Plasma high density lipoproteins (HDL) are small, dense, protein-rich particles compared with other lipoprotein classes; roughly half of total HDL mass is accounted for by lipid components. Phospholipids predominate in the HDL lipidome, accounting for 40–60% of total lipid, with lesser proportions of cholesteryl esters (30–40%), triglycerides (5–12%), and free cholesterol (5–10%). Lipidomic approaches have provided initial insights into the HDL lipidome with identification of over 200 individual molecular lipid species in normolipidemic HDL. Plasma HDL particles, however, reveal high levels of structural, compositional, and functional heterogeneity. Establishing direct relationships between HDL structure, composition, and atheroprotective functions bears the potential to identify clinically relevant HDL subpopulations. Furthermore, development of HDL-based therapies designed to target beneficial subpopulations within the circulating HDL pool can be facilitated using this approach. HDL lipidomics can equally contribute to the identification of biomarkers of both normal and deficient HDL functionality, which may prove useful as biomarkers of cardiovascular risk. However, numerous technical issues remain to be addressed in order to make such developments possible.

With all technical questions resolved, quantitative analysis of the molecular components of the HDL lipidome will contribute to expand our knowledge of cardiovascular and metabolic diseases.—Kontush, A., M. Lhomme, and M. J. Chapman. Unraveling the complexities of the HDL lipidome. J. Lipid Res. 2013. 54: 2950–2963.

High density lipoproteins (HDL) are small, dense, protein-rich particles compared with other plasma lipoprotein classes. Despite the elevated abundance of multiple structural and functional proteins in HDL particles (see other reviews in this Thematic Series), roughly half of total HDL mass is accounted for by lipid components.

For many years, HDL lipids were predominantly characterized in terms of the content of their major classes, notably phospholipids, unesterified (free) sterols (predominantly cholesterol), cholesteryl esters, and triglycerides. Cholesterol is the most characteristic component of the HDL lipidome as, in the form of HDL-cholesterol, it represents a major independent negative risk factor for cardiovascular disease (see other reviews in this Thematic Series). It is not cholesterol, however, but phospholipids that quantitatively predominate in the HDL lipidome, accounting, together with sphingomyelin (SM), for 40–60 wt% of total lipid, with lesser proportions of cholesteryl esters (30–40%), triglycerides (5–12%), and free cholesterol (5–10%). Structurally, individual HDL lipid classes fulfill distinct functions; phospholipids constitute the surface lipid monolayer of HDL, whereas cholesteryl esters and triglycerides form the hydrophobic lipid core. Unesterified sterols are predominantly located to the surface monolayer, partially penetrating the core.

Recent technological advances in mass spectrometry (MS) have enabled application of this powerful technique to provide a detailed identification and quantification of individual molecular species of lipids in the framework of the field known as lipidomics. Application of this approach to plasma lipoproteins has revealed complex profiles consisting of hundreds of molecular lipid species that have been quantified in major lipoprotein classes, including HDL (reviewed in Refs. 1–3). Our current knowledge of the HDL lipidome involves more than 200 individual molecular lipids species.
identified by recent lipidomic analyses (4, 5); this number is primarily limited by the sensitivity of the available technologies and will inevitably expand in the near future (6).

In this article, we will review data on the composition and functional significance of the HDL lipidome in health and disease, focusing on both traditional measurements of its content of major lipid classes and on modern lipidomic approaches to quantify individual lipid species.

MAJOR LIPID CLASSES PRESENT IN HDL

Phospholipids

Phospholipids represent the major component of the HDL lipidome, accounting for approximately half of all lipids on a weight basis (Table 1). Phosphatidylcholine (PC) predominates as the principal molecular class of phospholipids in HDL. In addition, HDL contains smaller but still significant amounts (>1 wt% of total HDL lipids) of lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and plasmalogens (4, 7, 8) (Table 1). Minor HDL phospholipids (<1 wt% of total HDL lipids) are represented by phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidic acid (PA) and cardiolipin (5, 9, 10).

Phospholipids are unequally distributed in the circulation across lipoproteins. As a result of enrichment in phospholipids, HDL represents a major carrier of PC, LPC, PE, and PE-derived plasmalogens, containing over 50% of each of these lipid classes present in human serum (4).

Phosphatidylcholine. PC is the key structural phospholipid of cell membranes and lipoproteins, and represents the principal plasma phospholipid that accounts for 33–45 wt% of total lipid in HDL (4) (Table 1). The 16:0/18:2, 18:0/18:2, 16:0/20:4, and 16:0/18:1 species constitute major molecular species of HDL PC (4, 9, 11–13). Relative to other lipoproteins, HDL is enriched in PC containing polyunsaturated fatty acid (PUFA) moieties (4). HDL PC can be of both hepatic (via formation of nascent lipoprotein particles) and extrahepatic [via the actions of phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP)] origin.

Lysophosphatidylcholine. LPC, which is the product of PC hydrolysis in the lecithin:cholesterol acyltransferase (LCAT) reaction, constitutes a quantitatively important subclass of phospholipids in HDL [up to 15 wt% in HDL isolated by fast performance liquid chromatography (FPLC)] compared with apolipoprotein (apo)B-containing lipoproteins (2–3 wt%) (4, 11) (Table 1). The preferential association of LPC with HDL is consistent with the predominant occurrence of the LCAT reaction in this lipoprotein (7). As considerable amounts of serum LPC are also associated with albumin (4), HDL contamination by the latter as typically occurs upon FPLC isolation can overestimate the amount of this lipid in the HDL fraction isolated by this approach.

Phosphatidylethanolamine. PE is another important structural phospholipid that accounts for approximately 1 wt% of total HDL lipid (4) (Table 1). Principal plasma PEs are represented by the 36:2 and 38:4 species, which are evenly distributed across VLDL, LDL, and HDL (7).

Phosphatidylethanolamine plasmalogens. PE plasmalogens are minor plasma phospholipids with antioxidative properties (15–17) present in HDL at approximately 1 wt% of total HDL lipids (4).

