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Lipidomics of Plasma High-Density Lipoprotein: Insights into Anti-Atherogenic Function

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Abstract

Low concentrations of high-density lipoprotein-cholesterol (HDL-C) represent a strong, independent risk factor for cardiovascular (CV) disease and atherosclerosis. The association between HDL-C and CV risk is thought to reflect multiple atheroprotective properties of HDL particles. The multiple biological functions of HDL particles are directly related to the presence of key bioactive lipid and protein components. Modern LC/MS/MS lipidomic approaches can be particularly useful to provide insights into molecular determinants of atheroprotective function of HDL. First comprehensive lipidomic studies of HDL particles in healthy subjects and in patients with CV disease or CV risk factors performed using modern lipidomic approaches have provided initial insights into lipid species profiles of human plasma HDL in health and disease. Such structure-function analyses of HDL bear the potential to identify clinically relevant, atheroprotective HDL components, which can contribute to the development of HDL-based therapies specifically designed to target beneficial subspecies of circulating HDL pool. Furthermore, HDL lipidomics can help identify novel biomarkers of HDL function, which may prove useful as biomarkers of cardiovascular risk superior to HDL-C levels.

Low concentrations of high-density lipoprotein-cholesterol (HDL-C) represent a strong, independent risk factor for cardiovascular (CV) disease and atherosclerosis [1]. The association between HDL-C and CV risk is thought to reflect multiple atheroprotective properties of HDL particles. Indeed, HDL can efflux cholesterol from lipid-loaded macrophages, reduce proinflammatory responses, decrease oxidative stress, attenuate cellular apoptosis, diminish platelet activation, improve beta-cell function and enhance vasodilation [2-6]. The multiple biological functions of HDL particles are directly related to the presence of key bioactive lipid and protein components [7]. The HDL lipidome is dominated by phospholipids (PLs) that contribute to 20-25% of total HDL mass, followed by cholesteryl esters (CEs; 14-18 wt %), sphingolipids (SLs; 3-4 wt %), triglycerides (TGs; 3-6 wt %) and free cholesterol (FC; 3-5 wt %) [8]. Phosphatidylcholine (PC) represents the major subclass of HDL PLs (15-18 wt %) followed by lysophosphatidylcholine (LPC; up to 3 wt %) and plasmalogens (1-2.5 wt %) [9-11]. In addition, HDL contains several low-abundance PLs (<1 wt %), including phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidic acid (PA) and cardiolipin [10-15]. Among SLs, sphingomyelin (SM) clearly prevails, providing >90% of the total mass of this subclass.

HDL particles feature a high level of structural, compositional and functional heterogeneity, differing in physical (shape, size, density, electrophoretic mobility) and biological properties. Interestingly, small, dense HDL3, which feature distinct structure [16,17] and are enriched in several bioactive lipids and proteins [10,18] display enhanced capacity to efflux cellular cholesterol via the ATP-binding cassette transporter A1 (ABCA1) [19,20], to reduce apoptosis in endothelial cells [21] and to protect low-density lipoprotein (LDL) from oxidative stress [22]. The content of major lipid classes per HDL particle typically diminishes with progressive increase in HDL density, reflecting depletion of total lipid relative to protein components [10]. By contrast, the proportions of most lipid classes relative to total lipid content remain relatively constant across plasma HDL subspecies [10]. Two remarkable exceptions from this rule are represented by SM and sphingosine-1-phosphate (S1P). Thus, HDL % content of SM is reduced in small, dense HDL, whereas that of S1P, a minor bioactive lipid, is elevated [10]. These data suggest that heterogeneity in HDL composition can impact biological function of HDL.

Modern LC/MS/MS lipidomic approaches can be particularly useful to provide insights into molecular determinants of atheroprotective function of HDL. The power of lipidomics results from the ability to provide quantitative data on individual lipid species, including low abundance molecules. Reference values for the lipidome of total HDL isolated from healthy normo lipidemic controls by FPLC were provided by Wiesner and colleagues [11]. More recently, we have characterized the heterogeneity of the HDL lipidome and reported the content of >160 species of PLs and SLs in five major HDL subpopulations isolated from healthy normo lipidemic subjects [23]. We found that when data were expressed relative to total lipid, the contents of LPC and of negatively-charged PS and PA increased progressively with increase in hydrated density of HDL, whereas the proportions of SM and ceramide decreased. When key biological activities of HDL, notably cholesterol efflux capacity from human THP-1 macrophages, antioxidative activity towards LDL oxidation, anti-thrombotic activity in human platelets, cell-free anti-inflammatory activity and anti-apoptotic activity in endothelial cells, were assessed, all of them were predominantly associated with small, dense, protein-rich HDL3. The biological activities of HDL particles were strongly intercorrelated, exhibiting significant correlations with multiple components of the HDL phosphosphingolipidome. Specifically, the content of PS revealed positive correlations with all metrics of HDL functionality, reflecting enrichment of PS in small, dense HDL3. Our structure-function analysis thereby suggested that the HDL lipidome, and primarily negatively-charged PS, may strongly impact atheroprotective HDL function. In order to obtain direct data on the role of PS for HDL function, HDL was enriched with PS in vitro; cholesterol efflux capacity of such PS-

