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Original Article

Low plasma HDL cholesterol is associated with greater risk for cardiovascular disease in subjects with metabolic syndrome

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ABSTRACT

Low plasma HDL cholesterol (HDL-c) is one of the features of metabolic syndrome (MetS) and is associated with an increased risk for cardiovascular disease (CVD). The objective of this study was to analyze whether low HDL-c and/or HDL functionality are associated with additional biomarkers of CVD in subjects with MetS. Forty subjects with MetS (11 men/29 women) were classified as having normal HDL-c (men $\geq$ 40 mg/dL, women $\geq$ 50 mg/dL, n = 13) or low HDL-c (men < 40 mg/dL and women < 50 mg/dL, n = 17). Anthropometric measurements, plasma lipids and glucose, hepatic enzymes, plasma insulin, glycosylated hemoglobin, and biomarkers of oxidative stress and inflammation as well as HDL functionality measurements including paraoxonase-1 (PON-1) and serum amyloid A1 (SAA1) as well as lipoprotein size and subfraction number were assessed. Participants with low HDL-c had higher systolic blood pressure ($p < 0.05$), higher triglycerides ($p < 0.05$), and lower total antioxidant capacity ($p < 0.05$) than those with normal HDL-c. Large VLDL, medium VLDL and small LDL were higher in the low HDL-c group ($p < 0.01$), while large and total HDL particles were higher in the normal HDL-c group ($p < 0.01$). Apolipoprotein A-1 concentrations and PON-1 activity were higher in the normal compared to the low HDL-c group ($p < 0.05$). These data indicate that men and women with MetS and low HDL-c have a more pronounced hyperlipidemia, higher concentrations of atherogenic lipoproteins, lower antioxidant capacity as well as a less functional HDL. These data suggest that low HDL-c, in combination with MetS, is associated with additional risk factors for CVD.

KEYWORDS: metabolic syndrome, HDL cholesterol, heart disease risk, inflammation, oxidative stress.

INTRODUCTION

Cardiovascular disease (CVD) is a major cause of mortality with about 31% people dying of CVD per year globally [1]. Metabolic syndrome (MetS) is a serious health condition associated with increased risk for several chronic diseases, and is defined by the presence of three or more of the following clinical risk factors: central adiposity, high blood pressure, hyperglycemia, high plasma triglycerides (TG), and low HDL-cholesterol (HDL-c) concentrations [2]. It has been estimated that nearly 35% of adults have MetS [3]. Low plasma HDL-c, one of the criteria for MetS, is inversely related with the risk for CVD, even in the setting of LDL-c levels below 70 mg/dL [4]. Observational studies have estimated that for each increment of 1 mg/dL in HDL-c, there is an approximate 2-3% reduction in CVD risk [5, 6]. The most known function of HDL is the efflux of

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cholesterol ester (CE) from peripheral cells and its transfer to the liver for excretion [7], in a process called reverse cholesterol transport (RCT). Other cardioprotective properties of HDL are: 1) inhibition of the expression of endothelial adhesion molecules and its action as anti-inflammatory agent; 2) antioxidative effects due to the association of HDL with several antioxidant enzymes such as paraoxonase-1 (PON-1); 3) prevention of oxidized LDL (Ox LDL) induced apoptosis, and 4) regulation of platelet adhesion [8, 9]. HDL particles are highly heterogeneous in size, shape, density, and content of cholesterol, phospholipids, and apolipoproteins [10]. In clinical settings, plasma HDL-c concentrations are often measured as a predictor and an independent risk parameter for CVD [11, 12]. However, recent data suggest that in addition to HDL-c concentration, HDL particle size is an important determinant of CVD mortality among MetS patients [12]; very large HDL particles may be a key driver of the cardioprotective properties of the functional HDL [13] and reduce cardiovascular death by 50% [13]. Interestingly, in two large trials of high-risk populations, it was shown that higher HDL particle number, but not plasma HDL-c concentrations, was significantly and inversely associated with the occurrence of cardiac events [14]. The aim of the present study was to examine whether low HDL-c and/or HDL functionality are associated with additional biomarkers of CVD in subjects classified with MetS. We hypothesized that subjects with MetS, and compared to subjects with normal HDL-c, those with low HDL-c, would have a greater risk for CVD as determined by clinical biomarkers.

MATERIALS AND METHODS

Study design

Data for this project were obtained from baseline measurements of subjects recruited for a clinical trial with men (n = 11) and women (n = 29) [15] who met the metabolic syndrome (MetS) criteria in accordance with NCEP:ATP III definition [2]. The study was approved by the University of Connecticut Institutional Review Board (IRB H14-278). All subjects provided their written informed consent prior to participating in the study. The study was registered at Clinicaltrials.gov, protocol # NCT02531334.

Participants had no current or history of heart disease, stroke, diabetes, liver disease, cancer, severe infections, or autoimmune diseases. Subjects with MetS were classified into two groups according to their HDL-c levels; Normal HDL-c group (men ≥ 40 mg/dL and women ≥ 50 mg/dL) (n = 23, 6 men and 17 women), and Low HDL-c group (men < 40 mg/dL and women < 50 mg/dL) (n = 17, 5 men and 12 women).

Diet and exercise records

Dietary assessment was conducted through the analysis of 3-day diet records completed by participants. Nutrition Data System for Research software (NDSR; 2013, University of Minnesota, Minneapolis, MN) was used to analyze dietary records [16]. Subjects also completed a 3-day exercise record.