Sphingolipids

Sphingomyelin. Sphingomyelin constitutes 5–10 wt% of HDL lipids, including the 18:1/16:0, 18:2/24:0, 18:1/24:1, 18:2/22:0, 18:1/22:1 species (4, 7, 38).

Cardiolipin. Cardiolipin constitutes approximately 0.2 wt% of total HDL lipids (10).

Steroids

Cholesterol. Cholesterol accounts for 30–40 wt% of total HDL lipids (36, 37).

Triacylglycerides

18:1/16:0/18:1, 18:2/16:0/18:1 (5, 38).

Cholesteryl ester

18:1/16:0/18:1, 18:2/16:0/18:1 (7, 38).

TABLE 1. Major components of the HDL lipidome

| Lipid Class          | HDL Content<sup>a</sup> (wt% of total HDL lipid) | Major Subspecies                                | Refs. |
|----------------------|-------------------------------------------------|-------------------------------------------------|-------|
| Phospholipids        | 35–50                                           | Phosphatidylcholine                             | (4, 7, 38) |
| Phosphatidylcholine  | 33–45                                           | 16:0/18:2, 18:0/18:2, 16:0/20:4, 16:0/18:1      | (4, 7, 38) |
| Lyso phosphatidylcholine | 0.5–5                                          | 16:0, 18:0, 18:1, 18:2                          | (4, 7, 39) |
| Phosphatidylethanolamine | 0.5–1.5                                        | 16:0/18:2, 18:0/18:2, 18:0/20:4                 | (4, 7, 38) |
| Phosphatidylinositol | 0.5–1.5                                         | 18:0/20:3, 18:0/20:4                            | (9, 13, 38) |
| Plasmalogens         | 0.5–1.5                                         | 18:0/20:4, 16:0/20:4, 18:1/20:4, 18:0/22:6      | (4)   |
| Phosphatidylserine   | 0.02–0.04                                       | 16:0/18:0                                      | (5, 10, 38) |
| Phosphatidylglycerol | ND                                              | 18:1/20:2                                      | (9)   |
| Phosphatic acid      | ND                                              | 20:0/20:4, 20:2/20:4, 18:2/20:1                 | (9)   |
| Cardiolipin          | 0.2                                             |                                                 | (10)  |
| Sphingolipids        | 5–10                                            | Sphingomyelin                                   | (4, 7, 38) |
| Sphingomyelin        | 5–10                                            | 18:1/16:0, 18:2/24:0, 18:1/24:1, 18:2/22:0, 18:1/22:1 | (4, 7, 38) |
| Ceramide             | 0.05                                            | 24:0, 24:1, 25:0, 22:0, 16:0                    | (4)   |
| S1P                  | 0.01–0.02                                       |                                                 | (7)   |
| Sphin gly coplcholine| 0.0005                                          |                                                 | (30)  |
| Steroids             | 5–10                                            | Cholesterol                                    | (36, 37) |
| Triacylglycerides    | 5–12                                            | 18:1/16:0/18:1, 18:2/16:0/18:1                  | (5, 38) |
| Cholesteryl ester    | 30–40                                           | Cholesteryl linolate                            | (7, 38) |

<sup>a</sup>Depending on the specific HDL subpopulation, lipids constitute from 30% to 70% of total HDL mass (145).
lipid (4) (Table 1). Species containing arachidonic acid residues predominate; interestingly, HDL is enriched in the 20:4 species relative to VLDL and LDL, in parallel to the depletion of species containing 18:1 and 18:2 residues.

Phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, and phosphatidic acid. PI, PS, PG, and PA are negatively charged phospholipids that are present in lipoproteins in low amounts (Table 1). These lipids may significantly impact on the net surface charge of HDL (9, 18, 19), thereby modulating charge-dependent interactions with lipases, lipid transfer proteins, extracellular matrix, and other protein components. Major molecular species of these lipids in HDL remain to be reported.

Cardiolipin. Cardiolipin is a minor anionic phospholipid with potent anticoagulant properties that is equally present in trace amounts in HDL (10) (Table 1).

Isoprostane-containing PCs. Isoprostane-containing PCs are stable, final products of nonenzymatic oxidation of PCs containing PUFA moieties. High stability of these lipids relative to the intermediate products of lipid peroxidation renders them useful biomarkers of oxidative stress (20). Isoprostane-containing PCs are present in the circulation at very low (nanomolar) concentrations and are largely associated with HDL (21). Major molecular species of isoprostane-containing PCs in the circulation include 5,6-epoxy-isoprostaglandine A_2-PC (EIPGA_2-PC) (36:3); 5,6-EIPGE_2-PC (36:4); IPGE_2/D-2-PC (36:4); IPGF-PC (36:4); IPGE_2/D-2-PC (38:4); and IPGF-PC (38:4) (13).

Sphingolipids
Sphingomyelin (SM) represents the major sphingolipid class in HDL, followed by ceramide and lysosphingolipids (Table 1). As SM contains a phosphate group in addition to the sphingosine residue, it is often considered a phospholipid. However, the major reason for the inclusion of SM in the sphingolipid class is presumably the presence of a choline group, which forms the basis for colorimetric determination of phospholipids by commercially available kits. Indeed, such kits predominantly employ choline esterase as a key enzyme; as a result, they primarily detect total concentration of PC, LPC, and SM.

Sphingomyelin. SM accounts for 5–10 wt % of total lipid in HDL (4) (Table 1). Major molecular species of HDL SM are the d18:1/16:0 and d18:2/24:0 species (4, 7, 11). SM content constitutes a critical factor in determining surface pressure in lipid membranes and lipoproteins, including HDL, enhancing rigidity and thereby influencing activity of embedded proteins (22, 23).

SM largely originates from triglyceride-rich lipoproteins and only to a minor extent from nascent HDL (24). Contrary to phospholipids, the largest amount of SM (50 mol% of total plasma SM) is carried in the circulation by LDL (4).

