SECTION 1

NORMAL FUNCTIONAL HIGH-DENSITY LIPOPROTEIN

Lipoproteins are plurimolecular, typically quasi-spherical, pseudomicellar complexes composed of polar and non-polar lipids solubilized by proteins with specialized structure and function, the apolipoproteins. Lipids and apolipoproteins thus constitute major building blocks of lipoproteins. The principal lipid-binding structural motifs of apolipoproteins include amphipathic alpha-helixes and beta-sheets. Major lipoproteins in human plasma are, in the order of increasing density and decreasing size, chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoprotein (LDL), lipoprotein (a) [Lp(a)] and high-density lipoprotein (HDL). Whereas chylomicrons, VLDL, IDL, LDL and Lp(a) are commonly regarded as large, light, lipid-rich particles, HDL represents a small, dense, protein-rich lipoprotein with a mean size of 8–10 nm and density of 1.063-1.21 g/ml (Fig. 1.1).
Figure 1.1 Plurimolecular, quasi-spherical, pseudomicellar HDL particle with major structural components. Apolipoprotein A-I (apoA-I) molecules are shown as ribbons, surface phospholipid molecules as light-blue spheres, surface free cholesterol molecules as yellow spheres, core cholesteryl ester molecules as dark-blue spheres and core triglyceride molecules as green spheres. The average number of protein and lipid molecules per HDL particle are as follows: apoA-I, 3–4; phospholipid, 80–120; free cholesterol, 20–40; cholesteryl ester, 100–160; triglyceride, 15–25 (see color plate section; see also Table 1.1).
As a result of protein enrichment, proteins form the major building blocks of HDL particles. HDL carries a particularly large number of proteins as compared to other lipoprotein species (Table 1.1). Heterogeneity of the HDL proteome was originally demonstrated as early as 1968–1969 [1–3], with most of the major proteins being discovered in the 1970s.

HDL proteins have traditionally been divided into four major subgroups: apolipoproteins, enzymes, lipid transfer proteins and minor proteins (<5% of total HDL protein; Table 1.1). Whereas apolipoproteins and enzymes are now recognized as key HDL components whose biologic importance is beyond doubt, the role of minor proteins, primarily those involved in complement regulation, protection from infections and the acute-phase response, has received increasing attention in recent years, mainly as a result of advances in proteomic technologies. Indeed, HDL particles have been long thought to contain only apolipoproteins and enzymes directly involved in lipid metabolism. The subsequent discovery of minor amounts of serum amyloid A (SAA), a major positive acute phase reactant, as a component of normal plasma HDL [4] was, at first, considered to be an exception. However, the recent development of proteomic technologies has significantly enhanced the sensitivity of protein detection, revealing that the protein cargo of HDL is much more diverse than previously realized [5–7] (Table 1.2). These studies have allowed the identification of more than 50 proteins in human HDL isolated by ultracentrifugation [5–7] (Table 1.2). Numerous proteins involved in the acute-phase response were unexpectedly found as
### TABLE 1.1 Major Components of the HDL Proteome