Anthropometrics

Weight was measured to the closest 0.1 kilogram (kg) on a portable scale and height was measured to the closest 0.5 centimeters with a stadiometer. Body mass index (BMI) was calculated as kg/m^2. Waist circumference (WC) was assessed with a flexible measuring tape placed at the top of the iliac crest. WC was averaged from 3 readings. BP was measured on the right arm using an Omron automated BP cuff (Omron HEM 7320-Z/HEM 7131-Z, Lake Forest, IL) after subjects had been allowed to rest sitting quietly for 1-3 minutes. An average of 3 readings was used to account for variability [17].

Blood sample collection

After a 12-hour fast, 40 ml of blood was collected from the antecubital vein into EDTA-containing tubes to prevent coagulation. A sample of whole blood (1 ml) was collected to measure glycosylated hemoglobin (HbA1c). Plasma was isolated by centrifugation at 2000 x g for 20 minutes, aliquoted and frozen at -80°C for further analysis.

Plasma lipids, glucose, glycosylated hemoglobin (HbA1c), liver enzymes and C-reactive protein

Plasma total cholesterol (TC), TG, glucose, HDL-c, CRP, liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and HbA1c were all determined using an automated

clinical chemistry analyzer (Cobas c-111, Roche Diagnostics, Indianapolis, IN). Plasma LDL-c was calculated using the Friedewald equation [18].

Plasma insulin

Plasma insulin was measured using an enzyme-linked immunosorbent assay (ELISA) (kit (Crystal Chem, Elk Grove Village, IL) and concentrations were calculated using a BioTek Synergy 2 Multi-Mode Microplate Reader with Gen5 Software (BioTek Instruments, Inc, Winnoski, VT) at a wavelength of 450 nm [19].

Lipoprotein size and subfractions

Lipoprotein particle number, size, and concentration were assessed using proton nuclear magnetic resonance (NMR) spectroscopy. NMR analysis was performed on a 400 MHz NMR analyzer (LipoScience, Inc., Raleigh, NC) as previously described [16].

Apolipoprotein (apo) analysis

Plasma apolipoproteins (apo A-I, apo A-II, apo B, apo C-II, apo C-III, and apo E) were quantified simultaneously using a commercially available human apolipoprotein multiplex assay kit (EMD Millipore, Billerica, MA) and analyzed by a Luminex MAGPIX analyzer (Luminex Corporation, Austin, Texas) [16].

Paraoxonase-1 (PON-1)

Plasma PON-1 activity was measured by a commercial fluorometric assay kit (BioVision, Inc, Milpitas, CA) [20]. The fluorescence levels were measured in kinetic mode for 60 minutes at 37 °C by using the Microplate Fluorescence Reader with Gen5 Software (BioTek Instruments, Inc.; Winooski, VT, USA).

Serum amyloid-1 (SAA1)

SAA1 was measured by a Human Immunoassay kit (Invitrogen, Vienna, Austria) [21] using Luminex Technology.

Plasma inflammatory biomarkers

Plasma tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), Interleukin (IL)-6 and IL-8, were measured by use of LINCOplex technology [22].

Plasma antioxidant biomarkers

Plasma glutathione peroxidase (GPx), superoxide dismutase (SOD), total antioxidant capacity (TAC), and catalase were measured using commercially available kits (Cayman Chemical Company, Ann Arbor, MI) as previously described [23-25].

Oxidized LDL (Ox LDL)

Ox LDL was measured using ELISA technology. Concentrations were measured by using a BioTek Synergy 2 Multi-Mode Microplate Reader with Gen5 Software at 450 nm.

Statistical analysis

Statistical analysis was performed using SPSS version 25 statistical software for Windows (SPSS, Inc., Chicago, IL). Significance was defined as $p < 0.05$. Values are represented as mean ± standard deviation. An un-paired t test was used to determine significant differences between the groups in all measured parameters. Pearson correlations were used to evaluate relationships between the measured parameters.

RESULTS

Diet and exercise records

Participants were divided into two groups based on HDL-c levels, into Normal HDL-c and Low HDL-c as previously defined. Dietary intake and exercise records of participants are shown in Table 1. There were no significant differences between groups in any of the analyzed nutrients including macronutrients, types of fat, glycemic index, fiber or carotenoids. Similarly, there were no significant differences in minutes of exercise/ day between groups.

Parameters of metabolic syndrome, plasma insulin and HbA1c

The age of participants ranged from 32-70 y, and average age did not differ between low HDL-c and normal HDL-c groups (Table 2). The majority of the subjects were classified as obese (n = 27), and the average BMI was similar between groups. The Low HDL-c group had significantly higher systolic BP and TG than the normal HDL-c group ($p < 0.01$); however, there were not significant differences in the other MetS parameters, plasma insulin or HbA1c between groups. A negative

Table 1. Dietary intake and physical activity in normal HDL-c and low HDL-c groups.