Ceramide. Ceramide is present in HDL at approximately 0.05 wt% relative to total lipids (Table 1). The pattern of ceramide species is distinct in HDL relative to other lipoproteins. Thus, the percentage of the 16:0 species is almost doubled and the proportion of the 24:0 species is reduced in HDL compared with VLDL and LDL (4, 11). Similar to SM, ceramides are preferentially carried by LDL (60 mol % of total plasma ceramides) compared with HDL (25 mol %) and VLDL (15 mol %) (4). These sphingolipids play a key role as signaling molecules involved in cellular survival, growth, and differentiation.

Lysosphingolipids. HDL carries several lysosphingolipids, which represent subclasses of sphingoid bases (Table 1). Among them, sphingosine-1-phosphate (S1P) is by far the most known for its role in vascular biology. Indeed, S1P functions as a ligand for G-protein-coupled S1P receptors in endothelial and smooth muscle cells, which regulate cell proliferation, motility, apoptosis, angiogenesis, wound healing, and immune response (25). HDL is the major carrier of S1P in plasma, which ensures its bioavailability (25). Indeed, more than 90 wt% of sphingoid base phosphates, including S1P, is found primarily in HDL as well as in albumin-containing fractions by liquid chromatography-mass spectrometry (LC-MS) (8). The abundance of S1P in HDL is however low (<1 mol/mol HDL); on the average, only one of 20–30 HDL particles contains a molecule of S1P (7, 26, 27). S1P is synthesized via phosphorylation of sphingosine by sphingosine kinases, which are expressed in erythrocytes, platelets, neutrophils, and mononuclear cells. Primary sources of plasma S1P are represented by erythrocytes and platelets (28, 29).

Other biologically active lysosphingolipids carried by HDL include sphingosylphosphorylcholine (lyosphingomyelin; Table 1) and lysosulfatide (30).

Minor sphingolipids. Other minor HDL sphingolipids are represented by glycosphingolipids, gangliosides, and sulfatides (31–35); lipidomic data on molecular species of these lipids are scarce. Hexosyl and lactosyl species constitute the major glycosphingolipids in plasma lipoproteins (8). Interestingly, HDL is depleted in glycosphingolipids and gangliosides compared with LDL (31). Gangliosides determine interactions with protein receptors and signal transduction, whereas sulfatides are involved in the regulation of insulin secretion, immune response, hemostasis, thrombosis, and infection. The physiological relevance of the presence of these lipids in HDL remains indeterminate.

Steroids
HDL steroids are dominated by cholesterol (5–10 wt% of lipid; Table 1), reflecting the key role of HDL in cholesterol transport through the circulation. Other sterols are present in HDL at much lower levels as exemplified by minor amounts of oxysterols (27-hydroxycholesterol, 24-hydroxycholesterol, cholesterol-5,6-β-epoxide, and 7-keto-cholesterol) (36); estrogens (largely present as esters) (37); and phytosterols.

Together with phospholipids, steroids are located in the surface lipid monolayer of HDL particles and contribute to regulation of its fluidity.
Unraveling the complexities of the HDL lipidome

HETEROGENEITY IN LIPIDS

HDL particles are highly heterogeneous in structure, intravascular metabolism, and biological activity. From a physicochemical point of view, plasma HDL is a heterogeneous group of small discoid and spherical particles (7–12 nm diameter) that differ in density, size, and electrophoretic mobility (Fig. 1).

HDL can be fractionated by different techniques into discrete subclasses according to physicochemical properties and chemical composition. Sequential ultracentrifugation allows separation of HDL into two main subfractions on the basis of density: large, light, lipid-rich HDL2 (d 1.063–1.125 g/ml) and small, dense, protein-rich HDL3 (d 1.125–1.21 g/ml) (43). HDL2 and HDL3 can be further subfractionated on nondenaturing polyacrylamide gradient gel electrophoresis into five distinct subpopulations of decreasing size: HDL2b, HDL2a, HDL3a, HDL3b, and HDL3c (43); equivalent subpopulations of increasing density can be quantitatively isolated using isopycnic density gradient ultracentrifugation (43) (Fig. 1). Such heterogeneity of HDL particles directly reflects differences in the relative content of proteins and lipids in HDL.

Phospholipids

HDL particle content of phospholipids, expressed as a percentage of total HDL mass, decreases approximately 2-fold with increase in hydrated density from large, light HDL2b to small, dense HDL3c (7), reflecting depletion of HDL lipid relative to protein (Fig. 2A). Similar differences in the particle content of major phospholipid subclasses, including PC, PE, PI, and LPC, were observed between HDL2b and HDL3c subpopulations (Fig. 2B). HDL particle content of total fatty acids and their subclasses, including saturated, monounsaturated, and polyunsaturated n-6 and n-3 moieties, followed these profiles. However, differences between HDL subspecies are attenuated to a minor degree when data for PC, PE, PI, LPC, and total fatty acids are expressed as a percentage of total lipids (Fig. 1), suggesting that their molecular species are in dynamic equilibrium between HDL subpopulations (7). Intriguingly, our recent studies suggest that minor phospholipid classes reveal highly unsymmetrical distribution across the HDL particle spectrum (L. Camont et al., unpublished data).

Sphingolipids

Similar to phospholipids, HDL content of SM, expressed as a percentage of total mass, decreases progressively in parallel with HDL density from 8.6% in HDL2b to 2.1% in HDL3c (7) (Fig. 2B). The depletion of SM in small versus large HDL is, however, more pronounced compared with that of phospholipid; as a result, HDL content of SM, expressed as a percentage of total lipids, decreases in parallel with HDL density from 12.8% in HDL2b to 6.2% in HDL3c (7). In parallel, the PC/SM ratio is elevated from 2.6 in HDL2b to 5.6 in HDL3c (Fig. 2C).

The relative depletion of SM in small versus large HDL3c suggests that the SM pool is not in equilibrium across HDL.