<table>
<thead>
<tr>
<th>Protein</th>
<th>Chromosome</th>
<th>Number of Amino Acid Residues</th>
<th>$M_r$, kDa</th>
<th>Major Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoA-I</td>
<td>11</td>
<td>243</td>
<td>28</td>
<td>Major structural and functional apolipoprotein, LCAT activator</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>1</td>
<td>77</td>
<td>17</td>
<td>Structural and functional apolipoprotein</td>
</tr>
<tr>
<td>ApoA-IV</td>
<td>11</td>
<td>376</td>
<td>46</td>
<td>Structural and functional apolipoprotein</td>
</tr>
<tr>
<td>ApoA-V</td>
<td>11</td>
<td>343</td>
<td>39</td>
<td>Activator of LPL, inhibitor of TG secretion</td>
</tr>
<tr>
<td>ApoC-I</td>
<td>19</td>
<td>57</td>
<td>6.6</td>
<td>Modulator of CETP activity</td>
</tr>
<tr>
<td>ApoC-II</td>
<td>19</td>
<td>79</td>
<td>8.8</td>
<td>Activator of LPL</td>
</tr>
<tr>
<td>ApoC-III</td>
<td>19</td>
<td>79</td>
<td>8.8</td>
<td>Inhibitor of LPL</td>
</tr>
<tr>
<td>ApoC-IV</td>
<td>19</td>
<td>101</td>
<td>11</td>
<td>Regulates TG metabolism</td>
</tr>
<tr>
<td>ApoD</td>
<td>3</td>
<td>169</td>
<td>19</td>
<td>Binding of small hydrophobic molecules</td>
</tr>
<tr>
<td>ApoE</td>
<td>19</td>
<td>299</td>
<td>34</td>
<td>Structural and functional apolipoprotein, ligand for LDL-R and LRP</td>
</tr>
<tr>
<td>ApoF</td>
<td>12</td>
<td>162</td>
<td>29</td>
<td>Inhibitor of CETP</td>
</tr>
<tr>
<td>ApoH</td>
<td>17</td>
<td>326</td>
<td>38</td>
<td>Binding of negatively charged molecules</td>
</tr>
<tr>
<td>ApoJ</td>
<td>8</td>
<td>427</td>
<td>70</td>
<td>Binding of hydrophobic molecules, interaction with cell receptors</td>
</tr>
<tr>
<td>Protein</td>
<td>Number</td>
<td>Molecular Weight (Da)</td>
<td>ID</td>
<td>Description</td>
</tr>
<tr>
<td>-------------</td>
<td>--------</td>
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<td>----</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>ApoL-I</td>
<td>22</td>
<td>371</td>
<td>44/46</td>
<td>Trypanolytic factor of human serum</td>
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<tr>
<td>ApoM</td>
<td>6</td>
<td>188</td>
<td>25</td>
<td>Binding of small hydrophobic molecules</td>
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<tr>
<td><strong>Enzymes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCAT</td>
<td>16</td>
<td>416</td>
<td>63</td>
<td>Esterification of cholesterol to cholesteryl esters</td>
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<td>PONI</td>
<td>7</td>
<td>354</td>
<td>43</td>
<td>Calcium-dependent lactonase</td>
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<td>PAF-AH (LpPLA&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>6</td>
<td>420</td>
<td>53</td>
<td>Hydrolysis of short-chain phospholipids</td>
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<td>GSPx-3</td>
<td>5</td>
<td>206</td>
<td>22</td>
<td>Reduction of hydroperoxides by glutathione</td>
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<td><strong>Lipid transfer proteins</strong></td>
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<td></td>
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<tr>
<td>PLTP</td>
<td>20</td>
<td>476</td>
<td>78</td>
<td>Conversion of HDL into larger and smaller particles, transport of LPS</td>
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<tr>
<td>CETP</td>
<td>16</td>
<td>476</td>
<td>74</td>
<td>Heteroexchange of CE and TG and homoexchange of PL between HDL and apoB-containing lipoproteins</td>
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<td><strong>Acute-phase proteins</strong></td>
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<tr>
<td>SAAI</td>
<td>11</td>
<td>104</td>
<td>12</td>
<td>Acute-phase reactant</td>
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<tr>
<td>C3</td>
<td>19</td>
<td>1663</td>
<td>187</td>
<td>Complement activation</td>
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<tr>
<td><strong>Proteinase inhibitors</strong></td>
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<td></td>
</tr>
<tr>
<td>Alpha-1-antitrypsin</td>
<td>14</td>
<td>418</td>
<td>52</td>
<td>Inhibitor of serine proteinases</td>
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</table>

CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; GSPx, glutathione selenoperoxidase; LCAT, lecithin:cholesterol acyltransferase; LDL-R, LDL receptor; LPL, lipoprotein lipase; LpPLA<sub>2</sub>, lipoprotein-associated phospholipase A2; LRP, LDL receptor-related protein; LPS, lipopolysaccharide; PAF-AH, platelet-activating factor-acetyl hydrolase; PL, phospholipid; PLTP, phospholipid transfer protein; PONI, paraoxonase 1; SAA, serum amyloid A; TG, triglyceride.
<table>
<thead>
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<th>Reference</th>
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<tr>
<td>Albumin</td>
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<tr>
<td>Alpha-1-acid glycoprotein 2</td>
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<tr>
<td>Alpha-1-antitrypsin</td>
<td>[8–12]</td>
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<tr>
<td>Alpha-1B-glycoprotein</td>
<td>[8, 11]</td>
</tr>
<tr>
<td>Alpha-2-antiplasmin</td>
<td>[8]</td>
</tr>
<tr>
<td>Alpha-2-HS-glycoprotein</td>
<td>[8, 9]</td>
</tr>
<tr>
<td>Alpha-2-macroglobulin</td>
<td>[9]</td>
</tr>
<tr>
<td>Alpha-amylase (salivary)</td>
<td>[12]</td>
</tr>
<tr>
<td>Angiotensinogen</td>
<td>[8]</td>
</tr>
<tr>
<td>Apo(a)</td>
<td>[10]</td>
</tr>
<tr>
<td>ApoA-I</td>
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</tr>
<tr>
<td>ApoA-II</td>
<td>[8–10, 12–14]</td>
</tr>
<tr>
<td>ApoA-IV</td>
<td>[8,10–12] [13, 14]</td>
</tr>
<tr>
<td>ApoB</td>
<td>[8, 10]</td>
</tr>
<tr>
<td>ApoC-I</td>
<td>[8–10, 12]</td>
</tr>
<tr>
<td>ApoC-II</td>
<td>[8, 10, 12, 13]</td>
</tr>
<tr>
<td>ApoC-III</td>
<td>[8–14]</td>
</tr>
<tr>
<td>ApoC-IV</td>
<td>[8, 10]</td>
</tr>
<tr>
<td>ApoD</td>
<td>[8–10, 13, 14]</td>
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<tr>
<td>ApoE</td>
<td>[8–10, 12–14]</td>
</tr>
<tr>
<td>ApoF</td>
<td>[8–10]</td>
</tr>
<tr>
<td>ApoH</td>
<td>[8]</td>
</tr>
<tr>
<td>ApoJ</td>
<td>[8–10, 13]</td>
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<tr>
<td>ApoL-I</td>
<td>[8,10–12] [13]</td>
</tr>
<tr>
<td>ApoM</td>
<td>[8, 10, 12, 13]</td>
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<td>Bikunin</td>
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<tr>
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<tr>
<td>Complement C3</td>
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<tr>
<td>Complement C4</td>
<td>[8]</td>
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<td>Complement C4b</td>
<td>[8]</td>
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<td>Complement C9</td>
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<tr>
<td>Fibrinogen</td>
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<td>Hrp</td>
<td>[8–10]</td>
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<tr>
<td>Haptoglobin</td>
<td>[13, 14]</td>
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<tr>
<td>Hemopexin</td>
<td>[8]</td>
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<tr>
<td>Inter-alpha-trypsin inhibitor chain H4</td>
<td>[8, 11]</td>
</tr>
<tr>
<td>Kininogen-1</td>
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<tr>
<td>LBP</td>
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<tr>
<td>LCAT</td>
<td>[8, 14]</td>
</tr>
<tr>
<td>PAF-AH (LpPLA₂)</td>
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<td>PLTP</td>
<td>[8, 10]</td>
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<tr>
<td>PON1</td>
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</tr>
<tr>
<td>PON3</td>
<td>[8, 10]</td>
</tr>
<tr>
<td>Prenylcysteine oxidase</td>
<td>[8]</td>
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<tr>
<td>Prothrombin</td>
<td>[10]</td>
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<tr>
<td>Retinol-binding protein</td>
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TABLE 1.2  (Continued)

<table>
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<tr>
<td>SAA1/2</td>
<td>[8, 10, 12]</td>
</tr>
<tr>
<td>SAA4</td>
<td>[8–10, 12]</td>
</tr>
<tr>
<td>Serpin F1</td>
<td>[8]</td>
</tr>
<tr>
<td>Transferrin</td>
<td>[8, 9, 13]</td>
</tr>
<tr>
<td>Transthyreitin</td>
<td>[8, 10, 11, 13]</td>
</tr>
<tr>
<td>Vitamin D-binding globulin</td>
<td>[8, 9]</td>
</tr>
<tr>
<td>Vitronectin</td>
<td>[8]</td>
</tr>
</tbody>
</table>

Modified from Reference 7. HDL was isolated by density gradient or sequential ultracentrifugation in salt solutions in all these studies except [14]. Hrp, haptoglobin-related protein; LBP, lipopolysaccharide-binding protein.

components of normal human plasma HDL. Furthermore, two other large families of HDL-associated proteins, notably those involved in complement regulation and protease inhibition, were discovered [8], raising the possibility that HDL may play a previously unsuspected role in host defence mechanisms and inflammation [7]. It is important to keep in mind, however, that the content of all these proteins in HDL is much lower as compared to that of major HDL apolipoproteins, i.e., apoA-I and apoA-II.