Parameter	Normal HDL-c[1] (n = 23)	Low HDL-c (n = 17)	P value
Total energy (kcal)	2051.4 ± 891.6	2025.1 ± 472.3	0.913
Carbohydrate (%)	38.3 ± 9.0	40.4 ± 6.7	0.408
Protein (%)	17.2 ± 2.9	18.3 ± 3.8	0.315
Fat (%)	41.2 ± 8.6	39.1 ± 7.4	0.428
SFA (g/day)	30.3 ± 12.2	31.4 ± 15.1	0.797
MUFA (g/day)	39.7 ± 41.8	32.9 ± 11.9	0.523
PUFA (g/day)	21.3 ± 17.5	19.5 ± 6.4	0.676
Trans fatty acids (g/day)	2.5 ± 2.3	2.1 ± 0.94	0.250
Cholesterol (mg/day)	311.0 ± 139.7	311.7 ± 193.2	0.990
Total fiber (g/day)	21.8 ± 14.8	24.1 ± 10.3	0.590
Soluble fiber (g/day)	6.7 ± 2.2	7.7 ± 2.4	0.224
Insoluble fiber (g/day)	14.9 ± 13.5	16.4 ± 8.5	0.700
Glycemic index	57.7 ± 6.1	56.6 ± 5.6	0.577
Glycemic load	101.7 ± 44.2	106.4 ± 34.5	0.720
β-Carotene (μg)	3423.4 ± 3255.9	3886.2 ± 3430.1	0.666
α-Carotene (μg)	577.7 ± 1045.2	645.8 ± 924.4	0.832
Lycopene (μg)	2390.8 ± 2108.9	3830.7 ± 4787.4	0.206
Lutein + Zeaxanthin (μg/day)	97.8 ± 122.9	331.7 ± 711.8	0.129
Exercise (minutes/week)	36.7 ± 29.8	53.8 ± 50	0.185

[1]Data are presented as mean ± standard deviation.

correlation was found between HDL-c and WC ($r = -0.418$, $p < 0.01$) (Figure 1, panel A) and between HDL-c and insulin ($r = -0.413$, $p < 0.025$) (Figure 1, panel B).

Lipoprotein size and subfractions

The total concentrations of the different lipoprotein subfractions are presented in Table 3. The number of large and medium VLDL as well as the small LDL particles were higher in the low HDL-c group ($p < 0.05$). In addition, large LDL particles were higher in the normal HDL-c group. Both total and large HDL particles were higher in the normal compared to the low HDL-c group ($p < 0.001$). The size of LDL and HDL particles was larger in the normal HDL-c group compared to the low HDL-c group while VLDL particles were not different between groups (Figure 2).

Apolipoproteins (apo)

Plasma apo A-I concentrations were higher in individuals with normal HDL-c values ($p < 0.01$) (Table 4). None of the other apolipoproteins differed between groups (Table 4). A positive correlation was found between plasma apo A-I concentrations and large and total HDL particles ($r = 0.530$, $p < 0.01$ (Figure 3, panel A) and $r = 0.671$, $p < 0.01$ (Figure 3, panel B), respectively). A positive correlation was found between plasma TG and apo CIII ($r = 0.549$, $p < 0.01$) (data not shown).

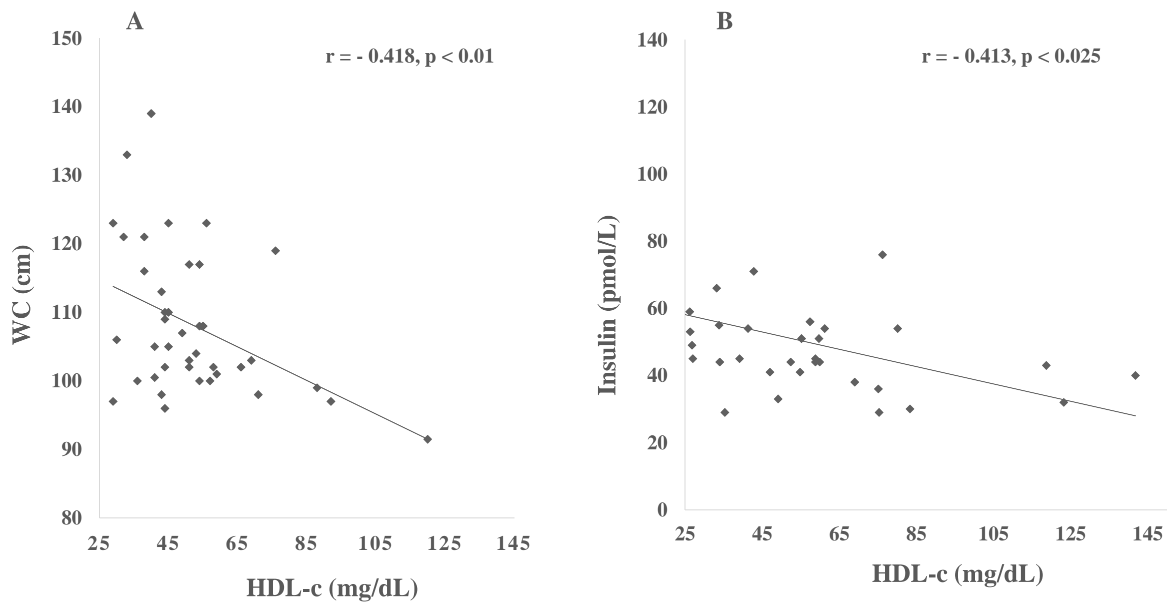
PON-1 and SAA1

PON-1 activity and plasma SAA-1 concentrations are presented in Figure 4. PON-1 activity was higher in the normal HDL-c compared to the low HDL-c group ($p = 0.022$) while plasma concentrations

Table 2. Anthropometrics, plasma lipids and glucose parameters in normal HDL-c and low HDL-c groups.