Minor lipids

Minor bioactive HDL lipids include diacylglycerides, monoacylglycerides, and free fatty acids (4, 31, 32, 38, 39). Lipidomic characterization of these lipid classes in HDL remains to be performed. HDL also contains minor amounts of oxidized lipids, lipophilic vitamins, and antioxidants, such as tocopherols, carotenes, and coenzyme Q10 (40–42).

Cholesteryl esters

Cholesteryl esters (30–40 wt% of HDL lipid; Table 1) are largely (up to 80%) formed in plasma HDL as a result of the reaction of trans-esterification between phospholipids and cholesterol catalyzed by LCAT. Because cholesteryl esters are highly hydrophobic, the LCAT reaction displaces the cholesterol moiety from the surface lipid monolayer into the lipid core of HDL. Subsequently, cholesteryl esters can be transferred to apoB-containing lipoproteins by CETP in exchange for triglycerides.

Most of HDL cholesteryl ester is present in the form of cholesteryl linoleate (7).

Triglycerides

HDL triglycerides (5–12 wt% of lipid; Table 1) are derived from apoB-containing triglycercide-rich lipoproteins, primarily VLDL, as a result of CETP-mediated heteroexchange with HDL-derived cholesteryl esters. Similar to cholesteryl esters, triglycerides are highly hydrophobic and are located in the HDL lipid core. Compared with cholesteryl esters, however, triglycerides form a more fluid phase.

HDL triglycerides are dominated by species containing oleic, palmitic, and linoleic acid moieties (4). High molecular diversity of plasma triglycerides has recently been reported (6), an observation that appears to be relevant to all plasma lipoproteins, including HDL (4, 11).

Fig. 1. Physicochemical properties and lipid class composition of HDL particle subpopulations. Particle diameter is proportional to the total number of lipid molecules present. Based on data from Refs. 4, 7, 38, 113, 114, 145, and 146.
subpopulations, consistent with the slow rate of transfer of SM through the aqueous phase (24). The low SM content of small plasma HDL is further consistent with the low SM content of small nascent HDL secreted by J774 macrophages. As such particles originate from the exofacial leaflet of the plasma membrane (44), the SM/PC ratio may reflect distinct metabolic origin(s) of individual HDL subpopulations.

Lipidomic differences between HDL subpopulations equally include those in minor bioactive sphingolipids. Thus, the abundance of S1P per HDL particle is asymmetric across the HDL pool, with preferential enrichment in HDL3 (40–50 mmol/mol HDL) compared with HDL2 particles (15–20 mmol/mol) (7, 26, 27). Such enrichment of small, dense HDL3 in S1P is potentially driven by apoM, a specific carrier for S1P (45) that is predominantly associated with HDL3 (46). A specific apoA-I-containing, apoM- and S1P-enriched HDL subclass may exist within the HDL3 subfraction, which contains a molar excess of apoM over S1P (47).

**Sterols**

Similarly to SM, the particle content of free cholesterol relative to both total HDL mass and total lipids decreases from HDL2b to HDL3c (7) (Fig. 2A). Interestingly, both SM and free cholesterol impact the physical properties of surface phospholipids, decreasing fluidity in the liquid-crystal phase. The low abundance of SM and free cholesterol in small, dense HDL may therefore result in elevated fluidity of surface lipids in this subpopulation, potentially enhancing functionality (7).

**Cholesteryl esters and triglycerides**

HDL particle content of cholesteryl esters and triglycerides progressively decreases with increase in hydrated density from large, light HDL2b to small, dense HDL3c (7) (Fig. 2A). As the decrease in the content of free cholesterol is more pronounced, the cholesteryl ester/free cholesterol ratio increases with HDL density (Fig. 2C). This finding reflects the contention that small HDL constitutes a major site of cholesterol esterification within the HDL particle spectrum (48). Elevated LCAT activity and increased PC/SM ratio in HDL3c support this proposal, as SM represents a physiological inhibitor of LCAT (49, 50).

Differences between HDL subpopulations in the abundance of cholesteryl ester and free cholesterol result in the pronounced enrichment of total cholesterol in large, light HDL. It is worth mentioning in this regard that routine clinical measurement of plasma HDL-cholesterol (HDL-C) primarily reflects levels of large, cholesterol-rich HDL and lacks sensitivity to provide information about small, cholesterol-poor particles. The marked heterogeneity and complexity of the HDL particle profile is, therefore, hardly reflected by a single measurement of HDL-C.

**FUNCTIONAL RELEVANCE OF THE HDL LIPIDOME**

HDL particles are distinguished from atherogenic apoB-containing lipoproteins by their capacity to exert a wide spectrum of antiatherogenic functional biological activities (see other reviews in this Thematic Series). Such biological functions of HDL directly reflect the composition and structure of HDL particles. Despite the fact that proteins are typically considered to constitute major functional components of HDL, lipids play key roles in antiatherogenic HDL functionality.

**Cellular cholesterol efflux**

The capacity of HDL to efflux cellular cholesterol is primarily determined by the properties of HDL surface lipids (Table 2). Thus, cholesterol efflux to HDL mediated by scavenger receptor class B type I (SR-BI) is proportional to the HDL content of phospholipids (51). The relevance of
the HDL lipidome to other pathways of cholesterol efflux, primarily those mediated by ATP-binding cassette transporters A1 and G1 (ABCA1 and ABCG1), remains to be established.

The impact of HDL phospholipids on the process of cholesterol efflux is not limited by their concentration in HDL. Qualitatively, the physical state of phospholipids represents an important determinant of the ability of HDL to accept cellular cholesterol. Indeed, liquid-crystal unsaturated phospholipids form more efficient cholesterol acceptor particles than their gel-phase saturated analogs (52). As a consequence, phase transition temperatures of molecular species of PC (53) are inversely related to the capacity of PC-containing reconstituted HDL to efflux cellular cholesterol (52), emphasizing the role of the lipid phase fluidity. The fluidity of HDL surface lipids is particularly important for cholesterol efflux mediated by SR-BI (51). Lipid packing in the surface lipid monolayer of HDL appears to underlie this relationship, with more fluid liquid-crystal lipids capable of better inserting exogenous lipid molecules (e.g., cholesterol) compared with their gel-phase counterparts. By contrast, the differential conformation of apoA-I observed in the liquid-crystal versus gel state does not contribute to differences in efflux (52).