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enriched HDL was elevated relative to control HDL, consistent with the functional role of PS [23]. Alternatively, elevated content of PS in small, dense HDL may serve as a biomarker of the presence in this subpopulation of multiple proteins with diverse biological functions [18].

Biological activities of HDL can be attenuated in patients with CV risk factors, potentially contributing to atherogenesis [2-5]. Such defective HDL functionality may reflect alterations in the composition and structure of HDL, primarily in the proteome and lipidome of this lipoprotein. Subnormal cholesterol content in HDL measured as reduced plasma levels of HDL-C is the most known example of such compositional alterations. To characterize the relationships between HDL lipidome and atherosclerosis, we evaluated the content of >160 molecular species of PLs and SLs in patients with CV disease as well as several other conditions associated with elevated CV risk.

First, the relationships between modifications in the molecular composition and the functionality of HDL subpopulations were assessed in patients with ST segment elevation myocardial infarction (STEMI) who were recruited within 24h after diagnosis (n=16) and featured low HDL-C (-31%, $p < 0.05$) and acute-phase inflammation (determined as marked elevations in C-reactive protein, serum amyloid A (SAA) and interleukin-6) [24]. STEMI plasma HDL and its subpopulations (HDL2b, 2a, 3a, 3b, 3c) displayed attenuated cholesterol efflux capacity from macrophagic lipid-loaded THP-1 cells (up to -32%, $p < 0.01$, on a unit PL mass basis) as compared to age- and sex-matched controls (n=10). In addition, plasma HDL and small, dense HDL3b and 3c subpopulations from STEMI patients exhibited reduced antioxidative activity (up to -68%, $p < 0.05$, on a unit HDL mass basis). In parallel, HDL lipidome was markedly affected by STEMI. Enrichment in two pro-inflammatory bioactive lipids, LPC (up to 3.0-fold, $p < 0.05$) and PA (up to 8.4-fold, $p < 0.05$), was the most systematic alteration observed across several HDL sub-populations (Figure 1). Furthermore, STEMI HDL was depleted in apolipoprotein A-I (apoA-I), the major HDL protein (up to -23%, $p < 0.05$) and enriched in SAA (up to +10.2-fold, $p < 0.05$). ApoA-I accounts for up to 70% of total HDL protein and provides principal contribution to virtually all known metrics of HDL functionality, including cholesterol efflux capacity together with antioxidative, anti-inflammatory, and immunomodulatory activities. It is therefore plausible that defective HDL functionality observed in STEMI was, at least in part, derived from the reduced content of apoA-I. Importantly however, in vitro HDL enrichment in both LPC and PA exerted deleterious effects on HDL functionality. These data document that in the early phase of STEMI, HDL subpopulations display concomitant alterations in both lipidome and proteome, which can lead to impaired HDL functionality. Such modifications may act synergistically to confer deleterious biological activities to STEMI HDL.

In another study, functional and compositional properties of HDL were evaluated in subjects from a kindred of genetic apoA-I deficiency (two homozygotes and six heterozygotes) with a nonsense mutation at APOA1 codon -2 known to be associated with increased CV risk [25]. The homozygotes displayed markedly reduced plasma levels of HDL-C (-95%) relative to age- and sex matched healthy controls (n=11); plasma apoA-I was undetectable. The heterozygotes displayed low HDL-C (21 ± 9 mg/dl), low apoA-I (79 ± 24 mg/dl), normal LDL-cholesterol (132 ± 25 mg/dl) and elevated TG (130 ± 45 mg/dl) levels. Cholesterol efflux capacity of HDL subpopulations was reduced (up to -25%, $p < 0.01$, on a PL basis) in heterozygotes vs. controls. Small, dense HDL3 and total HDL from heterozygotes exhibited diminished antioxidative activity (up to -48%, $p < 0.001$ on a total mass basis) relative to controls. In parallel, HDL subpopulations from both homozygotes and heterozygotes displayed altered chemical composition, with depletion in apoA-I, PL and CE, enrichment in apoA-II, FC and TG, and altered phosphosphingolipidome. HDL subpopulations isolated from heterozygous patients showed enrichment in products of PL hydrolysis, LPC (up to +310%, $p < 0.001$, in HDL3b) and PA (up to +786%, $p < 0.05$, in HDL2b), as well as in negatively charged lipids PI (up to +47%, $p < 0.05$, in HDL3a), PS (up to +426%, $p < 0.05$, in HDL3c) and PG (up to +512%, $p < 0.001$, in HDL2a) and in ceramide (up to +142%, $p < 0.001$, in HDL2b), In addition, HDL subpopulations were depleted in PC (up to -7%, $p < 0.05$, in HDL2b) (Figure 1). The defective atheroprotective activities of HDL were correlated with altered lipid and apolipoprotein composition. These data show that atheroprotective activities of HDL particles are impaired in homozygous and heterozygous apoA-I deficiency and are intimately related to marked alterations in protein and lipid composition.