In addition to proteins, HDL contains multiple molecular species of lipids (Table 1.3) [15, 16]. As is the case in other plasma lipoproteins, the HDL lipidome contains phospholipids, unesterified sterols (predominantly cholesterol), cholesteryl esters and triglycerides as its four major classes of lipid (Fig. 1.1). Phospholipids build the surface lipid monolayer of HDL, whereas cholesteryl esters and triglycerides form the hydrophobic lipid core (Fig. 1.1); unesterified (free) sterols are predominantly located in the surface monolayer, partially penetrating the core. Recent lipidomic analyses allowed the identification of more than 200 individual molecular species of lipids in the HDL lipidome [17, 18], the number of which is limited only by the sensitivity of available technologies [19]. Finally, HDL contains multiple sugar moieties as components of glycosylated proteins and has recently been shown to transport microRNA [20].

DEFINITIONS

**Lipoprotein:** plurimolecular, typically quasi-spherical, pseudomicellar complex composed of polar and non-polar lipids solubilized by proteins possessing specialized structure and function, the apolipoproteins.

**Apolipoprotein:** a specialized protein that binds and transports lipids in the form of lipoproteins in the circulatory and lymphatic systems; frequently targets lipids to receptors at sites of degradation, storage, transformation and recycling.

**Lipid transfer protein:** protein involved in the mass transfer and exchange of lipids between lipoprotein particles.
<table>
<thead>
<tr>
<th>Lipid class</th>
<th>HDL Content, % Total</th>
<th>Major Subspecies</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipids</td>
<td>20–30</td>
<td>Phosphatidylcholine, sphingomyelin</td>
<td>[15–17]</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>0.6–1.2</td>
<td>18:0/20:3, 18:0/20:4</td>
<td>[15, 21]</td>
</tr>
<tr>
<td>Lyso phosphatidylcholine</td>
<td>0.2–6</td>
<td>16:0, 18:0, 18:1, 18:2</td>
<td>[15–17]</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>0.2–0.6</td>
<td>18:2/16:0, 18:2/18:0, 18:0/20:4</td>
<td>[15–17]</td>
</tr>
<tr>
<td>Plasmalogens</td>
<td>0.2–0.6</td>
<td>18:0/20:4, 16:0/20:4, 18:1/20:4, 18:0/18:2, 16:0/22:6</td>
<td>[17]</td>
</tr>
<tr>
<td>Ceramide</td>
<td>0.1</td>
<td>24:0, 24:1, 23:0, 22:0, 16:0</td>
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<td>Phosphatidylserine</td>
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<td>16:0/18:0</td>
<td>[15, 18, 22]</td>
</tr>
<tr>
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<td>n.e.</td>
<td>18:1/20:2</td>
<td>[21]</td>
</tr>
<tr>
<td>Cardiolipin</td>
<td>0.10–0.15</td>
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<td>[22]</td>
</tr>
<tr>
<td>Sterols</td>
<td>3–5</td>
<td></td>
<td>[15, 23]</td>
</tr>
<tr>
<td>Triacylglycerides</td>
<td>3–6</td>
<td>18:1/16:0/18:1, 18:2/16:0/18:1</td>
<td>[15, 18]</td>
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<tr>
<td>Cholesterol ester</td>
<td>14–18</td>
<td></td>
<td>[15, 16]</td>
</tr>
</tbody>
</table>

*Depending on HDL subpopulation, lipids make 35% to 65% of total HDL mass [24]; n.e., not established.*
1.1 PROTEOME

Apolipoproteins

Apolipoprotein A-I. Discovered at the end of the 1960s, apolipoprotein A-I (apoA-I; Mr 28 kDa) is the major structural and functional HDL apolipoprotein (see Chapter 3) accounting for approximately 70% of total HDL protein [25]. Almost all HDL particles are believed to contain apoA-I [26, 27]. Major functions of apoA-I involve the activation of lecithin:cholesterol acyltransferase (LCAT), and interaction with cellular receptors; equally apoA-I endows HDL with multiple anti-atherogenic activities (Table 1.1). ApoA-I is synthesized as a 267-residue proprotein which is processed to release an 18-residue signal peptide and a 6-residue propeptide. Mature, circulating apoA-I contains 243 amino acid residues within a single polypeptide that lacks glycosylation or disulfide linkages and is encoded by exon 3 (residues 1–43) and exon 4 (residues 44–243) of a gene located on the long arm of chromosome 11. The apoA-I gene is a part of the APOA1/APOC3/APOA4 gene cluster. The N-terminal region of apoA-I is more highly conserved compared with the C-terminus, suggesting key biologic properties [28, 29].