The requirement of the fluid lipid phase for cholesterol efflux can be viewed in the context of the concept of HDL structural lability as a prerequisite for this process (54). According to this view, compositional changes that destabilize HDL particles may accelerate their metabolic remodeling, including that which occurs during cellular lipid efflux. Such compositional changes may include reduced chain length and an increased level of unsaturation of fatty acid residues in surface phospholipids (54, 55).

In a similar fashion, high SM content decreases cellular cholesterol efflux to HDL containing unsaturated lipids in a liquid-crystal state (56). Indeed, decrease in the fluidity of surrounding liquid-crystal lipids represents one of the major effects of SM. As a result of its high affinity for free cholesterol, SM, however, exerts dual effects on the cholesterol efflux capacity of HDL. Such direct interactions between SM and cholesterol can counteract the effects of diminished fluidity (56); as a consequence, the net effect of HDL enrichment in SM on the cholesterol efflux can become negligible (51) or even beneficial (57). This example demonstrates the role of specific lipid-lipid interactions as modulators of cholesterol efflux.

**Antioxidative activity**

HDL lipids can markedly influence antioxidative activity of HDL, primarily acting via modulation of the physical properties of the HDL surface lipids (Table 2). Indeed, the fluidity of the surface phospholipid monolayer is a key determinant of the transfer efficiency of phospholipid hydroperoxides (PLOOH) from LDL to HDL (58). The strong correlation of the PLOOH transfer rate from oxidized LDL to liposomes with liposomal membrane fluidity emphasizes the importance of physical properties in this process. Furthermore, both the capacity of reconstituted HDL to protect LDL from oxidation and the PLOOH transfer rate from oxidized LDL increase with the surface lipid fluidity of reconstituted HDL (58). Elevated lipid membrane fluidity equally enhances the transfer of lipid hydroperoxides (LOOH) to HDL from cell membranes (59). The surface lipid monolayer fluidity of HDL is primarily determined by the content of such lipids as SM (relative to PC), free cholesterol (relative to PC), and saturated and monounsaturated fatty acids (relative to polyunsaturated ones); decreasing relative content of each of them fluidifies the surface lipid monolayer of HDL (58).

The composition of the HDL lipid core also appears to impact the antioxidative activity of HDL, acting via altered conformation of apoA-I, the major determinant of the capacity of HDL to inactivate LOOH. Reduction of LOOHs by Met residues of apoA-I forms the basis for the antioxidative activity of this protein. In this regard, the ratio of cholesteryl ester to triglyceride in the HDL core influences the conformation of the central and C-terminal domains of apoA-I (60, 61), thereby potentially modifying the accessibility of Met residues to LOOH. In addition, modifications of the hydrophobic core modulate the net surface charge of HDL, which is determined by the exposure of charged amino acid residues of apoA-I (62). However, direct demonstration of the role of cholesteryl ester and triglyceride components for antioxidative properties of HDL is still lacking.

Finally, small amounts of lipophilic antioxidants, primarily tocopherols, may provide a minor contribution to the antioxidative properties of HDL (40–42) (Table 2).

**Anti-inflammatory activity**

The surface phospholipid component of HDL also impacts the anti-inflammatory actions of HDL (Table 2). Indeed, 1-palmitoyl-2-linoleoyl PC is capable of inhibiting

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**TABLE 2.** Major lipid components involved in biological activities of HDL

| Activity                     | Component                        | Role                                                   |
|------------------------------|----------------------------------|--------------------------------------------------------|
| Cholesterol efflux           | PL                               | Incorporation of cellular lipids                       |
| Protection from oxidative damage | PL fatty acid moieties, SM, FC | Modulation of surface lipid fluidity                   |
| Anti-inflammatory activities | PL fatty acid moieties           | Modulation of surface lipid fluidity                   |
| Cytoprotective activities    | S1P                              | Activation of intracellular signaling through S1P receptors |
| Antithrombotic activities    | Cardiolipin, PS, PI              | Inhibition of platelet activation                       |

Unraveling the complexities of the HDL lipidome 2955
proinflammatory signaling via the nuclear factor kappa B (NFκB) pathway and may thereby contribute to the capacity of HDL to inhibit inflammation in endothelial cells (63, 64). In addition, 1-palmitoyl-2-linoleoyl PC can account for the ability of HDL to inhibit the activation of T cells mediated by dendritic cells (65).

Interestingly, the anti-inflammatory activity of HDL depends on the fatty acid composition of PC (66). Specifically, reconstituted HDL particles containing 1-palmitoyl-2-linoleoyl PC and 1-palmitoyl-2-arachidonoyl PC inhibit vascular cell adhesion molecule-1 (VCAM-1) expression in activated endothelial cells to a greater degree than reconstituted HDL containing 1-palmitoyl-2-oyleoyl PC, whereas dipalmitoyl PC-containing particles do not inhibit at all. These differences, which cannot be explained by differential cellular binding of HDL (66), can be related to differences in physical properties of PCs. Indeed, as the fluidity of the PC phase increases with the degree of the unsaturation of its fatty acid moieties (53), enhanced anti-inflammatory properties of HDL containing PUFA residues can reflect accelerated efflux of cell-derived proinflammatory lipids to more fluid particles as an underlying mechanism.

HDL lysosphingolipids can also exert anti-inflammatory effects, acting via reduced aortic expression of monocyte chemotactic protein-1 (MCP-1) (67). By contrast, chemotactic effects of S1P toward macrophages may endow HDLs with proinflammatory activity (68); cellular S1P3 receptors are essential for this effect.