Finally, we studied the relationship between HDL composition and function in normolipidemic female patients with active rheumatoid arthritis (RA; n=12), a well-established CV risk factor, as compared to normolipidemic age-matched controls (n=10) [26]. Active RA patients exhibited a wide range of plasma high-sensitivity C-reactive protein (hsCRP) levels, which were elevated relative to controls (8.4[3.5-11.4] vs. 0.47[0.30-0.89] mg/l, $p < 0.001$). The lipidome of HDL3c particles was significantly modified in RA patients despite normal HDL-C levels, with depletion of PL (19 ± 2 vs. 21 ± 2 wt %; $p < 0.05$) and of FC (1.9 ± 0.4 vs. 2.2 ± 0.4 wt %; $p < 0.05$) relative to normolipidemic HDL3c and also showed specific alterations of the content of negatively-charged phospholipids including PI (-23%, $p < 0.05$), PG (-50%, $p < 0.05$) and PA (+137%, $p < 0.05$) (Figure 1). By contrast, antioxidative activity of small, dense HDL did not differ between RA patients and controls ($p > 0.05$). Nonetheless, subgroup analyses revealed that RA patients featuring high levels of inflammation (sCRP >10 mg/l) possessed small, dense HDL with reduced antioxidative activities (up to -23%, $p < 0.01$). Furthermore, antioxidative activity of HDL was inversely correlated with plasma hsCRP levels ($p < 0.01$). These data suggest that functional deficiency of small, dense HDL in normolipidemic inflammatory states requires high levels of inflammation. Interestingly, an altered lipidomic profile of HDL, including diminished content of ceramides and S1P, has been recently reported in patients with Type 1 diabetes displaying normal levels of HDL-C [27]. These findings additionally emphasise limitations of HDL-C as a biomarker of altered lipoprotein metabolism.

