.4. Univariable linear regression analysis between plasma nitrated lipoproteins and vascular outcomes

Univariate analysis showed that plasma concentrations of NT-HDL were significantly associated with VRI outcome in the total population and in patients with HbA1c ≤ 7% (β= -0.178, p= 0.034 and β= -0.265, p= 0.042, respectively; Table 5). In contrast, NT-LDL was significantly associated with CIMT outcome in total population and in patients with HbA1c >7% (β= -0.205, p= 0.022 and β= -0.240, p= 0.044, respectively; Table 5).

4.5. Multivariable linear regression analysis between plasma NT-HDL and VRI outcome

Multivariable linear regression analysis showed that plasma NT-HDL levels were independently associated with VRI in total population (β= -0.262, p= 0.028; Table 6) and in well-controlled patients with HbA1c≤7% (β= -0.383, p= 0.050; Table 6), but not in poorly-controlled patients with HbA1c>7% (β= 0.001, p= 0.996; Table 6), after adjusting the model with other independent variables such as gender, age , weight, hypertension, dyslipidemia, stroke, smoking, creatinine, total cholesterol, LDLc, HDLc, triglycerides and diabetes duration. Additionally, levels of total cholesterol and LDLc levels were found to be confounding factors affecting the

Table 3. Spearman correlation between NT-LDL, lipid parameters and vascular outcome in African-Americans diabetic patients

Variables	Total population		HbA1c ≤ 7.0%		HbA1c > 7.0%	
	R	p-value	R	p-value	R	p-value
Gender	-0.032	0.698	-0.173	0.184	0.112	0.307
Age	-0.128	0.124	-0.067	0.606	0.176	0.108
Weight	-0.026	0.754	0.088	0.502	0.045	0.685
PWV	-0.121	0.153	-0.154	0.253	0.047	0.676
VRI	-0.075	0.375	-0.091	0.494	0.060	0.588
CIMT	**-0.198**	**0.027**	-0.200	0.147	**0.252**	**0.034**
HbA1c	**-0.347**	**0.000**	0.038	0.770	**0.234**	**0.031**
Total cholesterol	0.031	0.716	0.064	0.629	0.021	0.851
LDL-c	0.142	0.092	0.029	0.830	0.197	0.075
HDL-c	-0.042	0.616	-0.051	0.703	0.203	0.066
Triglycerides	-0.096	0.253	-0.175	0.181	0.008	0.943
Diabetes duration	**-0.316**	**0.000**	-0.252	0.071	0.150	0.221
Creatinine	-0.030	0.733	-0.162	0.247	0.121	0.299
Nitric oxide	-0.135	0.105	0.197	0.128	**0.265**	**0.014**
Platelets count	-0.119	0.226	-0.168	0.289	0.066	0.604
NT-HDL	**0.259**	**0.002**	**0.250**	**0.052**	0.166	0.128

R: Rho coefficient. Statistical significance: p-values < 0.05 were considered statistically significant.

association between NT-HDL and VRI outcome in total population only (Table 6).

4.6. Multivariable linear regression analysis between plasma NT-LDL and CIMT outcome

Multivariable-adjusted regression analysis revealed that the association between NT-LDL and CIMT was lost in total population and in the poorly-controlled group. Sex and duration of diabetes were identified as the main confounding variables affecting the association between NT-LDL and CIMT (Table 7).

4.7. Association between plasma nitrated lipoproteins and total NO availability

To further examine the impact of NO availability, we included total NO as independent variable in the previous analysis models (Table 8). Total NO levels did not impact the association between NT-HDL levels and VRI outcome in total population and in the group of patients with HbA1c≤7% (β= -0.303, p= 0.010 and β= -0.416, p= 0.035, respectively; Model #1, Table 8). In contrast, the association between NT-LDL levels and CIMT remained insignificant in all groups even after addition of NO in the model (Model#2, Table 8). Moreover, NT-LDL, but not NT-HDL, independently predicted NO availability in total population even after adjusting to other independent variables (β= -0.290, p= 0.025; Model #3, Table 8).