Cytoprotective activity

The capacity of HDL to protect cells from apoptotic death depends on the HDL content of lysosphingolipids. Indeed, S1P, sphingosylphosphorylcholine and lysosulfatide contribute to HDL-mediated protection from apoptosis induced by growth factor deprivation (30, 69–72) (Table 2).

HDL-associated S1P potently protects several cell types from apoptosis induced by multiple agents. Antiapoptotic effects of HDL S1P on endothelial cells involve enhanced nitric oxide (NO) production (73). Survival benefits conferred by S1P to cardiomyocytes are mediated by intracellular signaling via both Akt and the mitogen-activated protein kinase kinase (MEK)1/2 - extracellular signal-regulated kinase (ERK)1/2 pathways and stimulation of the phosphorylation of glycogen synthase kinase-3β (74). Akt activation is mediated by S1P3 receptors, whereas the activation of ERK1/2 occurs through an S1P1 receptor-G-protein-dependent pathway. The cytoprotection induced by S1P can also involve S1P2 receptors, with subsequent activation of tyrosine kinase family Src and transcription factor Stat3 (75, 76). Acting via S1P receptors, S1P can also mediate effects of HDL on endothelial cell and smooth muscle cell migration (71, 77).

In a similar fashion, S1P3 receptors can be activated by sphingosylphosphorylcholine, resulting in reduced myocardial apoptosis (78). HDL-associated sphingosylphosphorylcholine and lysosulfatide also display mitogenic effects in fibroblasts (79).

Anti-infectious activity

Interestingly, some lipids may endow HDL with anti-infectious properties. Indeed, rHDL containing ganglioside monosialotetrahexosylganglioside (GM1), a component of the cholera toxin receptor complex, can bind the toxin and divert it away from healthy mammalian cells (80). Low levels of several biologically active gangliosides are present in human plasma HDL; their relevance to protection from infection remains to be established (33–35).

Vasodilatory activity

S1P is a key HDL-associated bioactive lipid which mediates multiple effects of HDL on the endothelium, including vasodilation. S1P interacts with several S1P receptors, activating intracellular signaling cascades which include the small G-protein Rac, Src kinase, PI3K, Akt, and mitogen-activated protein kinase (MAPK) (81) (Table 2). The S1P3 subclass of S1P receptors mediates stimulating effects of HDL on endothelial nitric oxide synthase (eNOS) (82, 83). The S1P2 and S1P3 subclasses participate in the activating effects of S1P on endothelial cell proliferation and tube formation mediated by Akt/ERK/NO (84). In addition, S1P2 and S1P3 receptors are involved in the stimulation of prostacyclin production by HDL-associated S1P, acting via upregulation of cyclooxygenase-2 in smooth muscle cells (85). Importantly, the effects of S1P on the endothelium do not involve modulation of cellular cholesterol efflux as S1P does not influence this process (84).

Other HDL-associated lipids exhibiting vasodilatory activity include estradiol and androsterone. HDL-derived estradiol stimulates endothelial relaxation through induction of eNOS (86), an effect that can be related to the stimulation of cellular cholesterol efflux (87). Another potent vascular relaxant, dehydroepiandrosterone, is present in HDL in its naturally occurring lipophilic esterified form. Such dehydroepiandrosterone fatty acyl esters can induce vascular relaxation via stimulation of eNOS (88).

Antithrombotic activity

Cardiolipin, a potent anionic anticoagulant, may contribute to the effects of HDL on coagulation and platelet aggregation (10). In addition, HDL-derived PS and PI, negatively charged phospholipids that play roles in platelet activation and apoptosis (89), possess a potential for modulating platelet function.

ALTERED HDL LIPIDOME UNDER PATHOPHYSIOLOGICAL CONDITIONS

Phospholipids

HDL phospholipids can be modified under metabolic conditions associated with elevated cardiovascular risk. Thus, subjects presenting with low HDL-C levels were reported to display diminished HDL content of LPC (5). By contrast, patients with isolated arterial hypertension present with reduced content of total HDL phospholipid and
altered phospholipid composition characterized by elevated content of LPC and PE and diminished content of PC (90). Consistent with these data, HDL obtained from patients on maintenance hemodialysis contained reduced amounts of phospholipid and elevated amounts of lyso-phospholipid (91). Total HDL phospholipid is equally diminished in patients with psoriasis (92). The fatty acid composition of HDL can equally be affected, potentially reflecting alteration in the phospholipid fraction, as occurs in patients with triple vessel coronary heart disease, in which HDL displays reduced content of PUFAs (93) paralleled by diminished content of PC.

The phospholipid content of HDL can be altered under acute-phase conditions, although the data are inconsistent (94). The reduction of total HDL phospholipids reported in some studies (95, 96) may reflect an elevated activity of secretory phospholipase A2 (sPLA2) as frequently observed in the acute phase (97–99). Acute-phase HDL contains reduced levels of PI, diacyl and ether-linked PEs paralleled by elevated levels of LPC and nonesterified fatty acids compared with normal HDL (13). In this study, several molecular species of LPC were affected by the induction of the acute phase, specifically, LPC 18:0, 18:2, 20:4, 20:5, and 22:6.

The phospholipid subclass profile is equally abnormal in HDL isolated from subjects with Niemann-Pick disease type B (NPD B), an autosomal disorder characterized by the lack of sphingomyelinase. This disease is associated with the accumulation of cholesterol and SM in multiple organs, including the liver and the spleen (100). HDL from NPD B subjects are enriched in PC compared with HDL from healthy controls. Major PC species enriched in NPD B HDL are PC 34:2, 36:4, 34:1, and 36:2 (100).