ApoA-I represents a typical amphipathic protein that contains 8 alpha-helical amphipathic domains of 22 amino acids and two repeats of 11 amino acids (Figs 1.2 and 1.3); as a consequence, apoA-I binds avidly to lipids. Such properties render apoA-I a potent detergent which forms stable micellar complexes with phospholipids, cholesterol, triglycerides, and cholesteryl esters. The elevated amphipacity of apoA-I underlies its capacity to move between lipoprotein particles. As a consequence, apoA-I is also found in chylomicrons and VLDL.

As for many plasma apolipoproteins, the main sites for apoA-I synthesis and secretion are the liver and small intestine, with the liver being the major contributor to the plasma apoA-I pool.

ApoA-II. ApoA-II (Mr 17 kDa) is the second major HDL apolipoprotein and represents approximately 15%–20% of total HDL protein. About half of HDL particles may contain apoA-II [43]. Synthesis of apoA-II resembles that of apoA-I, with a 100-residue proprotein processed to release an 18-residue signal peptide and a 5-residue propeptide. ApoA-II circulates as a homodimer composed of two identical polypeptide chains, each containing 77 amino acids [44, 45] (Table 1.1). The two polypeptide chains are connected by a single disulfide bridge at position 6 in the sequence [46] (Figs 1.2 and 1.3). The presence of a Cys residue allows apoA-II to form heterodimers with other cysteine-containing apolipoproteins, such as apoE and apoD [47].

The human apoA-II gene is located on chromosome 1. Despite displaying amphipathic properties, apoA-II is more hydrophobic than apoA-I. Similar to apoA-I, apoA-II is predominantly synthesized in the liver but also in the intestine [48].
Figure 1.2  Expermenteally-obtained structures of major HDL apolipoproteins. Crystal structures of apolipoproteins A-I, A-II, C-I, C-II, C-III, D, E and M are shown as visualized with Cn3D 4.1 (available at: http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtm; see the link for the colors) using data from [30–41] (see color plate section).

**ApoA-IV.** ApoA-IV is a 46 kDa O-linked glycoprotein, 376 amino acids in length [49] (Table 1.1). ApoA-IV is the most hydrophilic apolipoprotein; consequently, it readily exchanges between lipoproteins, primarily between chylomicrons, VLDL and HDL, and may also circulate in a free form. ApoA-IV contains thirteen 22-amino acid tandem repeats, nine of which are highly alpha-helical; many of these helices are amphipathic (Fig. 1.3). Such repeats may serve as lipid-binding domains.

In most mammals, including humans, apoA-IV is synthesized in the intestine; hepatic synthesis also occurs in rodents. ApoA-IV is secreted into the circulation on the surface of newly synthesized chylomicron particles and is particularly abundant in follicular fluid [50]. The APOA4 gene resides on chromosome 11 in close linkage to APOA1 and APOC3. The primary translation product of the gene is a 396-residue proprotein which undergoes proteolytic processing to cleave a 20-residue signal peptide and to yield mature apoA-IV.

**ApoA-V.** The APOA5 gene is located on chromosome 11 adjacent to the APOA1/APOC3/APOA4 gene cluster [51]. The human APOA5 gene is only expressed in the liver where it encodes a 366-amino acid protein containing a 23-residue signal sequence (Table 1.1). ApoA-V circulates as a 39 kDa protein which is predominantly located on triglyceride-rich particles, chylomicrons and VLDL, but also on HDL; the carboxyl-terminal segment of apoA-V ensures its
Figure 1.3 Model structures of major HDL proteins. Structures of apolipoproteins A-I, A-II, C-I, C-II, C-III, D, E and M (upper panel), as well as of apoA-IV, apoJ, apoL-I, LCAT, PON1, PAF-AH, PLTP and CETP (lower panel) calculated by The Protein Model Portal (www.proteinmodelportal.org) are shown [42]. The structures are generated using Molscript; colours describe protein structures according to Jmol viewer (see color plate section).
binding to lipids [52]. ApoA-V functions as an activator of lipoprotein lipase (LPL) and as an inhibitor of hepatic production and secretion of triglyceride.