4.8. Effects of medication use on the association between plasma nitrated lipoproteins and vascular outcomes

We then attempted to assess the impact of various medication used by the patients. Multiregression analysis resulted in identification of potential confounding factors (such as calcium channel blockers and ACE inhibitors) that could affect the association between NT-HDL levels and VRI outcome (Table 9). In contrast, none of the drugs used by participants seem to significantly

Table 4. Multiple linear regression analysis of the association between NT-HDL and NT-LDL in African-Americans diabetic patients

Variables	Total Population			HbA1c ≤ 7%			HbA1c > 7%		
	B	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
NT-HDL	**0.337**	**0.085 ; 0.406**	**0.003**	0.977	-0.093 ; 0.267	0.329	**0.571**	**0.234 ; 0.842**	**0.001**
Gender	-0.085	-0.327 ; 0.181	0.570	-0.411	-0.487 ; 0.326	0.685	0.135	-0.226 ; 0.470	0.478
Age	**-0.243**	**-0.460 ; -0.002**	**0.048**	-0.674	-0.406 ; 0.207	0.507	**-0.487**	**-0.858 ; -0.137**	**0.008**
Weight	-0.228	-0.457 ; 0.011	0.062	0.746	-0.227 ; 0.483	0.463	**-0.513**	**-0.859 ; -0.175**	**0.004**
Hypertension	-0.071	-0.268 ; 0.135	0.535	-0.091	-0.260 ; 0.238	0.928	-0.102	-0.391 ; 0.191	0.487
Stroke	0.003	-0.162 ; 0.166	0.981	-0.558	-0.266 ; 0.153	0.582	0.055	-0.191 ; 0.283	0.694
Dyslipidemia	0.046	-0.162 ; 0.236	0.713	1.592	-0.064 ; 0.489	0.125	-0.068	-0.411 ; 0.270	0.675
Smoking	0.179	-0.067 ; 0.371	0.170	0.745	-0.222 ; 0.471	0.464	0.140	-0.168 ; 0.428	0.381
Creatinine	0.021	-0.221 ; 0.260	0.874	-1.423	-0.774 ; 0.143	0.168	0.004	-0.281 ; 0.288	0.982
Cholesterol	-1.079	-2.210 ; 0.236	0.112	-1.010	-34.022; 11.700	0.323	-0.433	-2.010 ; 1.277	0.652
LDL-c	0.907	-0.272 ; 2.011	0.133	1.016	-10.087; 29.571	0.320	0.312	-1.282 ; 1.856	0.711
HDL-c	0.332	-0.287 ; 0.908	0.304	1.006	-4.972 ; 14.381	0.325	0.010	-0.862 ; 0.882	0.981
Triglycerides	0.418	-0.278; 0.661	0.278	1.003	-3.109 ; 8.967	0.326	0.030	-0.837 ; 0.763	0.926
Diabetes duration	0.117	-0.296 ; 0.102	0.335	-0.718	-0.662 ; 0.321	0.480	0.132	-0.149 ; 0.358	0.408

Dependent variable: NT-LDL. Data are expressed as regression coefficient (β) and 95% confidence intervals (CI). p-values < 0.05 were considered statistically significant.

Table 5. Univariate analysis of associations between nitrated lipoproteins and vascular outcome in African-Americans diabetic patients

Variables	Total Population			HbA1c ≤ 7%			HbA1c > 7%		
	β	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
NT-HDL[1]	**-0.178**	**-0.001 ; 0.001**	**0.034**	**-0.265**	**-0.001 ; 0.001**	**0.042**	-0.005	-0.001; 0.001	0.965
NT-LDL[2]	**-0.205**	**-0.082 ; -0.007**	**0.022**	0.205	-0.109 ; 0.015	0.138	**-0.240**	**-0.105 ; -0.002**	**0.044**

1: VRI dependent variable; 2: CIMT dependent variable. Data are expressed as regression coefficient (β) and 95% confidence intervals (CI).
p-values < 0.05 were considered statistically significant.

affect the association between plasma NT-LDL levels and CIMT (Table 9).