Importantly, the HDL phospholipid moiety can be oxidized in vivo with formation of biologically active oxidized compounds. Thus, HDL oxidation by transition metal ions or free radicals produces a variety of molecular species of oxidized phospholipids that differ markedly in structure and biological activities (101, 102). HDL oxidation by HOCl produces 2-chlorohexadecanal, a chlorinated fatty aldehyde formed upon oxidative cleavage of plasmalogen, which exerts inhibitory actions on eNOS (103). In the circulation, HDL represents a major source of lipid hydroperoxides compared with other lipoproteins (104, 105). In addition, HDL carries significant amounts of circulating isoprostanes (21) in which content can be altered in the acute phase (13).

Sphingolipids

The HDL content of SM is reported to be diminished in low HDL-C subjects (5) and in patients with coronary heart disease (93). On the other hand, HDL SM is elevated in patients with isolated arterial hypertension (90) and with psoriasis (92). Interestingly, SM enrichment in HDL may selectively inhibit generation of specific molecular species of cholesteryl esters catalyzed by LCAT, altering the plasma profile of cholesteryl esters (106).

Acute-phase HDL is depleted of SM, and particularly of SM 33:1 and 38:1 species, compared with normolipidemic HDL (13). Total SM, and primarily the d18:1/16:0 SM species, are elevated in HDL isolated from NPD B patients (100). The SM/PC ratio is, however, not significantly different between NPD B and control HDL as PC increases in parallel to SM.

Ceramide levels were measured by LC-MS in HDL (obtained as apoB-depleted serum) from patients with ischemic heart disease (107). The abundance of ceramide 24:1 was inversely correlated with the occurrence of ischemic heart disease in this study (107).

In patients presenting with cardiac ischemia, HDL contents of both S1P and dihydro-S1P are inversely correlated with the occurrence of ischemic heart disease (107). Furthermore, plasma levels of S1P are decreased in monogenic disorders of HDL metabolism involving mutations in ApoA1, Lcat, or Abca1 genes compared with family controls (47). By contrast, HDL content of S1P does not appear to be modified in subjects with metabolic syndrome (27).

Sterols

The acute phase is well known to feature decreased LCAT activity (94); as a consequence, increased abundance of free cholesterol in HDL is frequently observed in inflammatory states. Similarly, HDL free cholesterol is elevated in genetic LCAT deficiency (108). By contrast, HDL content of cholesterol can be reduced in patients with psoriasis (92).

Cholesteryl esters and triglycerides

HDL enrichment in triglyceride with cholesteryl ester depletion is the most frequent abnormality of HDL lipid composition. Elevated CETP-mediated heteroexchange of triglyceride and cholesteryl ester between VLDL and HDL primarily accounts for this effect (109–112). Such modification of the HDL lipid core occurs in low HDL-C hypertriglyceridemic states associated with decreased activities of lipoprotein lipase (LPL), hepatic lipase, and/or LCAT, as well as with hyperinsulinemia of metabolic syndrome and type 2 diabetes (113–115). Similar elevation in HDL triglycerides and decrease in HDL-C are observed in patients with coronary heart disease (93). Such compositional modifications of the HDL core are frequently paralleled by low-grade, chronic inflammation.

In a similar fashion, conditions of acute phase and inflammation involve an enlarged pool of triglyceride-rich lipoproteins. Therefore, similar alterations to the HDL lipid core are typically observed in the acute phase and during inflammation (94, 116). Thus, acute-phase HDL obtained from patients following bypass surgery is depleted of cholesteryl ester and enriched in triglyceride (13). Furthermore, HDL3 from patients with myocardial infarction is enriched in triglyceride (95). Similarly, HDL from patients with malaria (117) and uremic patients on hemodialysis (91) displays elevated content of triglyceride, whereas HDL from patients with psoriasis is depleted in cholesteryl ester (92). Cytokine-induced increase in the activity of CETP paralleled by decreases in the activities of hepatic lipase, LPL, and LCAT can underlie hypertriglyceridemia,
low HDL-C, and altered HDL lipid composition as shown by studies in monkeys (116, 118, 119).

The composition of HDL can be equally abnormal in other forms of dyslipidemia. In familial hypercholesterolemia, HDL levels of triglyceride are elevated and the cholesteryl ester/triglyceride ratio is reduced (120, 121), reflecting accelerated cholesteryl ester transfer from LDL and HDL to triglyceride-rich lipoproteins as a result of increased concentrations and abnormal properties of plasma VLDL1 (120, 122, 123). By contrast, attenuation of cholesteryl ester transfer from LDL and HDL to triglyceride-rich lipoproteins, as observed in plasma of subjects with heterozygous CETP deficiency, results in elevated HDL cholesteryl ester content and reduced content of triglyceride (124).

FUNCTIONAL RELEVANCE OF ALTERATIONS IN THE HDL LIPIDOME

Altered HDL function primarily results from alterations in the apolipoprotein and enzymatic cargo of HDL. Defective biological activities of HDL are, however, considerably influenced by changes in lipid content.

Cellular cholesterol efflux

Abnormal lipid composition resulting from altered lipoprotein metabolism may impair cholesterol efflux properties of HDL.

Enrichment of HDL particles in triglyceride and depletion of phospholipid may render them deficient in their capacity to efflux cellular cholesteryl from lipid-loaded macrophages [primarily mediated by ABCA1 (125)] as observed in low HDL-C subjects relative to the high HDL-C group (126). Further along this line, HDL capacity to deliver cholesteryl ester to hepatic cells through interaction with SR-BI diminishes in parallel with HDL enrichment in triglyceride (127). These data suggest that alterations in the HDL lipidome can contribute to the impairment of cellular cholesterol efflux mediated by ABCA1 and SR-BI.

Excessive HDL enrichment in cholesteryl ester can also exert deleterious effects on cholesterol efflux, as demonstrated by the diminished capacity of large, cholesteryl ester-enriched HDL2 obtained from subjects with homozygous CETP deficiency to accept cholesteryl from lipid-loaded mouse peritoneal macrophages (128). As a consequence, normalization of the lipid composition of such HDL via removal of excess cholesteryl ester improves HDL cholesterol efflux capacity (129).