Parameter	Normal HDL-c[1] (n = 23)	Low HDL-c (n = 17)	P value
Age (years)	53.3 ± 9.4	51.2 ± 9.8	0.492
Weight (kg)	88.6 ± 17.3	93.6 ± 14.6	0.341
BMI (kg/m^2)	32 ± 4.1	32.7 ± 3.3	0.563
Waist circumference (cm)	114.2 ± 15.5 (men)	113.6 ± 11.5 (men)	0.948
	104.4 ± 8.3 (women)	108.5 ± 9.5 (women)	0.231
Systolic BP (mm Hg)	121.3 ± 14.2	129.9 ± 9.8	0.028
Diastolic BP (mm Hg)	84.8 ± 7.1	83.1 ± 9.1	0.507
Total cholesterol (mg/dL)	187.4 ± 36.3	178.6 ± 30.8	0.426
Triglycerides (mg/dL)	115.5 ± 56.8	165.7 ± 80.7	0.026
HDL-c (mg/dL)	47.2 ± 5.1 (men)	31.6 ± 3.8 (men)	0.000
	66.5 ± 18.6 (women)	41.4 ± 3.8 (women)	0.000
LDL-c (mg/dL)	102.7 ± 31.7	107 ± 29.6	0.662
Glucose (mg/dL)	106.2 ± 10.1	105.1 ± 9.9	0.733
Insulin (pmol/L)	45.6 ± 29.9	60.3 ± 29.1	0.141
HbA1c (%)	5.6 ± 0.45	5.6 ± 0.58	0.815

[1]Data are presented as mean ± standard deviation.



Negative correlation between HDL-c concentrations and waist circumference (WC)

Figure 1. Correlations between HDL-c and waist circumference (WC) ($r = -0.418$, $p < 0.001$) (panel A) and between HDL-c and plasma insulin ($r = -0.413$, $p < 0.025$) (panel B).

Table 3. Particle size and subfraction distribution of large, medium, and small lipoprotein particles in normal HDL-c and low HDL-c groups.

Lipoprotein particles size	Normal HDL-c[1] (n = 23)	Low HDL-c (n = 17)	P Value
VLDL particles			
Large (60-100 nm), nmol/L	5.6 ± 4.4	9.4 ± 6	0.028
Medium (40-60 nm), nmol/L	15.8 ± 8.2	25.1 ± 11.3	0.005
Small (30-40 nm), nmol/L	22.2 ± 13.3	17.9 ± 9.2	0.258
Total	43.6 ± 22.2	52.3 ± 16.1	0.178
Mean particle size, nm	53.4 ± 7.6	57.6 ± 10.6	0.151
LDL particles			
Large (23-30 nm), nmol/L	330.4 ± 215.6	165 ± 164	0.012
Small (18-23 nm), nmol/L	522.4 ± 288.2	822.5 ± 283	0.002
Total, nmol/L	1158.5 ± 355.5	1264.7 ± 342	0.349
Mean particle size, nm	20.8 ± 0.54	20.2 ± 0.68	0.008
HDL particles			
Large (10-13 nm), µmol/L	8.2 ± 4.1	4.3 ± 1.9	0.001
Medium (8.2-10 nm), µmol/L	13.2 ± 6.1	10.8 ± 5.2	0.208
Small (7.3-8.2 nm), µmol/L	16.1 ± 5.1	15.7 ± 5.5	0.805
Total, µmol/L	37.5 ± 4.4	30.8 ± 4.8	0.000
Mean particle size, nm	9.4 ± 0.58	9.1 ± 0.35	0.024

[1]Data are presented as mean ± standard deviation.

of SAA-1 were not different between groups. PON-1 activity correlated positively with total number of HDL particles (data not shown).

Inflammatory and oxidative stress biomarkers

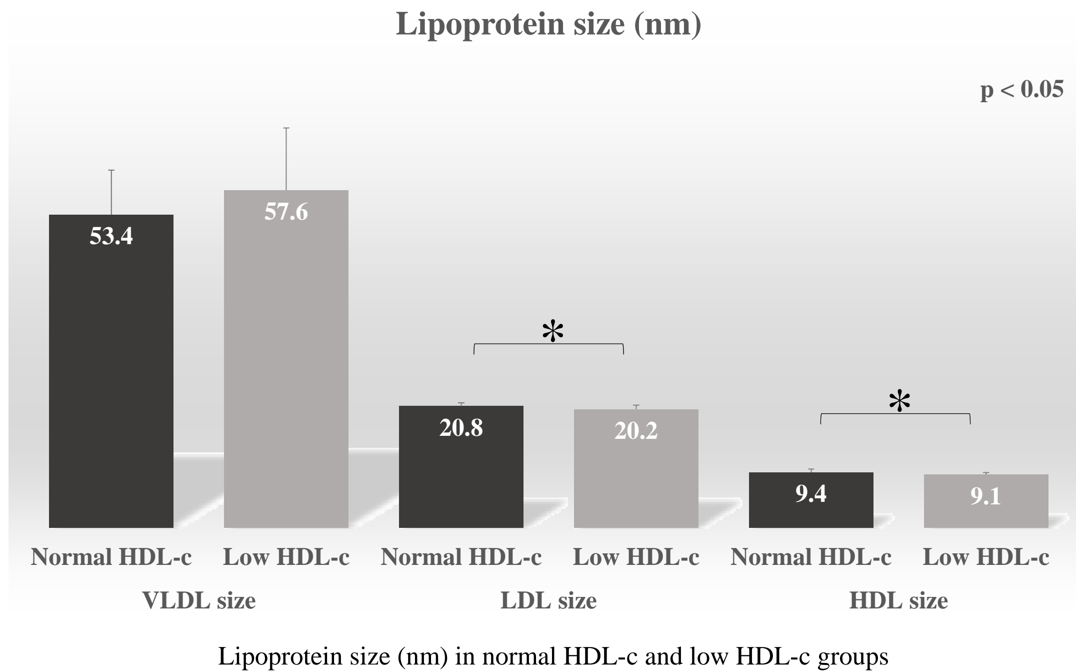
Plasma inflammatory and oxidative stress biomarkers were not different between groups (Table 5) with the exception of TAC which was higher in the normal HDL-c group ($p < 0.05$).

