Weight Loss and Exercise Alter the High-Density Lipoprotein Lipidome and Improve High-Density Lipoprotein Functionality in Metabolic Syndrome

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Objective—High-density lipoprotein (HDL) lipid composition and function may better reflect cardiovascular risk than HDL cholesterol concentration. This study characterized the relationships between HDL composition, metabolism, and function in metabolic syndrome (MetS) patients and how changes in composition after weight loss (WL) and exercise treatments are related to function.

Approach and Results—Plasma samples from MetS patients (n=95) and healthy individuals (n=40) were used in this study. Subsets of the MetS group underwent 12 weeks of no treatment (n=17), WL (n=19), or WL plus exercise (WLEX; n=17). HDL was isolated using density-gradient ultracentrifugation. The HDL lipidome was analyzed by mass spectrometry, and particle size determined by nuclear magnetic resonance. Cholesteryl ester transfer protein activity and ex vivo HDL cholesterol efflux capacity (CEC) were assessed. The HDL lipidome in the MetS patients was substantially different from that in healthy individuals, mean particle size was smaller, and CEC was lower. Several HDL phospholipid and sphingolipid species were associated with HDL diameter and CEC. The HDL lipidome and particle size were modified toward the healthy individuals after WL and WLEX treatments, with greater effects observed in the latter group. Cholesteryl ester transfer protein activity was reduced after WL and WLEX, and CEC was improved after WLEX.

Conclusions—WLEX treatment in MetS patients normalizes the HDL lipidome and particle size profile and enhances CEC. HDL lipids associated with diminished CEC may represent novel biomarkers for early prediction of HDL dysfunction and disease risk and may represent potential therapeutic targets for future HDL therapies.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00163943

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Key Words: cardiovascular diseases ■ cholesterol ester transfer proteins ■ cholesterol, HDL ■ metabolic syndrome ■ weight loss

In recent years, the conflicting results of multiple CETP (cholesteryl ester transfer protein) inhibitor trials, all of which led to an increase in high-density lipoprotein cholesterol (HDL-C) in response to treatment,[1,2] have called into question the nature of the relationship between HDL-C and cardiovascular disease (CVD) risk. Recent studies have shown that plasma levels of HDL-C do not always reflect CVD risk[3] and that HDL-C efflux capacity is correlated with incident type-2 diabetes (T2D) mellitus and CVD independent of either HDL-C or apolipoprotein A-I (apoA-I).[4] Such studies have suggested that HDL-cholesterol efflux capacity (CEC), as a measurement of HDL atheroprotective function, may be a better biomarker for CVD risk.[4–6]

Metabolic syndrome (MetS) incorporating obesity, elevated plasma glucose, hypertension, and dyslipidemia represents a series of features that are risk factors for the development of T2D, CVD, and other metabolic sequelae. MetS is characterized by abnormalities in HDL structure and function,[7] which may contribute to this increased disease risk. As a consequence of these observations, the current focus of HDL studies has shifted from HDL-C to relationships between HDL composition, particle size, and functionality to better understand their potential in the prediction and management of CVD risk.

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Nonstandard Abbreviations and Acronyms	
apoA-I	apolipoprotein A-I
CEC	cholesterol efflux capacity
CETP	cholesteryl ester transfer protein
CVD	cardiovascular disease
HDL-C	high-density lipoprotein cholesterol
LCAT	lecithin–cholesterol acyltransferase
LDL	low-density lipoprotein
MetS	metabolic syndrome
T2D	type-2 diabetes
WL	weight loss
WLEX	weight loss plus exercise

HDL particle number and size, determined using nuclear magnetic resonance spectroscopy, can reveal abnormalities in HDL metabolism which may associate with CVD risk.[8] Lipidomic studies of HDL, using mass spectrometry, can provide detailed information about lipid composition of HDL particles that can correlate with functional measurements.[9] Several recent reports have demonstrated that abnormalities of the HDL lipidome in metabolic diseases may adversely impact on HDL function and that specific lipids (such as sphingomyelin, plasmalogen, and triacylglycerol) may represent specific biomarkers to predict HDL function and potential therapeutic targets to modify that function.[10]

Changing lifestyle behaviors, through improved diet and increased exercise, is the first-line treatment for 26 different chronic diseases,[11] including for people at high risk of metabolic diseases such as obesity, MetS, T2D, and CVD. However, the effects of lifestyle modification (weight loss [WL] or exercise) on the HDL lipidome and how these changes are related to HDL metabolism and function are poorly understood. We hypothesized that (1) the HDL lipidome is interdependent with HDL structure and function, (2) the HDL lipidome will differ in individuals with MetS compared with healthy individuals, and (3) in individuals with MetS, WL and regular physical activity will normalize the HDL lipidome leading to changes in HDL structure and improved HDL function.

Here, we have applied lipidomics, nuclear magnetic resonance, and CEC measurements to characterize HDL structure–function relationships in MetS and define the effect of dietary WL and dietary WL plus exercise (WLEX) interventions in a group of MetS individuals.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Study Design

The analyses presented here used samples from 2 previous randomized controlled trials examining the effect of WL or WLEX (n=53) and pioglitazone (n=42) on sympathetic nervous activity.[12,13] Patients gave written consent for samples to be stored and used in future studies.

To define associations between the HDL lipidome and HDL function, we analyzed all available baseline plasma samples (n=95) from these 2 studies. The combined 95 MetS participants were overweight/obese men and postmenopausal women, who met National Cholesterol Education Program–Third Adult Treatment Panel criteria for the diagnosis of MetS. Participants were not receiving routine medication, in particular no antihypertensive or lipid-lowering therapies. Diabetic participants (fasting glucose ≥7 mmol/L) and those with renal, hepatic, or thyroid impairment were excluded. To provide a healthy comparator group, plasma samples from a group of 40 healthy individuals (defined as complete physical, mental, and social well-being, not receiving routine medication, with no history of diabetes mellitus or CVD) whose stored plasmas were obtained from the Baker Heart and Diabetes Institute Biobank. The mean age and sex ratio in the healthy group were not significantly different from the MetS group.