Impaired cholesterol efflux from macrophage-derived foam cells can also result from the accumulation in HDL of oxidized forms of cholesterol, including 7-ketocholesteryl (130, 131). Such oxysterols can act through alterations in cell membrane properties or in intracellular cholesterol trafficking. In a similar fashion, accumulation of oxidized lipids may underlie impairment in cholesterol efflux properties of HDL by cigarette smoke (132, 133). In addition, specific oxidized phospholipidoids, which serve as high-affinity ligands for the SR-B1, are capable of preventing binding of HDL to SR-B1 and can thereby interfere with SR-B1-mediated selective transfer of cholesteryl esters between HDL and hepatocytes (134).

Finally, alterations in physical properties of the surface lipid monolayer can impact cholesterol efflux to HDL. In this regard, both an elevated content of SM (relative to PC) and a decreased content of PUFAs (relative to other fatty acids) can be of pathological significance, as such modifications reduce HDL surface fluidity and thereby decrease both LCAT activity (49) and cholesterol efflux capacity of HDL (52).

Antioxidative activity

Altered lipid composition of HDL particles is associated with antioxidative deficiency of HDL in several types of low HDL-C dyslipidemias (113, 114, 135, 136). Such functionally deficient HDLs are enriched in triglyceride and depleted in cholesteryl ester in normotriglyceridemic low HDL-C phenotype, metabolic syndrome, type 2 diabetes, and postmenopausal women (113, 114, 135–137), reflecting elevated CETP activity (112). Mechanistically, the correlation between triglyceride enrichment of HDL particles and impairment of antioxidative activity might be accounted for by ineffective reduction of lipid hydroperoxides by Met residues of apoA-I secondary to alterations in the conformation of its central and C-terminal domains (60, 61). ApoA-I conformation can also be altered by oxidized phospholipids, which modify physicochemical properties of the lipid domain in reconstituted HDL, decreasing the stability of apoA-I (138).

Alterations at the level of the HDL lipidome that can impact the antioxidative properties of HDL might involve enrichment in SM and in saturated fatty acids, both resulting in elevated rigidity of the phospholipid surface monolayer of HDL (58). Such structural anomalies may cause deficiency in the capacity of HDL to acquire LDL-derived oxidized lipids and to protect LDL from free radical-induced oxidative damage (58).

Anti-inflammatory activity

Defective anti-inflammatory activity of HDL in dyslipidemia is associated with alterations in the HDL lipidome. The content of phospholipids in HDL3 is reduced in patients with familial hypercholesterolemia relative to healthy controls; in parallel, serum intercellular adhesion molecule-1 (ICAM-1) levels are elevated (139). Moreover, HDL3 from patients with familial hypercholesterolemia and premature coronary artery disease contains elevated levels of triglycerides compared with patients with familial hypercholesterolemia but without coronary artery disease. The anti-inflammatory activity of such dyslipidemic HDL3 is negatively correlated with its content of triglyceride (139).

Vasodilatory activity

HDL content of triglyceride is significantly elevated in patients with type 2 diabetes compared with control subjects.
and is highly inversely correlated with the vasodilatory activity of HDL (140). The deleterious role of oxidized lipids is consistent with the finding of elevated lipid peroxidation in HDL in type 2 diabetes (141). By contrast, HDL-associated S1P appears to play a beneficial role, as suggested by a positive correlation between its content in HDL from patients with ischemic heart disease and the capacity of HDL to induce endothelial cell barrier signaling (107).

CONCLUSIONS AND PERSPECTIVES

Modern lipidomic approaches have provided our initial insights into the lipid species profiles of human plasma HDL. Such studies are, however, few and were predominantly performed in HDL obtained from healthy normolipidemic subjects (4, 5, 8, 9). Thus, results of comprehensive lipidomic analysis of HDL in disease states are eagerly awaited. Such studies are essential to understand molecular mechanisms underlying development of atherosclerosis and other lipidoprotein-related disorders. Furthermore, available studies are largely confined to the measurements of lipid sum compositions and do not cover all aspects of hierarchical categorization of analytical lipid outputs (142); indeed, structurally defined molecular lipids need to be characterized to a larger extent. Plasma HDL particles reveal high levels of structural and compositional heterogeneity, which are linked to their heterogeneity in biological activities. Establishing direct relationships between distinct HDL structure and composition on the one hand and specific athero-protective functions on the other requires further study. Such structure-function analysis of HDL particles bears the potential to identify clinically relevant, atheroprotective HDL subpopulations. Furthermore, development of HDL-based therapies specifically designed to target beneficial sub- species of the circulating HDL pool can be facilitated using this approach.

HDL lipidomics can equally contribute to the identification of biomarkers of both normal and deficient HDL functionality, which may in turn prove useful as biomarkers of cardiovascular risk. It remains to be shown whether such novel HDL-based lipidomic biomarkers can be superior relative to HDL-C levels.

However, numerous technical issues remain to be thoroughly addressed to make such scientific developments possible. Major among them are appropriate system calibration for individual lipid species, proper choice of internal standards, optimization of chromatographic separation, and correction for matrix effects. Such a strategy was applied to the quantification of molecular species of PC and SM but not of other lipid classes (100). Extraction procedures and internal standards, which vary markedly from one study to another, require standardization. Despite recent advances, chromatographic separation of isomeric lipids differing in the positions of fatty acid residues and double bonds in fatty acid chains should be improved. The effects of variable biological matrices and ion suppression by coeluting molecular species should be better evaluated.

The final challenge to be fully addressed represents the handling of the vast amount of data produced by lipidomic analyses. Different aspects of this bottleneck issue primarily involve lack of tools for interpreting the results, such as metabolic pathway investigation, molecular dynamic simulation, and statistical analysis, and equally, processing of raw data, including automatic peak selection and quantification (143, 144). With all technical questions resolved, quantitative analysis of the molecular components of the HDL lipidome will undoubtedly contribute to expand our knowledge of cardiovascular and metabolic diseases.

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