_ApoC-I, ApoC-II, ApoC-III, ApoC-IV._ ApoC-I, apoc-II, apoc-III and apoc-IV form a family of small, exchangeable apolipoproteins (Table 1.1). Genes coding for apoc-I, apoc-II and apoc-IV are located close to each other on chromosome 19, forming a gene cluster together with the apoE gene. ApoC proteins are primarily synthesized in the liver and secreted in the circulation.

_ApoC-I_ is the smallest apolipoprotein (M_r 6.6 kDa), containing 57 amino acids [53] (Figs 1.2 and 1.3). ApoC-I associates with both HDL and VLDL and can readily exchange between them. ApoC-I carries a strong positive charge and can thereby bind free fatty acids and modulate activities of several proteins involved in HDL metabolism. In addition, apoc-I modulates the interaction of apoE with VLDL and inhibits binding of VLDL to the LDL receptor-related protein. It is mainly synthesized in the liver and, to a minor degree, in the intestine. ApoC-I synthesis starts with an 83-residue proprotein which is processed to cleave a 26-residue signal peptide. ApoC-I is involved in the activation of LCAT and inhibition of hepatic lipase and cholesteryl ester transfer protein (CETP).

_ApoC-II_ is an 8.8 kDa protein, 79 residues in length, which functions as an activator of several triacylglycerol lipases [54] (Figs 1.2 and 1.3). Similar to apoc-I, apoc-II is associated with HDL and VLDL and can exchange between their surfaces. Region 43–51 of the protein is involved in lipid binding, whereas region 55–78 ensures lipase activation. ApoC-II is produced as a 101-amino acid proprotein cleaved to release a 22-amino acid signal peptide.

_ApoC-III_ is an 8.8 kDa protein containing 79 amino acids [55] (Figs 1.2 and 1.3). As a major VLDL protein, apoc-III is predominantly present in VLDL, with small amounts found in HDL. The protein inhibits LPL and hepatic lipase and decreases the uptake of lymph chylomicrons by hepatic cells. Region 68–99 of apoc-III accounts for lipid binding. The apoC-III gene is a part of the APOAI/APOC3/APOA4 gene cluster on chromosome 11. The proprotein of apoc-III contains 99 amino acid residues which include a 20-residue signal peptide. ApoC-III exists in three different isoforms according to the degree of sialylation of the protein, which differ in their structure, metabolism and effects on triglyceride hydrolysis [56].

Recently discovered in 1996 [57], _apoc-IV_ is an 11 kDa protein containing 101 amino acids produced from a 127-residue proprotein. ApoC-IV is expressed in the liver and displays a predicted protein structure characteristic of the other genes in this family. The function of apoc-IV is largely unknown; when overexpressed, it induces hypertriglyceridemia in mice [58, 59]. In normolipidemic plasma, more than 80% of the protein resides in VLDL, with most of the remainder residing in HDL particles. The HDL content of apoc-IV is much lower compared with other apolipoproteins of this family, making apoc-IV a low prevalence HDL apolipoprotein.

_ApoD._ ApoD is a 19 kDa glycoprotein, 169 amino acids in length, mainly associated with HDL [60] (Table 1.1). The protein does not possess a typical
apolipoprotein structure and belongs to the lipocalin family, which also includes retinol-binding protein, lactoglobulin and uteroglobin. Lipocalins form a large, multifunctional family of small lipid-transfer proteins (15–25 kDa) with very limited amino acid sequence identity (often below 20%), but with a common tertiary structure. Lipocalins share a structurally conserved beta-barrel fold which, in many lipocalins, bind hydrophobic ligands (Figs 1.2 and 1.3). The lipocalin fold is followed by an alpha-helix at the C-terminus and surrounds a central cavity lined with hydrophobic aromatic residues that enable binding of small hydrophobic molecules [61]. As a result, apoD transports small hydrophobic ligands, with a high affinity for arachidonic acid [62]. In plasma, apoD forms disulfide-linked homodimers and heterodimers with apoA-II.