In one of the previous studies,[9] a controlled interventional study design was used to randomly allocate 53 MetS patients to 1 of 3 interventions for a 12-week period: no treatment (n=17), dietary WL (n=19), or dietary WLEX (n=17). Samples from baseline and the 12-week follow-up time point were stored at −80°C. To assess the effect of dietary WL and dietary WLEX on the HDL lipidome and HDL function, the 12-week follow-up samples from these participants were also analyzed in this study.

Results

Baseline and Clinical Characteristics

The MetS individuals had higher systolic blood pressure, body mass index, fasting glucose, triglycerides, total cholesterol and non–HDL-C, and lower HDL-C and apoA-I levels than the healthy individuals (Table 1). There were no significant differences in baseline characteristics between the 3 MetS intervention groups (MetS control, WL, and WLEX; Table III in the online-only Data Supplement). After 12 weeks of WL and WLEX treatments, there were significant reductions in body weight, body mass index, fasting insulin, and homeostatic model assessment-estimated insulin resistance total cholesterol measurements in comparison with the MetS control group. However, systolic blood pressure, triglycerides, and non–HDL-C levels decreased significantly only after the WLEX intervention ($P<0.05$; Table 2).

Abnormalities of the HDL Lipidome Associated With MetS

The percentage differences in HDL lipid classes and species (normalized to total phosphatidylcholine) between healthy individuals and MetS groups were calculated (Figure 1; Tables IV and V in the online-only Data Supplement). Before intervention, 16 of 22 lipid classes and subclasses were significantly different between the MetS and healthy individuals after correction for multiple comparisons ($P<0.05$). Most sphingolipids and phospholipids were lower in the MetS group while lysoalkylphosphatidylcholine, lysophosphatidylinositol, phosphatidylglycerol, and di- and triacylglycerol lipids were significantly higher. Of 333 lipid species, 81 lipid species were significantly higher and 127 lower in the MetS group compared with healthy individuals (corrected $P<0.05$; Table V in the online-only Data Supplement).

Effect of WL and Exercise on the HDL Lipidome

Multiple lipid classes, subclasses, and species that were significantly different in the MetS patients compared with the healthy individuals were modified, toward healthy individuals, by the WL and WLEX interventions. Greater normalization was observed after the WLEX intervention compared with WL alone although this was not significant. There was normalization in the majority of surface HDL lipids with significant changes in dihexosylceramide and phosphatidylinositol (19%

Table 1. Clinical Characteristics of Study Participants

Variable	Healthy (n=40)*	Metabolic Syndrome (n=95)*	*P* Value†
Age, y	54±4	55±6	1.4E-01
Sex, males/females	19/21	56/39	4.2E-01
SBP, mm Hg	121 (118, 129)	136 (126, 148)	9.2E-05‡
BMI, kg/m^2	24 (22, 24)	32 (30, 35)	4.4E-21‡
Fasting glucose, mmol/L	4.8 (4.6, 5.3)	5.8 (5.6, 6.2)	2.7E-13‡
Triglycerides, mmol/L	0.60 (0.50, 1.00)	1.50 (1.20, 2.20)	3.8E-14‡
Total cholesterol, mmol/L	4.90 (4.59, 5.01)	5.50 (4.90, 6.20)	1.5E-05‡
HDL-C, mmol/L	1.70 (1.50, 1.90)	1.20 (1.00, 1.40)	2.2E-16‡
Non–HDL-C, mmol/L	3.15 (2.80, 3.50)	4.20 (3.80, 5.00)	1.2E-13‡
ApoA-I, g/L	1.69±0.22	1.43±0.24	6.8E-08‡

ApoA-I indicates apolipoprotein A-I; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; and SBP, systolic blood pressure.

*Values expressed as mean±SDs (normal distribution) or median (1st, 3rd quartile, non-normal distribution).

†*P* values calculated using Student *t* test (normal distribution) or Mann–Whitney *U* test (non-normal distribution) for continuous variables and χ^2 analysis for categorical variables.

‡*P*<0.05 was considered statistically significant.

and 3%, respectively, corrected *P*<0.05) after WL intervention compared with the changes observed in the control intervention (Figure 1; Table IV in the online-only Data Supplement). Corresponding changes also occurred in 34 of 333 lipid species (17 increased, 17 decreased, corrected *P*<0.05; Table V in the online-only Data Supplement). Significant elevations were observed in the di- and trihexosylceramide classes (44% and 25%, respectively, corrected *P*<0.05) after the WLEX intervention in comparison with the MetS control intervention. At the lipid species level, 40 of 333 lipids (23 increased, 17 decreased) were significantly changed after the WLEX intervention compared with changes observed in the control intervention group (corrected *P*<0.05). Although the mean % change in WLEX group was typically greater than the WL group (35 of 40 lipid species showing greater changes), there were no significant differences between the WL and WLEX groups. Analyses of the fatty acids within the phosphatidylcholine, cholesteryl ester, diacylglycerol, and triacylglycerol classes showed slight differences between MetS and control groups, but only within triacylglycerol did we observe a normalization of the saturated fatty acids in the WLEX group (Figure I in the online-only Data Supplement).