5. DISCUSSION

The present study aimed to test the hypothesis that plasma nitrated lipoproteins levels may be positively related to vascular dysfunction in patients with T2DM. To our knowledge, this is the first study addressing the impact of nitrated lipoproteins (both NT-HDL and NT-LDL) on vascular dysfunction in T2DM patients. Here, we found that the concentrations of plasma NT-LDL were much lower than NT-HDL (~ 10 times less). This is in accordance with our previously published reports and others (16, 19, 29) showing that apoA-

Table 6. Multiple linear regression analysis of the association between NT-HDL and VRI outcome in African-Americans diabetic patients

Variables	Total Population			HbA1c ≤ 7%			HbA1c > 7%		
	B	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
NT-HDL	**-0.262**	**-0.426 ; -0.025**	**0.028**	**-0.383**	**-0.519 ; 0.001**	**0.050**	0.001	-0.386 ; 0.388	0.996
Gender	0.304	-0.152 ; 0.480	0.304	-0.162	-0.750 ; 0.452	0.613	0.292	-0.159 ; 0.728	0.200
Age	0.001	-0.284 ; 0.286	0.995	-0.274	-0.714 ; 0.171	0.217	0.045	-0.410 ; 0.508	0.829
Weight	-0.034	-0.331 ; 0.253	0.791	-0.322	-0.881 ; 0.184	0.188	-0.108	-0.552 ; 0.319	0.589
Hypertension	-0.133	-0.380 ; 0.109	0.273	0.118	-0.261 ; 0.462	0.570	-0.315	-0.705 ; 0.037	0.076
Stroke	-0.150	-0.337 ; 0.073	0.202	-0.247	-0.488 ; 0.120	0.222	-0.042	-0.340 ; 0.264	0.798
Dyslipidemia	-0.059	-0.304 ; 0.191	0.651	-0.278	-0.608 ; 0.190	0.290	-0.018	-0.454 ; 0.413	0.924
Smoking	0.064	-0.210 ; 0.341	0.638	0.086	-0.422 ; 0.578	0.750	0.134	-0.246 ; 0.513	0.477
Creatinine	-0.091	-0.399 ; 0.200	0.508	0.140	-0.496 ; 0.828	0.609	-0.121	-0.477 ; 0.248	0.524
Cholesterol	**1.579**	**0.188 ; 3.234**	**0.028**	-0.491	-33.728 ; 32.505	0.970	1.305	-0.904 ; 3.282	0.256
LDL-c	**-1.385**	**-2.993 ; -0.151**	**0.031**	0.459	-28.178 ; 29.271	0.969	-1.137	-3.126 ; 0.871	0.259
HDL-c	-0.501	-1.298 ; 0.189	0.141	0.434	-13.571 ; 14.450	0.949	-0.131	-1.249 ; 0.972	0.801
Triglycerides	-0.384	-1.075 ; 0.101	0.103	0.314	-8.430 ; 9.088	0.939	-0.229	-1.320 ; 0.718	0.551
Diabetes duration	0.144	-0.108 ; 0.391	0.261	0.186	-0.480 ; 1.003	0.472	0.067	-0.266 ; 0.320	0.721

Dependent variable: VRI. Data are expressed as regression coefficient (β) and 95% confidence intervals (CI). p-values < 0.05 were considered statistically significant.

I containing HDL particles is indeed the most preferred target for MPO- mediated nitration (15). Moreover, despite the differences in the degree of nitration on respective apolipoproteins ApoA-I and apoB in these particles, our study revealed a strong positive correlation between NT- HDL and NT-LDL levels. Regression analysis showed that the association between NT-HDL and NT-LDL was partially attributable to age factor that was found a confounding variable in our base model. In fact, age dependent proteins nitration has been previously reported in many diseases (30). It is also known that nitration of tyrosine residues of apoB-LDL makes LDL a very potent ligand for macrophages scavenger receptors leading to cholesterol accumulation and foam cell formation (31). This process has been well recognized as a major key event in the initiation and progression of atherogenesis. Based on the structural and functional differences between HDL and LDL particles, we hypothesized that circulating NT-LDL and NT-HDL levels may differentially influence vascular function in T2DM diabetic patients.