DISCUSSION

In this study we found that individuals with MetS and low HDL-c had higher TG and systolic BP and lower plasma TAC compared to those with normal HDL-c. We also found that subjects in the low HDL-c group had more atherogenic lipoprotein profiles, characterized by higher concentrations of large VLDL, medium VLDL and small LDL. In addition, plasma apo A-I concentration and PON-1 activity were lower in the low-HDL group suggesting a more diminished HDL functionality in these individuals.

Diet and metabolic syndrome parameters

Obesity is a key feature of metabolic dysfunction that increases the metabolic complications among individuals with MetS including CVD, insulin resistance, T2D, hyperlipidemia, and hypertension [26]. Unhealthy diets and physical inactivity are associated with obesity [27]. In the current study the dietary and exercise records showed a high intake of saturated fat and low dietary fiber in addition to low physical activity level. All participants in this study had high concentrations of central adiposity, a common feature in metabolic syndrome [28], strongly associated with insulin resistance [29]. Palaniappan *et al.* [30] have shown that a 11 cm increase in WC is associated



Lipoprotein size (nm) in normal HDL-c and low HDL-c groups

Figure 2. VLDL, LDL and HDL size in normal HDL-c (dark bar) and low HDL-c (gray bar) groups. * indicates significantly different ($p < 0.05$).

Table 4. Plasma apolipoproteins (Apo) concentrations in normal HDL-c and low HDL-c groups.

Parameter	Normal HDL-c[1] (n= 23)	Low HDL-c (n = 17)	P value
Apo A-I (mg/L)	985.4 ± 274.2	790.7 ± 188.1	0.019
Apo A-II (mg/L)	599.0 ± 159.4	586.4 ± 173.9	0.813
Apo C-II (mg/L)	243.4 ± 161.1	283.8 ± 212.5	0.497
Apo C-III (mg/L)	474.7 ± 274.1	520.1 ± 330.8	0.639
Apo E (mg/L)	106.2 ± 60.4	125.3 ± 62.9	0.339
Apo B (mg/L)	1707.1 ± 704.4	1908.8 ± 598.0	0.346

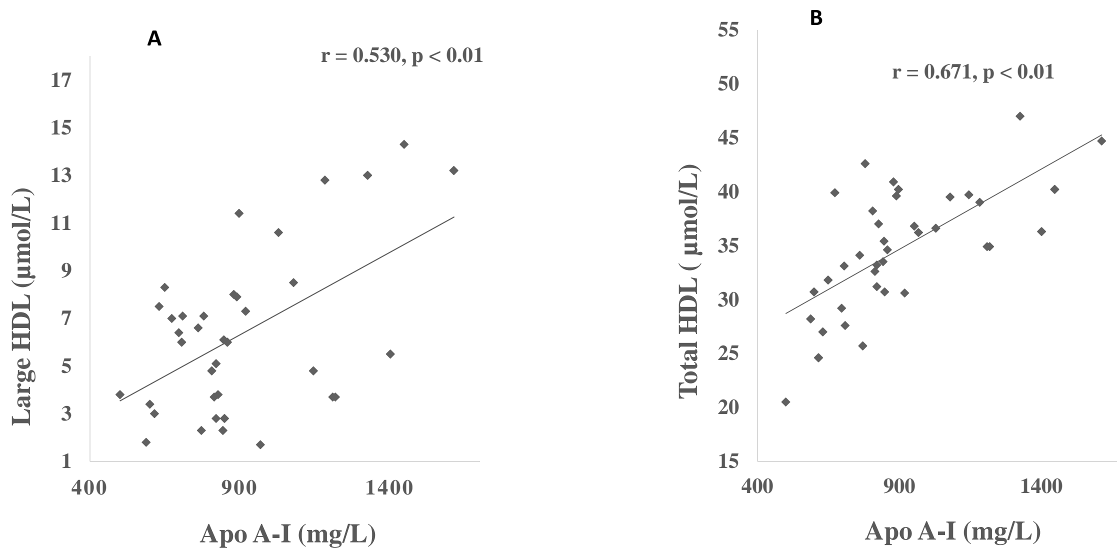
[1]Data are presented as mean ± standard deviation.

with an adjusted 80% increased risk of developing MetS condition.

Systolic BP and plasma TG were higher in the low HDL-c group. Elevated BP is one key CVD risk factor, and it has been estimated that a 5 mm Hg reduction in systolic BP can result in overall reduction of 14% in stroke mortality and 9% in coronary heart disease mortality [30]. Elevated TG concentrations are strongly correlated with increased risk of CVD. Insulin resistance results either from a genetic defect or obesity, and it may lead to elevation in TG as well as BP and reduction of HDL-c [30], an association confirmed in this study. Low HDL-c values have been linked to CVD and diabetes [31]. In contrast, normal HDL-c levels are considered to be protective against cardiovascular disease and insulin resistance.