Effects of WL and Exercise on CETP Activity

CETP activity was slightly elevated in the MetS group compared with that in healthy individuals (mean±SD, 0.251±0.085 versus 0.227±0.064 pmol/μL per hour) although this was not significant. CETP activity decreased significantly after WL and WLEX interventions (*P*<0.05; Figure 2A). However, the changes in the CETP activity in the MetS control, WL, and WLEX intervention groups were not significantly different.

Effect of WL and Exercise on HDL-CEC

CEC of isolated HDL was lower in the MetS group compared with that in the healthy individuals (−13%; *P*<0.05; Figure 2B;

Table 2. Clinical Characteristics of Study Participants Before and After Weight Loss and Weight Loss Plus Exercise Interventions

		Control MetS† (n=17)		Weight Loss† (n=19)		Weight Loss Plus Exercise† (n=17)	
Variable	*P* Value*	Baseline‡	12 Wk	Baseline‡	12 Wk	Baseline‡	12 Wk
Age, y	…	55±6		55±6		54±4	
Sex, males, females	…	9/8		11/8		10/6	
SBP, mm Hg	3.2E-02*	140±15	135±18	137±18	123±17	137±16	121±16
BMI, kg/m^2	4.2E-12*	33±4	33±4	33±4	30±4§	32±4	29±4§
Weight, kg	1.0E-11*	96±17	99±17	97±13	87±8§	96±15	85±11§
Fasting insulin, mU/L	1.4E-04*	16.0±4.4	18.2±9.3	18.9±4.5	13.3±4.4§	15.5±6.3	12.6±5.8§
HOMA-IR	9.9E-05*	4.1±1.4	4.7±2.6	5.0±1.3	3.4±1.2§	4.0±1.9	3.1±1.5§
Triglycerides, mmol/L	3.5E-01	2.24±1.45	2.11±1.48	1.86±1.45	1.32±0.86	2.00±0.85	1.41±0.85
Total cholesterol, mmol/L	2.8E-04*	5.88±1.01	5.81±1.04	5.48±0.89	4.88±0.92§	5.98±1.04	5.08±1.22§
Non–HDL-C, mmol/L	1.2E-04*	4.65±0.93	4.57±1.01	4.28±0.94	3.77±0.94§	4.68±0.77	3.78±0.95§
HDL-C, mmol/L	1.6E-01	1.20±0.27	1.19±0.28	1.20±0.25	1.11±0.22	1.25±0.33	1.16±0.27
ApoA-I, g/L	4.4E-01	1.45±0.28	1.41±0.22	1.41±0.18	1.30±0.23	1.42±0.25	1.39±0.23

ApoA-I indicates apolipoprotein A-I; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment-estimated insulin resistance; MetS, metabolic syndrome; and SBP, systolic blood pressure.

**P* values from 1-way ANOVA were calculated from the delta (between baseline and 12 weeks); *P*<0.05 considered statistically significant.

†Values are means±SDs.

‡There were no significant differences of mean baseline values for all variables between the 3 groups (ANOVA).

§The change post-intervention is significantly different compared with the control group. Post hoc analysis (Student *t* test) was used to determine significance; *P* values were corrected for multiple comparisons by the method of Dunn–Sidak, *P*<0.05 considered statistically significant.