Strict glycemic control has been proposed as an important factor to lower the risk of both microvascular and macrovascular complications of T2DM. We assessed the relationship between glycemic control (HbA1c levels) and nitrated lipoproteins and their impact on vascular function. Unexpectedly, we found an inverse correlation between HbA1c levels and nitrated lipoproteins in total population and in poorly-controlled patients with HbA1c > 7%. Furthermore, HbA1c levels correlated positively with VRI outcome in total population. HbA1c levels were also positively correlated with NO levels in well-controlled patients with HbA1c ≤ 7%. A possible explanation for these paradoxical findings could be related to intra-individual variability in HbA1c measurements among participants and/or

Table 7. Multiple linear regression analysis of the association between NT-LDL and CIMT outcome in African-Americans diabetic patients

Variables	Total Population			HbA1c ≤ 7%			HbA1c > 7%		
	B	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
NT-LDL	-0.094	-0.311 ; 0.145	0.467	-0.048	-0.583 ; 0.465	0.816	-0.221	-0.481 ; 0.129	0.245
Gender	**0.392**	**0.036 ; 0.547**	**0.026**	0.286	-0.244 ; 0.693	0.328	0.435	-0.047 ; 0.659	0.086
Age	0.020	-0.203 ; 0.158	0.885	0.115	-0.266 ; 0.466	0.572	-0.082	-0.403 ; 0.279	0.709
Weight	-0.065	-0.263 ; 0.101	0.620	0.046	-0.375 ; 0.458	0.837	-0.169	-0.404 ; 0.159	0.379
Hypertension	-0.208	-0.371 ; 0.037	0.107	-0.311	-0.593 ; 0.109	0.164	-0.107	-0.398 ; 0.223	0.566
Stroke	0.064	-0.110 ; 0.188	0.522	0.268	-0.079 ; 0.405	0.175	-0.083	-0.264 ; 0.165	0.638
Dyslipidemia	-0.173	-0.314 ; 0.073	0.217	0.188	-0.217 ; 0.458	0.463	-0.349	-0.635 ; 0.071	0.112
Smoking	-0.003	-0.224 ; 0.219	0.983	0.227	-0.238 ; 0.590	0.384	- 0.088	-0.372 ; 0.240	0.660
Creatinine	-0.100	-0.305 ; 0.148	0.490	-0.539	-1.193 ; 0.025	0.059	0.054	-0.233 ; 0.302	0.792
Cholesterol	-0.159	-1.673 ; 1.398	0.858	-8.280	-36.846 ; 18.192	0.486	-0.673	-2.747 ; 1.771	0.659
LDL-c	0.136	-1.246 ; 1.487	0.860	6.802	-15.733 ; 31.973	0.484	0.598	-1.601 ; 2.473	0.662
HDL-c	0.066	-0.669 ; 0.778	0.881	3.963	-7.943 ; 15.349	0.513	0.304	-0.904 ; 1.360	0.681
Triglycerides	0.136	-0.416 ; 0.695	0.618	2.898	-4.575 ; 9.930	0.448	0.165	-0.914 ; 1.295	0.725
Diabetes duration	**0.270**	**-0.005 ; 0.381**	**0.055**	0.121	-0.491 ; 0.768	0.650	0.338	-0.037 ; 0.437	0.094

Dependent variable: CIMT. Data are expressed as regression coefficient (β) and 95% confidence intervals (CI). P-values < 0.05 were considered statistically significant.