Together, the results of our first comprehensive lipidomic studies of HDL particles in healthy subjects and in patients with CV disease or CV risk factors performed using modern lipidomic approaches have provided initial insights into the lipid species profiles of human plasma HDL in health and disease. Such structure-function analyses of HDL bear the potential to identify clinically relevant, atheroprotective HDL components, which can contribute to the development of HDL-based therapies specifically designed to target beneficial subspecies of the circulating HDL pool [28]. HDL infusion therapies potently raise circulating concentrations of HDL particles and enhance their anti-atherosclerotic functions. HDL formulations used for this approach are prepared by reconstituting apoA-I with phospholipids, most often with PC. Our data identify PS as an important functional component that can enhance antiatherosclerotic activities of HDL. These data suggest that PS-containing reconstituted HDL bears a potential to reduce atherosclerosis in man. Furthermore, HDL lipidomics can help identify novel biomarkers of HDL function, which may prove useful as biomarkers of CV risk superior to HDL-C levels [29]. Elevated HDL content of LPC and PA can be useful in this regard, providing analytical biomarkers of impaired HDL function.
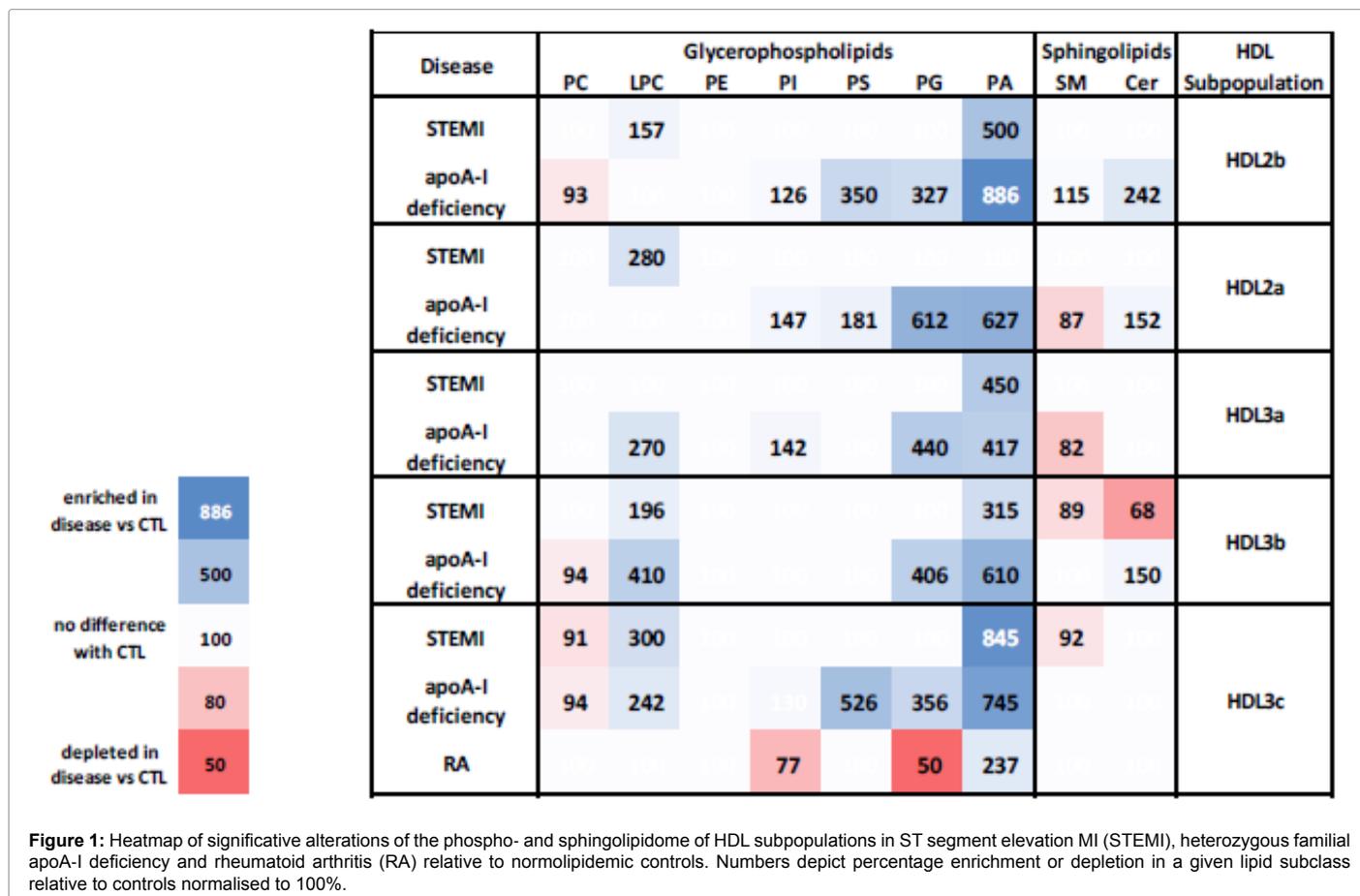
Disease	Glycerophospholipids							Sphingolipids		HDL Subpopulation
	PC	LPC	PE	PI	PS	PG	PA	SM	Cer	
STEMI		157					500			HDL2b
apoA-I deficiency	93			126	350	327	886	115	242	
STEMI		280								HDL2a
apoA-I deficiency				147	181	612	627	87	152	
STEMI							450			HDL3a
apoA-I deficiency		270		142		440	417	82		
STEMI		196					315	89	68	HDL3b
apoA-I deficiency	94	410				406	610		150	
STEMI	91	300					845	92		HDL3c
apoA-I deficiency	94	242		130	526	356	745			
RA				77		50	237			

Legend:
- enriched in disease vs CTL: 886, 500
- no difference with CTL: 100, 80
- depleted in disease vs CTL: 50

Figure 1: Heatmap of significative alterations of the phospho- and sphingolipidome of HDL subpopulations in ST segment elevation MI (STEMI), heterozygous familial apoA-I deficiency and rheumatoid arthritis (RA) relative to normolipidemic controls. Numbers depict percentage enrichment or depletion in a given lipid subclass relative to controls normalised to 100%.

We should be aware of limitations of the lipidomic approach, which primarily involve requirements for appropriate calibration for individual lipid species, adequate choice of internal standards, standardisation of lipid extraction, optimisation of chromatographic separation, correction for matrix effects, and optimal data handling and interpretation. With all the technical limitations properly addressed, analyses of the HDL lipidome will become a useful tool to improve our knowledge of CV and metabolic disorders. Contributions to the development of HDL-based therapies and biomarkers constitute potential clinical relevance of the HDL lipidomics; this statement can be considered as a take-home message of this article. Future research should expand our cross-sectional studies to prospective investigations of the relationships between the HDL lipidome and CV risk as well as to the evaluation of the effects of HDL-raising therapies.

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