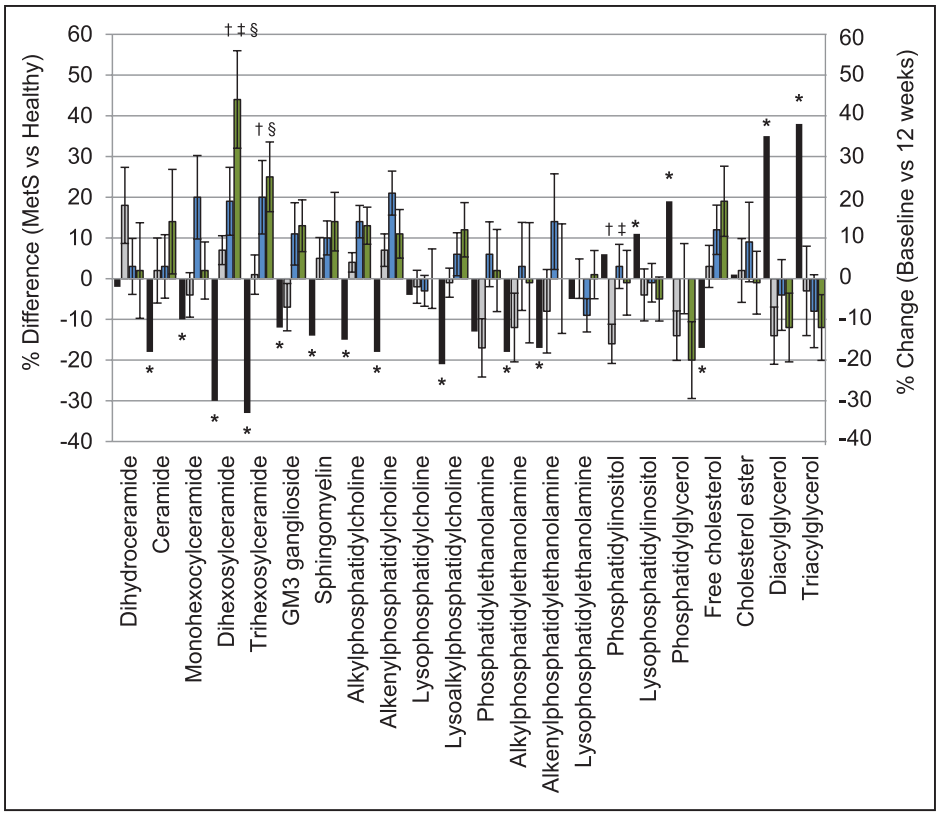

Figure 1. The effect of weight loss and weight loss plus exercise interventions on high-density lipoprotein (HDL) lipid classes in metabolic syndrome. HDL lipids were normalized to phosphatidylcholine, and the data were log transformed. The % difference of the means between the metabolic syndrome group and the healthy group was calculated (black bars), significant differences were determined using Student *t* tests and *P* values were corrected for multiple comparisons (* indicates corrected $P<0.05$). The mean % changes (±SEM) for the control (gray bars), weight loss (blue bars), and weight loss plus exercise (green bars) groups were calculated. Kruskal–Wallis analyses w performed on the delta (change between baseline and post-intervention) of lipid classes († indicated uncorrected $P<0.05$), no lipid classes reached significance after correction for multiple comparisons. Post hoc analysis (Mann–Whitney *U* test), corrected by the method of Dunn–Sidak, was performed to determine significance between groups (‡ indicates the change after the weight loss intervention was different to the change in the control group ($P<0.05$), § indicates the change after the weight loss plus exercise intervention was different to the change in the control group ($P<0.05$).

mean±SD, 5.82±1.31% versus 5.09±1.43% efflux/10 µg apoA-I). After WL and WLEX interventions, CEC was increased in the MetS groups (16% and 25%, respectively) reaching significance in the WLEX intervention only ($P<0.05$). However, the changes in the HDL-CEC in the MetS control, WL, and WLEX intervention groups were not significantly different.

Effect of WL and Exercise on HDL Particle Size

The mean diameter of HDL particles was significantly lower (−3.1%) in the MetS patients compared with healthy individuals (mean±SD, 9.84±0.14 versus 10.16±0.15 nm; $P=4.50E-22$). This lower HDL particle diameter in those with MetS was the result of substantially lower concentrations of the very large particles (−40%; $P=3.9E-22$) and large particles (−37%; $P=9.8E-14$). There was a higher concentration of small HDL particles (10%; $P=6.3E-10$) in those with MetS in comparison with healthy individuals (Table 3).

No significant change was observed in HDL diameter and particle profile after the WL intervention. The mean diameter of HDL was not significantly changed after either intervention. However, after the WLEX intervention, there was a significant decrease in the concentration of medium and small sized particles (−9.5% and −7.9%, respectively) compared with the MetS control group ($P<0.05$) and a significant reduction (−5.6%) in the total number of HDL particles after WLEX intervention compared with the change observed in the MetS control group ($P<0.05$). In addition, the decrease in the concentration of small HDL particles was also significant after the WLEX group compared with changes observed in the WL group (−7.9±1.6 versus −2.7±0.9, respectively; $P<0.05$; Table 3).

Associations Between the HDL Lipidome and Particle Size

After normalizing HDL lipids to total phosphatidylcholine, ceramide, di- and trihexosylceramide, sphingomyelin, alkyl- and alkenylphosphatidylcholine, lysoalkylphosphatidylcholine, alkyl- and alkenylphatidylethanolamine, and free cholesterol lipid classes were positively associated with HDL diameter (corrected $P<0.05$; Figure 3; Table VI in the online-only Data Supplement). In contrast, lysophosphatidylinositol and phosphatidylglycerol were negatively associated. Similarly, di- and triacylglycerol, located in the inner core of HDL, showed negative associations with HDL diameter.

Of the 333 lipid species, 209 were associated with HDL diameter (corrected $P<0.05$; Table VII in the online-only Data

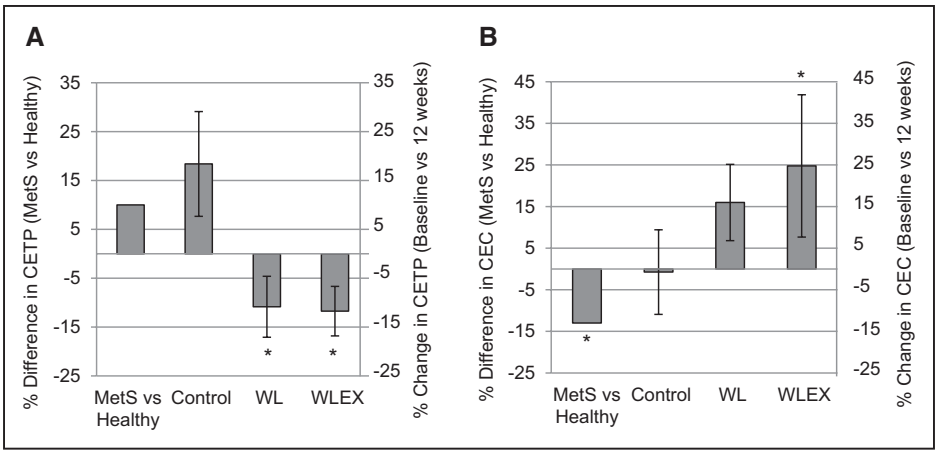

Figure 2. The effect of weight loss and weight loss plus exercise on plasma CETP (cholesteryl ester transfer protein) activity and high-density lipoprotein (HDL) cholesterol efflux capacity. Data represent the % difference of the means between the metabolic syndrome (MetS) and the healthy groups (using healthy as reference), and the mean of % changes in the control MetS, weight loss (WL), and weight loss plus exercise (WLEX) groups (baseline vs 12 wk)±SEMs for plasma CETP activity (pmol/μL per hour; **A**) and HDL cholesterol efflux capacity (%; **B**). The significance of the % difference and the mean of % changes were determined by Student *t* test and paired Student *t* test, respectively (**P*<0.05) using log-transformed data. The differences between groups (control MetS, WL, and WLEX) in the changes in the CETP activity and HDL cholesterol efflux capacity (CEC) were also assessed by Kruskal–Wallis analysis (*P*<0.05 was considered significant), and no significant difference was observed.