effect of other possible contributing factors that could influence NO bioavailability in diabetes. In fact, hypertension and dyslipidemia were shown to be important modifiable risk factors contributing to the pathogenesis of cardiovascular events in T2DM (32). Also, it is not possible to exclude possible effect of other residual confounding factors for known and unknown risks such as fasting blood glucose or inflammatory markers, which were not reported in the current study. Several paradoxical relationships between glycemic control and clinical outcomes has been reported. Unexpected inverse relationship has been reported between HbA1c levels and mortality in patients with diabetes and advanced systolic heart failure (33). Positive linear relationship has been also reported between HbA1c and all-cause mortality (34). Moreover, both low normal HbA1c level and high HbA1c level (J- or U-shaped curve) have been associated as well with increased risk of all-cause mortality (35). Most of all these effects were mainly attributable to known episode of severe hypoglycemia and/or the type of medication in T2DM. In the present study, we couldn't obtain information about frequency of hypoglycemia events from our participants. Poor glycemic control is related to vascular events in patients with T2DM, but the presence of advanced vascular disease might also influence associations between variables. HbA1c level measurements were not repeated in this current study; nevertheless, to avoid any possible bias with recording inconsistent HbA1c values, we have used an average of HbA1c values of the most recent measurements recorded within 6-12 months prior patients vascular testing.

It is well known that uncontrolled glycemia leads to impairment of NO production which may result in accelerated vascular complications in diabetic patients. NO has been shown to be involved in the defective insulin-mediated stimulation of blood flow in T2DM as well as in the pathogenesis of diabetic nephropathy (24). We found that both NT-HDL and NT-LDL negatively correlated with total NO levels, especially in patients with poor glycemic control, suggesting a possible interplay between the role of glucose homeostasis in the production of NO

Table 8. Linear regression of associations between NO availability, nitrated lipoproteins and vascular outcome in African-Americans diabetic patients

Variables	Total Population			HbA1c ≤ 7%			HbA1c > 7%		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
NT-HDL[1] NO[1]	**-0.303**	**-0.458 ; -0.064**	**0.010**	**-0.416**	**-0.542 ; -0.021**	**0.035**	-0.092	-0.491 ; 0.304	0.634
	0.223	-0.371 ; 0.004	0.054	-0.192	-2.186 ; 0.808	0.350	0.297	-0.410 ;0.052	0.124
NT-LDL[2] NO[2]	-0.141	-0.369 ; -0.118	0.306	-0.045	-0.598 ; 0.487	0.832	-0.174	-0.476 ; 0.197	0.401
	0.051	-0.122 ; 0.180	0.702	0.039	-1.186 ; 1.416	0.186	0.126	-0.129 ; 0.231	0.564
NT-HDL[3] NT-LDL[3]	-0.042	-0.303 ; 0.215	0.734	-0.182	-0.112 ; 0.043	0.362	-0.162	-0.979 ; 0.434	0.437
	-0.290	**-0.780 ; -0.054**	**0.025**	0.068	-0.154 ; 0.208	0.758	-0.266	-1.189 ; 0.240	0.185

1: Model #1; VRI used as dependent variable in this model. The model was adjusted for other independents variables mentioned in table 5. 2: Model #2; CIMT used as dependent variable in this model. The model was adjusted for other independents variables mentioned in table 6. 3: Model #3; NO used as dependent variable in this model. The model was adjusted for other independents variables mentioned in tables 5 & 6. Data are expressed as regression coefficient (β) and 95% confidence intervals(CI). p-values < 0.05 were considered statistically significant.

and circulating lipoproteins. In fact, it is well known that modified LDL (e.g. oxidized LDL) and NO exert contradictory actions within the vascular endothelium microenvironment which may influence key events in atherogenesis (36). HDL is thought to exert at least some parts of its antiatherogenic properties via stimulation of endothelial NO production, nearby inhibiting oxidative stress and inflammation (37). Based on available data linking diabetes to endothelial dysfunction, and the fact that HDL can protect endothelium by stimulating production of NO, it is possible that NT-HDL particles might not be fully functional and capable of modulating NO availability. Controversies exist concerning the effect of insulin on nitric oxide synthase (NOS) activity and NO generation. Studies with cultured endothelial cells suggest that insulin stimulates NO formation, whereas elevated glucose levels inhibit NO synthesis (38).