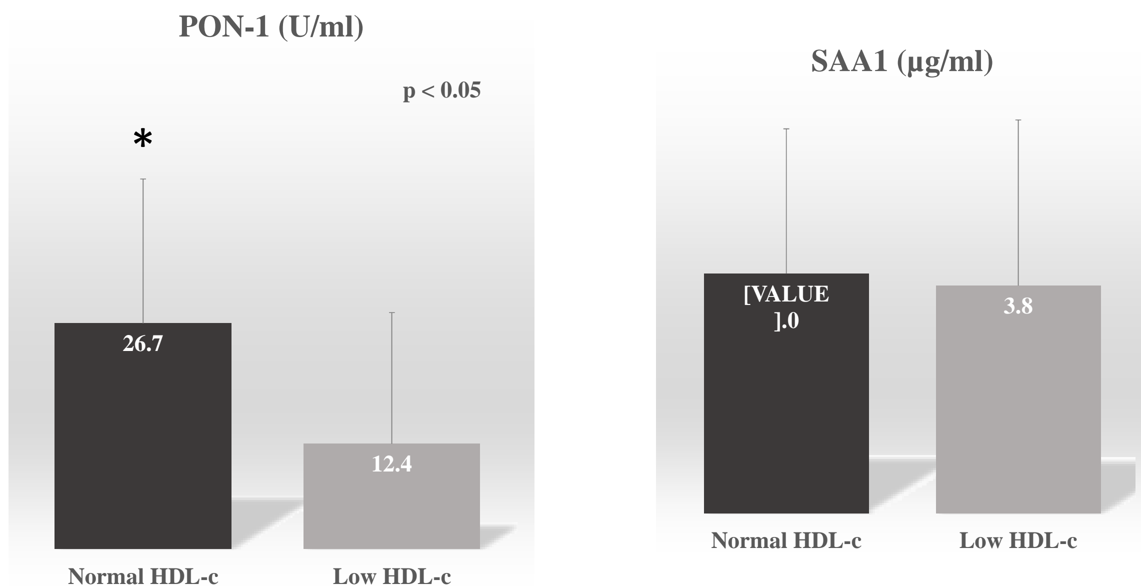
Lipoprotein distribution and size

Recently, interest has shifted from not only examining HDL-c, but also assessing HDL particle number



Positive correlation between large HDL and Apo A-I

Figure 3. Correlations between plasma apo A-1 and large HDL particles (r = 0.530, $p < 0.01$) (panel A) and plasma apo A-1 and total HDL particles (r = 0.671, $p < 0.01$) (panel B).



Paraoxonase-1 (PON-1) activity in normal HDL-c and low HDL-c groups

Figure 4. Plasma paraoxonase-1 (PON-1) activity in participants with normal HDL-c (black bar) and low HDL-c (gray bar) and serum amyloid A1 (SAA1). * indicates significantly different at $p < 0.05$.

and HDL quality [31]. HDL exhibits antiatherogenic functions through its role in the RCT process, and by acting as an anti-inflammatory, antioxidant, and antithrombotic agent, all of which may contribute to protection against atherosclerosis [31]. These functions may by impaired when HDL particles become dysfunctional. Therefore, lipoprotein particle size and number are important metrics for

Table 5. Plasma concentrations of Inflammatory and oxidative stress biomarkers and liver enzymes in normal HDL-c and low HDL-c groups.

Parameter	Normal HDL-c[1] (n =23)	Low HDL-c (n = 17)	P value
AST (U/L)	25.0 ± 6.1	26.2 ± 7.4	0.598
ALT (U/L)	29.2 ± 10.7	29.6 ± 10.8	0.915
C-reactive protein (mg/dL)	0.39 ± 0.39	0.31 ± 0.29	0.456
TNF-α (pg/mL)	6.2 ± 2.4	6.9 ± 3.9	0.508
MCP-1 (pg/mL)	129.6 ± 66.6	122.2 ± 29.0	0.676
IL-6 (pg/mL)	5.5 ± 0.78	5.99 ± 1.3	0.185
IL-8 (pg/mL)	9.7 ± 10.3	7.9 ± 1.2	0.479
TBARS (μM)	0.16 ± 0.03	0.14 ± 0.03	0.087
TAC (mM trolox equivalents)	2.1 ± 1.5	1.2 ± 0.9	0.044
SOD (U/mL)	2.3 ± 1.2	1.9 ± 1.2	0.361
GPx (nmol/min/mL)	150.6 ± 23.9	141.5 ± 20.2	0.220
CAT (nmol/min/mL)	19.4 ± 11.7	17.2 ± 7.0	0.507
Oxidized LDL (ng/ML)	201.2 ± 14.5	212.6 ± 62.9	0.405

[1]Data are presented as mean ± standard deviation.

better understanding lipoprotein metabolism [32]. Lipoprotein particles, which are considered atherogenic are present in increasing numbers in the MetS population [33]. The number of these particles has been shown to be positively associated with CVD risk. In the current study, NMR, a well-known and useful method that helps to link specific lipoproteins to CVD was used [33]. Our findings indicate that MetS subjects with low HDL-c had higher numbers of large and medium VLDL and small LDL while large LDL particles were lower in number as well as the mean size of LDL compared with the normal HDL-c group. Large LDL particles are known to be less susceptible to oxidation and subendothelial retention than small LDL particles [34]. LDL-c values do not provide a comprehensive assessment of CVD risk, while measuring LDL particles provides more information for subjects regarding atherogenic lipoproteins. In contrast, the number of large HDL particles as well as the mean size of HDL were higher among subjects in the normal HDL-c group also indicating a more efficient RCT in this group and in addition in agreement with our data, a negative correlation between HDL particles numbers and CVD has been reported [4].