Supplement). In general, lipid species present on the surface of the HDL were positively correlated with HDL diameter whereas di- and triacylglycerol species present within the hydrophobic core of the particles were negatively associated.

Associations Between the HDL Lipidome and CEC

After normalization of both CEC and HDL lipids to the apoA-I concentration, di- and trihexosylceramide, sphingomyelin, phosphatidylcholine, alkyl- and alkenylphosphatidylcholine, lysophosphatidylcholine, lysoalkylphosphatidylcholine, phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylinositol, and free cholesterol lipids located on the surface of HDL were positively associated with CEC (corrected *P*<0.05; Figure 4; Table VI in the online-only Data Supplement). By contrast, di- and triacylglycerol within the HDL particles were negatively associated with CEC, but only the diacylglycerols lipid remained significant after correcting for multiple comparisons.

Of 333 lipid species, 139 lipids (phospholipids and sphingolipids) present on the surface of the HDL were positively associated with CEC while 26 species mainly from di- and triacylglycerols with saturated and mono-unsaturated fatty acids were negatively associated (corrected *P*<0.05; Table VII in the online-only Data Supplement).

Table 3. The Effect of Weight Loss and Weight Loss Plus Exercise Interventions on HDL Particle Size

		MetS vs Healthy		Effect of Treatments			
					Mean % Change§		
Parameter	Healthy*	% Difference†	*P* Value†	*P* Value‡	Control‡	Weight Loss‡	Weight Loss Plus Exercise‡
Very large HDL particles	597±136 nmol/L	−40.3†	3.9E-22†	9.4E-02	6.7±3.5	−0.9±5.3	13.3±5.8
Large HDL particles	1350±357 nmol/L	−36.8†	9.8E-14†	3.8E-01	8.7±5.1	2.8±2.3	3.9±5.3
Medium HDL particles	1903±305 nmol/L	4.5	1.4E-01	3.8E-02‡	−0.04±2.7	−2.3±2.2	−9.5±2.2‖
Small HDL particles	4403±341 nmol/L	10.1†	6.3E-10†	5.6E-03‡	−0.8±1.5	−2.7±0.9	−7.9±1.6‖,¶
Total HDL (particles/mL)	4.97E15±5.54E14	−2.4	2.4E-01	4.0E-02‡	1.0±7.2	−1.6±6.9	−5.6±5.9‖
HDL diameter	10.16±0.15 nm	−3.1†	4.5E-22†	9.8E-02	0.4±0.2	0.2±0.3	0.7±0.2

HDL indicates high-density lipoprotein; and MetS, metabolic syndrome.

*Data represent mean±SD.

†% difference of the means between the MetS and the healthy individuals (healthy as a reference). Significance was determined by Student *t* test (MetS vs healthy individuals), *P*<0.05 considered statistically significant.

‡*P* values were calculated from the delta (between baseline and 12 weeks) by Kruskal–Wallis analysis; *P*<0.05 considered statistically significant. Post hoc analysis (Student *t* test) was used to determine significance; *P* values were corrected for multiple comparisons by the method of Dunn–Sidak. *P*<0.05 considered statistically significant.

§Mean of % change ± SEM (baseline vs 12 weeks, taking baseline as reference).

‖The change after intervention was statistically significant compared with the change in the control MetS group, *P*<0.05.

¶The change after intervention was statistically significant compared with the change in the weight loss group, *P*<0.05.

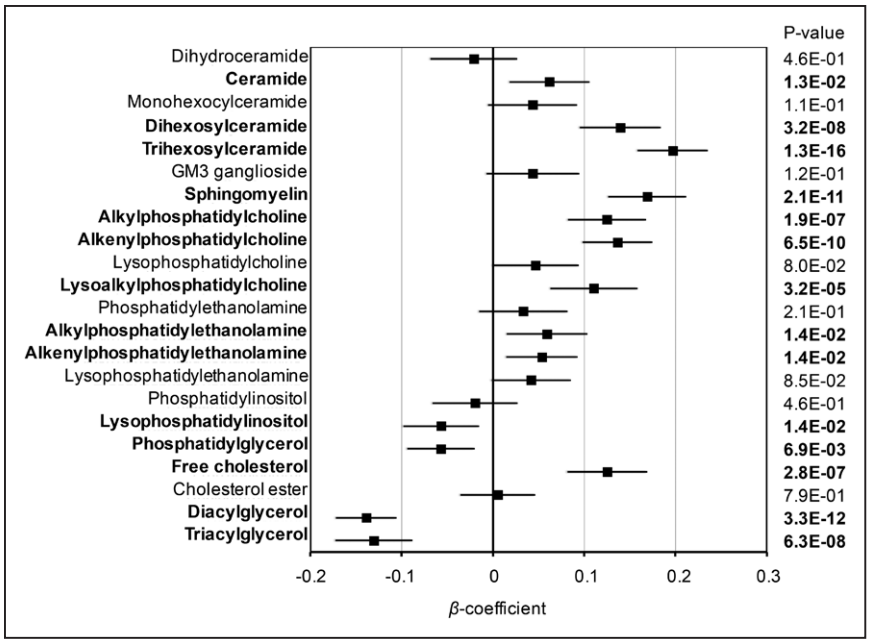

Figure 3. Association between high-density lipoprotein (HDL) lipid classes and diameter. Linear regression of the HDL lipidome normalized to phosphatidylcholine (pmol/μmol phosphatidylcholine) against HDL diameter (nm). β-coefficient (95% confidence intervals) based on an interquartile range increases in predictor HDL lipid class. The HDL lipidome data from the metabolic syndrome (pre-intervention) and the healthy individuals were included and normalized to the interquartile range for ease of interpretation. Lipids data were log transformed, and *P* values were corrected for multiple comparisons using the Benjamini–Hochberg approach. Bold type indicates significant classes (corrected $P<0.05$).