Several studies reported the existence of free-nitrotyrosine and nitrated lipoproteins enrichment in human atherosclerotic lesions using immunochemical methods and mass spectrometry (39). We have previously shown that higher NT-apoAI/apoAI ratio was significantly elevated in diabetic patients with CAD, and this ratio was negatively correlated with apoAI-HDL dependent macrophages cholesterol efflux (29). The present study validates and provides a rational evidence to our previous observations using an *in vitro* macrophages cholesterol efflux assay to assess nitrated apoAI-HDL functionality. Thus, we believe the actual report is the first physiological evidence addressing the relationship between circulating plasma nitrated lipoproteins and vascular dysfunction in diabetic patients. Nevertheless, additional investigations are warrant in order to highlight exact mechanisms underlying the observed differential effects of circulating NT-HDL and NT-LDL on vascular dysfunction in T2DM.

Diabetic micro- and macro vascular complications seem to be strongly interconnected with microvascular diseases promoting atherosclerosis development. Currently, it is still not well understood whether microvascular complications distinctly precede macrovascular complications, or both could progress simultaneously. In the current study, we found that NT-HDL independently predict VRI outcome but not PWV nor CIMT, suggesting that NT-HDL may play a role in early stages of diabetic microvascular complications. The pathology of macrovascular disease includes large vessels occlusion and transmural inflammation of the vessel wall leading to tissue damage (40). Traditional Framingham risk factors may influence the determination of vascular

Table 9. Effect of medication use on the associations between nitrated lipoproteins and vascular outcomes in African-Americans diabetic patients

Variables	Total Population			HbA1c ≤ 7%			HbA1c > 7%		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
NT-HDL[1]	-0.186	-0.001 ; -0.000	0.066	**-0.322**	**-0.001 ; 0.000**	**0.040**	0.048	-0.001 ; 0.001	0.724
Insulin	-0.047	-0.162 ; 0.261	0.641	0.303	-0.031 ; 0.702	0.071	0.018	-0.240 ; 0.274	0.894
Sulfonylurea	-0.046	-0.301 ; 0.189	0.652	0.258	0.138 ; 0.966	0.137	-0.250	-0.530 ; 0.027	0.075
Metformin	-0.077	-0.402 ; 0.198	0.501	0.471	0.099 ; 1.497	0.084	-0.223	-0.586 ; 0.070	0.120
DDP-4 inhibitors	0.021	-0.228 ; 0.185	0.840	-0.014	-0.335; 0.304	0.923	-0.025	-0.297 ; 0.250	0.865
GLP-1 agonists	-0.069	-1.359 ; 0.643	0.479	-0.069	-1.252 ; 0.776	0.636	ND	ND	ND
Meglitinide	-0.057	-0.792 ; 0.446	0.580	-0.332	-2.347 ; 0.068	0.064	-0.123	-1.035; 0.404	0.382
Calcium Channel Blockers	**0.239**	**0.028 ; 0.472**	**0.028**	0.260	-0.077 ; 0.606	0.124	**0.422**	**0.120 ; 0.719**	**0.007**
ACE inhibitors	-0.044	-0.252; 0.163	0.673	**-0.401**	**-0.770 ; -0.067**	**0.021**	0.054	-0.207 ; 0.308	0.694
Beta blockers	-0.113	-0.402 ; 0.145	0.352	-0.035	-0.601 ; 0.505	0.861	-0.010	-0.358 ; 0.337	0.953
Diuretics	-0.153	-0.409 ; 0.067	0.157	0.260	-0.157 ; 0.709	0.203	-0.282	-0.628 ; 0.011	0.058
Nitrates	0.179	-0.064 ; 0.837	0.092	ND	ND	ND	0.225	-0.092 ;0.785	0.119
Anti-platelets	-0.013	-0.230 ; 0.203	0.902	0.040	-0.290 ; 0.374	0.797	-0.131	-0.421 ; 0.167	0.389
Statins	0.052	-0.169 ; 0.277	0.630	-0.027	-0.346 ; 0.291	0.862	-0.080	-0.392 ; 0.231	0.604
NT-LDL[2]	**-0.321**	**-0.105 ; -0.026**	**0.001**	-0.331	-0.164 ; 0.005	0.065	**-0.431**	**-0.142 ; -0.027**	**0.005**
Insulin	-0.108	-0.095 ; 0.026	0.261	-0.166	-0.193 ; 0.074	0.370	-0.147	-0.119 ; 0.033	0.261
Sulfonylurea	-0.040	-0.053 ; 0.081	0.679	-0.166	-0.246 ; 0.086	0.335	0.217	-0.015 ; 0.146	0.107
Metformin	0.133	-0.033 ; 0.140	0.221	0.014	-0.254 ; 0.267	0.959	0.133	-0.051 ; 0.144	0.339
DDP-4 inhibitors	0.128	-0.019 ; 0.098	0.185	0.040	-0.091; 0.118	0.791	0.171	-0.033 ; 0.130	0.239
GLP-1 agonists	-0.123	-0.480 ; 0.095	0.186	-0.203	-0.565 ; 0.112	0.183	ND	ND	ND
Meglitinide	0.054	-0.129 ; 0.227	0.585	-0.291	-0.083 ; 0.731	0.115	-0.034	-0.249; 0.195	0.808
Calcium Channel Blockers	0.053	-0.047 ; 0.080	0.606	0.080	-0.085 ; 0.137	0.639	0.010	-0.090 ; 0.096	0.947
ACE inhibitors	-0.144	-0.104; 0.016	0.152	-0.036	-0.126 ; -0.102	0.830	-0.146	-0.119 ; 0.036	0.288
Beta blockers	0.101	-0.043 ; 0.113	0.375	-0.127	-0.132 ; 0.247	0.541	0.092	-0.077 ; 0.132	0.599
Diuretics	-0.117	-0.106 ; 0.028	0.251	-0.235	-0.223 ; 0.062	0.259	-0.098	-0.122 ; 0.058	0.482
Nitrates	0.104	-0.196 ; 0.060	0.294	0.225	-0.092 ;0.785	0.119	-0.134	-0.193 ; 0.067	0.335
Anti-platelets	0.106	-0.029 ; 0.096	0.293	0.090	-0.078 ; 0.139	0.575	0.141	-0.047 ; 0.130	0.351
Statins	-0.178	-0.119 ; 0.007	0.081	-0.127	-0.148 ; 0.066	0.441	-0.145	-0.138 ; 0.049	0.341