Apolipoproteins

Apo A-I is mainly present in HDL particles and higher levels of this apolipoprotein are inversely associated with CVD risk. Apo A-I has different major functions in RCT including activating lecithin cholesterol acyl transferase (LCAT), and being a ligand for SR-B1 in the liver and for ABCA1 in the extra hepatic tissue to facilitate removal of cholesterol [35]. In contrast it is known that apo C-III has proinflammatory and prothrombotic effects [31] while plasma apo B concentrations are highly correlated with cardiovascular disease [36]. In our study, we found increases in apo A-I while apo C-III and apo B did not differ between groups. In addition, there was a positive correlation between apo A-I and both large HDL and total HDL, which further highlights the importance of apo A-I in HDL functionality.

PON-1 and SAA1

The measuring of HDL functionality markers is important to provide a better view of the atheroprotective lipoprotein HDL. Dysfunctional HDL exhibit decreases in cholesterol efflux, increases in inflammation and thrombosis [31]. There are some markers indicative of HDL dysfunctionality, including apo C-III and SAA1. In the current study, apo C-III was positively correlated with TG levels. HDL functionality can be evaluated through measuring apo A-I, and PON-1. In the current study, apo A-I and PON-I activity were higher in the normal HDL-c group. Lower PON-1 activity is related to CVD, and MetS has been shown to reduce the enzymatic capacity of PON-1 [37]. The normal HDL-c group had higher numbers of large HDL particles and HDL-c levels. Thus, subjects in this study with normal HDL-c, who had greater number of large HDL particles, also exhibited greater HDL functionality as assessed through apo A1 and PON-I activity, suggesting a possible association between large HDL particle size and HDL functionality in this population.

Another marker for HDL functionality is SAA1. SAA1 is an apolipoprotein of HDL in the acute-phase, which causes HDL remodeling and dysfunctionality [38]. It has been documented that SAA1 plays a major role in lipid metabolism, but its impact remains incompletely understood [38]. In this study, we did not find significant differences in SAA1 between groups.

Inflammatory and antioxidants biomarkers

MetS has been linked to low-grade inflammation and increased oxidative stress biomarkers including oxidized LDL [39]. In addition, studies have shown that individuals with MetS have higher values of liver enzymes compared with healthy population [40]. However, we did not find differences between groups in these parameters in the current study except for TAC, which was higher in the normal HDL-c group. Because antioxidants are mostly carried in circulation through HDL, the higher number of HDL particles observed in the normal HDL-c group might have had a role in the more efficient transportation of these antioxidants.

CONCLUSIONS

These data suggest that in men and women with MetS, measuring both HDL-c concentrations and number of HDL particles can provide important information regarding levels and functionality of the protective HDL. Subjects with low HDL-c appear to have a higher risk for biomarkers associated with CVD including high plasma TG, higher concentrations of atherogenic lipoproteins, increased systolic BP, and lower antioxidant capacity. In addition, the data also suggest that subjects in the normal HDL-c group have better HDL functionality as determined by higher PON-1 activity and higher concentrations of apo A1.

CONFLICT OF INTEREST STATEMENT

Hana M. Al-Yousef, Bruno S. Lemos, Minu S. Thomas, Melissa Melough, Ock K. Chun and Maria Luz Fernandez have no competing financial interests.

REFERENCES

1. WHO | Cardiovascular diseases (CVDs). WHO. 2018, http://www.who.int/cardiovascular_diseases/en/ Accessed April 30, 2018.
2. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. 2001, JAMA, 285, 2486.
3. Aguilar, M., Bhuket, T., Torres, S., Liu, B. and Wong, R. J. 2015, JAMA, 313, 1973.
4. Kontush, A. 2015, Front. Pharmacol., 6, 218.
5. Gordon, D. J., Probstfield, J. L., Garrison, R. J., Neaton, J. D., Castelli, W. P., Knoke, J. D., Jacobs, D. R. Jr., Bangdiwala, S. and Tyroler, H. A. 1989, Circulation, 79, 8.
6. Kosmas, C. E., Martinez, I., Sourlas, A., Bouza, K. V., Campos, F. N., Torres, V., Montano, P. D. and Guzman, E. 2018, Drugs Context, 7, 1.
7. Tall, A. R., Yvan-Charvet, L., Terasaka, N., Pagler, T. and Wang, N. 2008, Cell Metab., 7, 365.
8. Camont, L., Chapman, M. J. and Kontush, A. 2011, Trends Mol. Med., 17, 594-603.
9. Mahdy Ali, K., Wonnerth, A., Huber, K. and Wojta, J. 2012, Br. J. Pharmacol., 167, 1177.
10. Boden, W. E. 2000, Am. J. Cardiol., 86, 19L.