Discussion

The concept that HDL functionality is a better predictor of disease risk and potentially a better therapeutic target than HDL-C is gaining support. That HDL functionality is tightly linked to lipid composition is a logical postulate, but to date, there are limited data to support this contention. Here, we report for the first time detailed analyses of HDL lipid composition, particle size, and function in MetS and healthy groups and changes that occur after WL and WLEX interventions. We show that the change in the HDL lipidome in MetS is associated with a decrease in HDL diameter and CEC (an indicator of HDL atheroprotective function).[6] Importantly, the HDL lipidome was shifted toward the healthy individuals after both WL and WLEX interventions, with greater effects observed in the WLEX group. These changes were also combined with a shift in HDL particle size toward large particles and an improvement in HDL-CEC. These data suggest that the HDL lipidome, particle size, and function are interdependent, and the changes observed in the HDL lipidome may mediate some of the changes in HDL function[5,10] and ultimately in CVD risk reduction that has been documented to occur with WLEX.[14] The study also raises the possibility of developing alternate strategies to alter the HDL lipidome to reduce the risk associated with metabolic disease.

HDL Lipidome Differs Between Healthy and MetS Patients

Our observations of the differences in the HDL lipidome between MetS and healthy individuals are aligned with

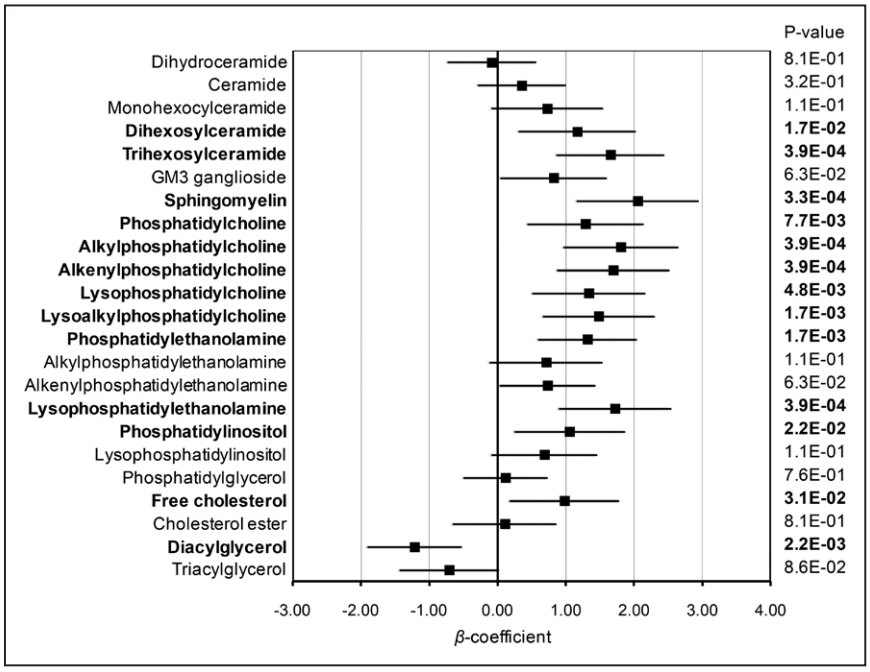

Figure 4. Association between high-density lipoprotein (HDL) lipid classes and cholesterol efflux capacity. Linear regression of the HDL lipidome (pmol/mg apolipoprotein A-I [apoA-I]) against HDL cholesterol efflux capacity (% efflux/10 μg apoA-I). β-coefficient (95% confidence intervals) based on an interquartile range increases in predictor HDL lipid class. The HDL lipidome data from the metabolic syndrome (pre-intervention) and the healthy individuals were included and normalized to the interquartile range for ease of interpretation. Lipids data were log transformed, and *P* values were corrected for multiple comparisons using the Benjamini–Hochberg approach. Bold type indicates significant association between highlighted lipid class and cholesterol efflux capacity, $P<0.05$.

previous studies examining HDL isolated from type-1[15] and type-2 diabetic patients, indicating such changes occur relatively earlier in the disease course.[16] Furthermore, the differences in the HDL lipidome in MetS, compared with the healthy group, resemble to some extent those observed in coronary artery disease (CAD),[5] as well as to our own previous studies on whole plasma from diabetic and prediabetic patients.[17] After normalization to phosphatidylcholine, the MetS individuals displayed a lower level of other lipids present in the HDL surface, including ether phospholipids (alkyl- and alkenylphosphatidylcholine and alkyl- and alkenylphosphatidylethanolamine), sphingolipids (ceramide, sphingomyelin, and glycosphingolipids), and an elevated level of di- and triacylglycerol present within the hydrophobic core of HDL. Denimal et al[18] recently reported lower proportions of plasmalogens (alkenylphosphatidylethanolamine and alkenylphosphatidylcholine) and sphingomyelin (relative to total phospholipids and sphingolipids) in HDL2 and HDL3 isolated from MetS patients compared with levels in healthy individuals. Lower levels of HDL sphingomyelin and elevated levels of triacylglycerol have also been reported in individuals with low levels of HDL-C compared with individuals with high levels of HDL-C.[19] Moreover, the lower level of HDL ether phospholipids (60% of plasma ether phospholipids are present in HDL particles[7]) may result from the higher oxidative stress in the MetS patient because the vinyl ether linkage in the ether phospholipids is susceptible to oxidation.[17]

Likely contributors to the altered HDL lipidome are enzyme activity changes characteristic of MetS, including lower activity of lipoprotein lipase and hepatic lipase or an increase in lecithin–cholesterol acyltransferase (LCAT) activity.[7] Higher concentrations of triacylglycerol-rich lipoproteins (within very-low-density lipoprotein and low-density lipoprotein [LDL]) in MetS contribute to heightened CETP activity. This drives exchange of triglyceride with cholesterol ester from apolipoprotein B–containing lipoproteins to HDL particles resulting in triacylglycerol enrichment. These triglyceride-enriched HDL particles are rapidly cleared from circulation, leading to an overall reduction in the average HDL diameter and changes in the HDL lipidome, surface charge, and biological properties.[20] The accelerated catabolism and slow maturation of HDL eventually result in lower HDL-C levels and decreased function.