1: VRI was used as dependent variable; 2: CIMT was used as dependent variable. Data are expressed as regression coefficient (β) and 95% confidence intervals(CI). P-values < 0.05 were considered statistically significant. ND: not determined.

function (41). The assessment of CIMT has been well accepted as an indicator of atherosclerosis. Our study showed that, in contrast to NT-HDL, NT-LDL was negatively associated with CIMT. This unexpected finding is somewhat intriguing. One plausible explanation is that similar to oxidized and

glycated LDLs, NT-LDL may be able to bind to other receptors than LDL receptors (LDLR), and start to be taken up by arterial cells, accumulate in the arterial intima, and display its proatherogenic effect on vascular cells for prolonged period of time. Therefore, this process may result in net depletion of circulating plasma NT-LDL levels due to faster clearance. In fact, Hamilton *et al.,* using *in vitro* binding experiments, demonstrated that both native LDL and peroxinitrite-treated LDL bind to LDLR, and the uptake of NT-LDL was stronger than native LDL, suggesting an alternate mechanism for cellular uptake involving macrophages LOX-1, CD36, and SR-A receptors (42). Faster clearance of modified LDL has been well demonstrated (43). Similarly, LDL nitration has been shown to be associated with enhanced monocyte/macrophage uptake when compared with oxidized LDL isolated from rheumatoid and osteoarthritis patients with CVD (44). Furthermore, a significantly higher amount of nitrotyrosine residues containing lipoproteins has been found in atherosclerotic site than in plasma (45). Modified LDL isolated from aortic atherosclerotic intima had 90-fold higher levels of 3-nitrotyrosine as compared to healthy subjects (46). Taken together, our findings are in good agreement with our previously published work and others (12, 15, 16, 19, 45).