11. Nissen, S. E., Tardif, J-C., Nicholls, S. J., Revkin, J. H., Shear, C. L., Duggarn, W. T., Ruzyllo, W., Bachinisky, W. B., Lsala, G. P. and Tuzcu, E. M. 2007, N. Engl. J. Med., 356, 1304.
12. Yvan-Charvet, L., Matsuura, F. and Wang, N. 2007, Arterioscler Thromb. Vasc. Biol., 27, 1132.
13. Kuller, L. H., Grandits, G., Cohen, J. D., Neaton, J. D. and Prineas, R. 2007, Atherosclerosis, 195, 122.
14. Parish, S., Offer, A., Clarke, R., Hopewell, J. C., Hill, M. R., Otvos, J. D., Armitage, J. and Collins, R. 2012, Circulation, 125, 2469.
15. Fernandez, M. L., Thomas, M. S., Lemos, B. S., DiMarco, D. M., Missimer, A., Melough, M., Chun, O. K., Murillo, A. G., Alyousef, H. M. and Medina-Vera, I. 2018, Curr. Pharm. Des., 24, 1905.
16. DiMarco, D. M., Norris, G. H., Millar, C. L., Blesso, C. N. and Fernandez, M. L. 2017, J. Nutr., 147, 323.
17. Pickering, T. G., Hall, J. E., Appel, L. J., Falkner, B. E., Graves, J. W., Hill, M. N., Jones, D. H., Kurtz, T., Sheps, S. G. and Roccella, E. J. 2005, Circulation, 111, 697.
18. Knopfholz, J., Disserol, C. C., Pierin, A. J., Schirr, F. L., Streisky, L., Takito, L. L., Ledesma, P. M., Faria-Neto, J. R., Olandoski, M., Pereira de Cuna, C. L. and Banderia, A. M. 2014, Cholesterol, 2014, 261878.
19. Jeffery, N., Richardson, S., Beall, C. and Harries, L. W. 2017, Exp. Cell Res., 361, 284.
20. Connelly, P. W., Maguire, G. F., Picardo, C. M., Teiber, J. F. and Draganov, D. 2008, J. Lipid Res., 49, 245.
21. Cocco, E., Bellone, S., El-Sahwi, K., Cargnelutti, M., Buza, N., Tavassoli, F. A., Schwartz, P. E., Rutherford, T. J., Pecorelli, S. and Santin, A. D. 2010, Cancer, 116, 843.
22. Al-Sarraj, T., Saadi, H., Volek, J. S. and Fernandez, M. L. 2010, Metab. Syndr. Relat. Disord., 8, 39.
23. Vance, T. M., Azabdaftari, G., Pop, E. A., Lee, S. G., Su, J. L., Fontham, E. T., Bensen, J. T., Steck, S. E., Arab, L., Mohler, J. L., Chen, M. H., Koo, S. I. and Chun, O. K. 2015, Prostate Cancer, 2015, 728046.
24. Lee, S. G., Wang, T., Vance, T. M., Hubert, P., Kim, D. O., Koo, S. I. and Chun, O. K. 2017, J. Microbiol. Biotechnol., 27, 388.
25. Ng, Y-W. and Say, Y-H. 2018, Peer J., 6, e4696.
26. Mozaffarian, D. 2016, Circulation, 133, 187.
27. Millen, B. E., Abrams, S., Adams-Campbell, L., Anderson, C. A., Brenna, J. T., Campbell, W. W., Clinton, S., Hu, F., Nelson, M., Neuhouser, M. L., Perez-Escamilla, R., Siega-Riz, A. M., Story, M. and Lichtenstein, A. H. 2016, Adv. Nutr., 7(3), 438-444.
28. Nolan, P. B., Carrick-Ranson, G., Stinear, J. W., Reading, S. A. and Dalleck, L. C. 2017, Prev. Med. Reports, 7, 211.
29. Kaur, J. A. 2014, Cardiol. Res. Pract., 2014, 943162.
30. Palaniappan, L., Carnethon, M. R., Wang, Y., Hanley, A. J., Fortmann, S. P., Haffner, S. M. and Wagenknecht, L. 2004, Diabetes Care, 27, 788.
31. Lubitz, S. A., Yin, X., McManus, D. D., Weng, L. C., Aparicio, H. J., Wakey, A. J., Rafael Romero, J., Kase, C. S., Ellinor, P. T., Wolf, P. A., Seshadri, S. and Benjamain, E. 2017, Stroke, 48, e142.
32. Davidson, W. S. 2014, Clin. Chem., 60, e1.
33. Andersen, C. J., Blesso, C. N., Lee, J., Barona, J., Sha, D, Thomas, M. J. and Fernandez, M. L. 2013, Lipids, 48, 557.
34. Ference, B. A., Ginsberg, H. N., Graham, I., Ray, K. K., Packard, C. J., Bruckert, E., Hegele, R. A., Krauss, R. M., Raal, F. J., Schunker, H., Watts, G. F., Boren, J., Fazio, S., Horton, J. D., Masana, L., Nicholls, S. J., Nordetsgaard, B. G., van de Sluis, B., Askinene, M. R., Tokgozoglu, L., Lndmesser, E., Laufs, U., Wiklund, O., Stock, J. K., Chapman, M. J. and Catapano, A. L. 2017, Eur. Heart J., 38, 2459.
35. Kalantari-Namiri, R., Gao, F., Cahhopadhayay, A., Wheeler, A. A., Navab, K. D., Farias-Eisner, R. and Reddy, S. T. 2015, Biofactors, 41, 153.
36. Fernandez, M. L and Webb, D. 2008, J. Am. Coll. Nutr., 27, 1.

37. Sentí, M., Tomás, M., Fitó, M. Weinbrenenr, T., Covas, M. I., Sala, J., Msia, R. and Mrrugat, J. 2003, J. Clin. Endocrinol. Metab., 88, 5422.
38. Sun, L. and Ye, R. D. 2016, Gene, 583, 48.
39. van Guilder, G. P., Hoetzer, G. L., Greiner, J. J., Stauffer, B. L. and DeSouza, C. A. 2006, Obesity, 14, 2127.
40. Zhang, L., Ma, X., Jiang, Z., Zhang, K., Zhang, M., Li, Y., Zhao, Z. and Xiong, H. 2015, Oncotarget, 6, 26782.