HDL Lipidome Is Normalized After WL and WLEX

We observed a striking global normalization of the HDL lipidome (toward healthy individuals) after WL and WLEX intervention, with stronger effects observed after WLEX. Many of the lipid concentrations altered by the interventions also correlated with HDL diameter and CEC, suggesting a direct or indirect link between the observed changes in the HDL lipidome and functional improvement after WL and WLEX interventions. For example, di- and trihexosylceramide were lower in MetS patients compared with healthy individuals and increased after WL and WLEX interventions. These lipids were positively associated with HDL diameter and CEC, supporting a structure–function link. In addition, because HDL has a smaller proportion of di- and trihexosylceramide compared with LDL, the increase in these HDL lipids after WLEX may possibly indicate higher transfer of surface lipids or oxidized lipid in particular from LDL to HDL.[21] In a similar fashion, elevation of the negatively charged HDL lipid, phosphatidylinositol after WL treatment was also associated with an increase in HDL-mediated CEC, as described above.

We showed that CEC was lower in the MetS patients compared with healthy individuals, an observation that is likely linked to accelerated HDL metabolism and altered lipid composition. Contradictory associations of CEC with MetS and diabetes mellitus have been previously reported,[22] possibly because of the lack of a standardized assay procedure, the use of different sample types (plasma versus isolated HDL), and variations in data handling (normalization to apoA-I).[23] A further relevant factor seems to be the presence of insulin resistance; in a subgroup of the present participants, Nestel et al[24] found that those who were insulin resistant but not those who were insulin sensitive showed increased CEC in the presence of whole plasma. The elevated plasma CEC can be explained by elevated levels of apolipoprotein B lipoproteins or increased LCAT and CETP activities in the plasma.

HDL-CEC Is Improved After WLEX

Consistent with our finding, Koba et al[14] have recently reported higher CEC, measured using apolipoprotein B–depleted serum, in patients with acute coronary syndrome who have exercised for 6 months compared with nontrained controls. The improvement in CEC after WLEX treatment may relate directly or indirectly to normalization of both the HDL lipidome and HDL catabolism. The decreased CETP activity and improvement of HDL particle size profile (discussed below) might also contribute to the improvement in CEC because large HDL particles may indicate longer HDL half-life (or decelerated catabolism) compared with smaller HDL particles.

Furthermore, HDL surface lipids mediate HDL binding to proteins, enzymes, and receptors, which will eventually impact on HDL metabolism and functionality. For example, CETP has been found to bind to HDL via hydrophobic interactions rather than protein–protein mechanisms.[25]

The exact mechanisms by which the WL and WLEX interventions are able to influence the HDL lipidome and thereby HDL functionality are not fully defined. However, the reduced calorie intake (low-fat diet) and the greater oxidation of fatty acids in muscle during exercise are likely to result in modulation of specific lipid metabolic pathways leading to initial changes. Similarly, the reduced activity of CETP[26] and hepatic lipase[27] and increased lipoprotein lipase,[28] LCAT,[29] and PON1 (paraoxonase 1)[30] are likely contributors to the altered HDL metabolism and ultimately to the HDL lipidome.

HDL Diameter Is Decreased in MetS and Normalized by WLEX

Compared with the healthy individuals, the HDL diameter was shifted in the MetS patients toward smaller particles, and particle size increased in the WLEX group. Du et al[8] have demonstrated that small, dense HDL subfractions were the most efficient mediators of cholesterol efflux from ABCA1 (ATP-binding cassette subfamily A member 1) while Lucero et al[22] also observed increased efflux capacity in individuals with MetS, linked to increased pre–β-1 HDL. Here, we see

the opposite effect with the larger HDL particles in the healthy group or after WL and exercise providing the most efficient CEC. The answer to this apparent contradiction may lie in the altered HDL lipidome in MetS counteracting any benefits of the smaller HDL particles on CEC. Tan et al[31] found, in diabetic subjects, that CEC to macrophages in the presence of apolipoprotein B lipoprotein-depleted plasma was correlated with the concentration of total and medium-sized HDL and not with that of the smallest particles, further supporting a different relationship between HDL particle size and CEC in patients with an altered HDL lipidome.

Our study demonstrated a strong association between specific HDL surface phospholipids and sphingolipids (polar lipids) and CEC and an inverse association with the di- and triacylglycerol lipids in the hydrophobic core. Similar associations between the HDL phospholipids and CEC have been reported.[5] Reduced levels of HDL phospholipids and sphingomyelin with elevated levels of triacylglycerol were identified as the primary lipids associated with HDL dysfunctionality and CVD.[32,33] The associations between the HDL lipidome and particle size were similar, thereby providing a potential mechanistic link between the HDL lipidome, particle size, and CEC.