Unlike NT-HDL, the association between NT-LDL and CIMT outcome becomes insignificant after inclusion of other independent variables into the base model. Potential confounding variables were identified, and sex was the most significant contributing factor in the CIMT variance, especially in the poorly-controlled patients' group. The explanation for sex difference in this report is unknown. This could be linked in part to hormonal differences between women and men. In fact, it is well known that estrogen upregulates endothelial NOS which may result in changes of NO production and association between NT-LDL and CIMT (47). Another plausible explanation is that CIMT may represent adaptive changes to biomechanical parameters such as media hypertrophy, aging and others, and therefore this parameter may not be a direct indicator of vascular atherosclerotic changes. The lack of association between NT-LDL and CIMT outcome suggest that plasma NT-LDL may not be considered a direct powerful measure of vascular atherosclerotic process in diabetic patients.

Use of drugs such as statins, beta-blockers and ACE inhibitors has been shown to reduce MPO levels in patients with acute coronary syndrome, but not in patients with stable CAD (48). In this study, we did not measure plasma MPO levels; however our study revealed that use of calcium channel blockers and ACE inhibitors significantly affected the association between NT- HDL levels and VRI outcome most likely by suppression of tyrosine nitration of proteins as shown in diabetes and chronic renal diseases (49, 50).

There are some limitations to be considered in the present study. First, the relatively small sample size from a single center may have limited the power to detect weak correlations among different stratified groups; however, the sample was sufficient to demonstrate significance among the groups. Second, the ELISA allowed us to measure total NT-LDL and NT-HDL particles; therefore, we were unable to distinguish between various nitrated subclasses of lipoproteins. In fact, most of published reports showed either circulating free nitrotyrosine or total nitrated apolipoproteins-containing lipoproteins by ELISAs and mass spectrophotometry techniques. Third, we did not exclude patients with prior lipid lowering medication which may have biased the prognostic value of nitrated lipoproteins levels. Our study did not, however, show any significant effect of known lipid modulating drugs such as statins. Lastly, the study population represents a relatively heterogeneous multi-ethnic community-based T2D cohort which may have contributed to individual intra-variability measurements. We are aware of differences in race and study population selection could account for discrepancies among studies; and therefore, this may limit clarification of causality between plasma NT-HDL and VRI outcome. This cross-sectional study provides strong evidence for differential effects between circulating NT-HDL and NT-LDL in the impairment of microvascular dysfunction in T2DM. Therefore, caution should be taken when interpreting data from quantitative measurements of nitrated lipoproteins to assess vascular disease state.

In conclusion, our study demonstrated that plasma levels of NT-HDL, but not NT-LDL, independently predicted VRI outcome in diabetic patients especially in well-controlled patients with HbA1c ≤ 7%. In addition, total NO availability does not seem to significantly influence the association between NT-HDL and VRI, suggesting that circulating NT-HDL action on the vasculature may occur via NO-independent pathway. Additionally, our data demonstrated that plasma NT-HDL is more reliable biomarker than NT-LDL and could be used as a predictor of early microvascular function in diabetes. This finding may have important clinical implication despite unknown exact mechanisms. Therefore, our study is worthy of further investigations and must be confirmed in larger cohorts.

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Abbreviations: ApoA-I: apolipoprotein A-I; ApoB: apolipoprotein B; cf-PWV: carotid-femoral pulse wave velocity; CIMT: Carotid intima-media thickness; CAD: coronary artery disease; CVD: cardiovascular disease; HbA1c: Hemoglobin A1c; HDL: high density lipoprotein; LDL: low density lipoprotein; LDL-c: low density lipoprotein cholesterol; LDLR: low density lipoprotein receptor; NO: nitric oxide; NOS: nitric oxide synthase; NT-HDL: nitrated high-density lipoprotein; PWV: pulse wave velocity; sdLDL: small dense low-density lipoprotein; T2DM: type 2 diabetes mellitus; VRI: vascular reactivity index

Key Words: African-Americans, Biomarkers, Cardiovascular Disease, Endothelium Function, Lipoproteins, Myeloperoxidase, Nitration, Nitric Oxide, Vascular Complications, Type 2 diabetes

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