The smaller HDL particle size in the MetS participants may reflect not only an altered HDL lipidome but also an accelerated metabolism and clearance of larger HDL particles from the circulation (potentially also driven by the altered HDL lipidome) and ultimately a greater CVD risk.[8,34] Similar observations were reported in obese MetS patients with insulin resistance compared with healthy obese individuals.[35] Würtz et al[36] also reported strong inverse associations of the concentration of large particles and HDL diameter with body mass index in adolescents and young adults. In our study, the concentrations of very large HDL particles increased (not significant), in parallel with a significant reduction in the concentration of medium and small HDL particles leading to an overall shift in HDL diameter toward larger particles after WLEX treatment. This supports a recent meta-analysis, where exercise intervention was positively associated with HDL diameter and the concentration of large particles.[37] WL has also been positively associated with HDL diameter and the concentration of large HDL particles.[38]

HDL Lipid Composition Influences Function

Sphingomyelin was positively associated with CEC. Sphingomyelin strongly impacts on the fluidity of HDL surface lipids, which is inversely correlated with the ratio of sphingomyelin to phosphatidylcholine.[9] Reduced levels of HDL sphingomyelin could contribute to higher hepatic lipase[39] and LCAT[40] activities leading to accelerated HDL catabolism, a characteristic of MetS. Previous studies have shown a strong association between low levels of HDL sphingomyelin and CAD[41] thought to be because of poor CEC. Furthermore, reconstituted HDL enriched with sphingomyelin was associated with a 20% increase in CEC as compared with HDL enriched with phosphatidylcholine,[42] which may relate to the high affinity of sphingomyelin for free cholesterol.[7] We also observed di- and trihexosylceramide associated with CEC. We have previously reported an inverse association between plasma dihexosylceramide and plasma glucose level and a negative association between di- and trihexosylceramide species with diabetes mellitus and pre-diabetes.[17] However, it is important to note that HDL contains only a small proportion of plasma glycosphingolipids with the majority carried by LDL.[7] Although the biological significance of HDL glycosphingolipids is still unclear, these lipids could represent specific biomarkers for HDL functionality at the early stages of metabolic disease.

Ether phospholipids (alkyl- and alkenylphosphatidylcholine) were positively associated with CEC. Alkenylphosphatidylcholine (plasmalogen) has antioxidative properties.[43] We have previously reported on the negative association of plasma alkyl- and alkenylphosphatidylcholine with T2D[17] and CAD[44] while Sutter et al[45] also reported an inverse association of HDL plasmalogens with future CAD and HDL antiapoptotic capacity. We recently reported that the modulation of plasma plasmalogen levels in an apolipoprotein E mouse model of atherosclerosis attenuated the development of atherosclerosis,[43] which may at least partially relate to HDL functional improvement.

We observed a positive association between CEC and lysophosphatidylcholine, lysoalkylphosphatidylcholine, and lysophosphatidylethanolamine. Lysophosphatidylcholine is enriched in small, dense HDL[46] and synthesized from the hydrolysis of phosphatidylcholine via LCAT and hepatic lipase. Lysoalkylphosphatidylcholine, also known as lyso-platelet-activating factor, is synthesized by the action of platelet-activating factor-acetyl hydrolase, alternatively known as lipoprotein phospholipase A2, on alkylphosphatidylcholine and platelet-activating factor. Although serum platelet-activating factor-acetyl hydrolase/lipoprotein phospholipase A2 activity, which is predominant in oxidized LDL, is known to be elevated in MetS, T2D, and type-1 diabetes mellitus patients and associated directly with CAD, HDL-associated platelet-activating factor-acetyl hydrolase/lipoprotein phospholipase A2 activity has been reported to be lower in MetS and T2D patients compared with healthy individuals.[47]

In the current study, a positive correlation between CEC and phosphatidylinositol, a minor negatively charged lipid in HDL, was identified. High levels of HDL phosphatidylinositol can contribute to the ability of HDL to uptake cellular cholesterol by improving HDL interaction with scavenger receptor class B member 1[9] and ultimately the amount of cholesterol efflux mediated by scavenger receptor class B member 1. Consistent with our findings, a direct link between HDL negatively charged lipids and HDL function has been reported previously.[46]

Limitations

The main limitation of this study was the small sample size, and larger numbers may have strengthened trends found with the present cohort. In addition, despite the interventional nature of the study with comprehensive clinically characterized, and mean age- and sex ratio-alignment of the participants, the associations observed between HDL structure/function and the lipidome are observational in nature and cannot be used to prove causality. Future biochemical studies enriching HDL with specific lipids will be necessary to fully assess causality and mechanisms of action.

Conclusion

The current study has provided insight about the modifications to HDL induced by WL and exercise interventions. Our results suggest that alterations in lipid metabolism and subsequently the lipid composition of HDL particles may contribute to their dysfunction and heightened cardiometabolic risk in MetS. The HDL lipidome, particle size CETP activity, and CEC in the MetS patients were normalized toward healthy levels after 12 weeks of WL and WLEX, with a trend to greater effects observed after WLEX, suggesting an attenuation of cardiovascular risk.

The assessment of HDL structure–function relationships may identify specific lipid biomarkers as surrogate measures of HDL metabolism and function and identify novel targets for future HDL-based therapies.

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Disclosures

None.

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Highlights

- The high-density lipoprotein lipidome in metabolic syndrome patients was substantially different from that in healthy individuals, and mean particle size was smaller and cholesterol efflux capacity was lower.
- The high-density lipoprotein lipidome and particle size were modified toward the healthy individuals after weight loss and weight loss plus exercise treatments.
- Cholesteryl ester transfer protein activity was reduced after weight loss and weight loss plus exercise, and cholesterol efflux capacity was improved significantly after the weight loss plus exercise.
- High-density lipoprotein lipids associated with diminished cholesterol efflux capacity may represent novel biomarkers for early prediction of high-density lipoprotein dysfunctionality and disease risk.