The protein is expressed in many tissues, including liver, intestine, pancreas, kidney, placenta, adrenal, spleen, fetal brain and tears. Synthesis of apoD involves the production of a 189-residue precursor and cleavage of a 20-residue signal peptide. The apoD gene is located on chromosome 3.

ApoE. ApoE, a 34 kDa glycoprotein, is a key structural and functional component of HDL despite its much lower content in HDL particles compared with apoA-I [63] (Table 1.1). The major fraction of circulating apoE is carried by triglyceride-containing lipoproteins whose apoE mediates their receptor binding, internalization and catabolism. As apoE possesses the LDL receptor-binding and heparin-binding domains, the apolipoprotein serves as a ligand for the LDL receptor, the LDL receptor-related protein and VLDL receptor, and ensures lipoprotein binding to cell-surface glycosaminoglycans.

ApoE is produced as a 317-residue molecule which is cleaved to release an 18-residue signal peptide. The mature apoE molecule contains 299 amino acids and is extensively glycosylated and sialylated at multiple sites, including Thr194 and Ser290 [64]. Similar to apoA-I and apoA-II, apoE contains eight amphipathic alpha-helical repeats and displays detergent-like properties towards vesicular phospholipids [65] (Figs 1.2 and 1.3).

ApoE is synthesized in multiple tissues and cell types, including liver, endocrine tissues, central nervous system (mainly in astrocytes) and macrophages. The apoE gene resides on chromosome 19. Interestingly, apoE is the evolutionary precursor of mammalian apoA-I; the latter appeared after the divergence of the tetrapod and teleost lineages [66]. ApoE possesses a long, highly conserved region in the amino terminal two-thirds of the protein between residues 22 and 182. Another region of highly conserved sequence is located at the carboxyl terminus [28].

Three common APOE alleles have been identified, APOE2, APOE3, and APOE4, which differ by amino acid substitutions at positions 130 and 176. Specifically, the E2 allele contains Cys residues at positions 130 and 176, the E3 allele displays Cys130 and Arg76, and the E4 allele possesses Arg at both positions. The presence of cysteine residues allows apoE2 and apoE3 to form heterodimers with apoA-II. Human apoE3 preferentially binds to HDL, while apoE4 preferentially binds to VLDL [67]. The stronger lipid-binding ability of
apoE4 relative to that of apoE3, together with differences in the nature of the surfaces of VLDL and HDL particles, the former being largely covered with phospholipid and the latter with protein, account for such differential binding properties [67].

**ApoF.** ApoF is a 29 kDa sialoglycoprotein present in human HDL and LDL [68] (Table 1.1). ApoF is also known as lipid transfer inhibitor protein (LTIP) because of its ability to inhibit CETP. ApoF is synthesized in the liver from a gene located on chromosome 12 as a 308-amino acid precursor protein, which contains a signal peptide of 18 amino acids followed by a large proprotein of 290 amino acids. The proprotein is further cleaved to release the 162-amino acid C-terminal fragment which makes up the mature secreted form of the protein.

ApoF is heavily glycosylated with both O- and N-linked sugar groups. Such glycosylation renders the protein highly acidic with an isoelectric point of 4.5, resulting in a molecular mass some 40% greater than predicted [69].

**ApoH.** ApoH, also known as beta-2-glycoprotein 1, is a multifunctional cardioliopin-binding, N- and O-glycosylated protein of Mr 38 kDa (Table 1.1). In addition to cardiolipin, apoH binds to various types of negatively charged substances, such as heparin and dextran sulfate and may prevent activation of the intrinsic blood coagulation cascade by binding to phospholipids on the surface of damaged cells. Such binding properties are related to the presence of a positively charged domain at position 282–287. ApoH regulates platelet aggregation, inhibiting the generation of factor Xa and the activation of both factor XIIa and protein C. ApoH is expressed by the liver as a 345-residue proprotein and is secreted in plasma following cleavage of a signal peptide as a 326-residue protein. The apoH gene is located on chromosome 17.

**ApoJ.** ApoJ (also called clusterin and complement-associated protein SP-40,40) is a 70 kDa antiparallel disulfide-linked heterodimeric glycoprotein (Table 1.1). Human apoJ consists of two subunits designated alpha (34–36 kDa) and beta (36–39 kDa) which share limited homology [70]. The two subunits are linked by five disulfide bonds to form an antiparallel ladder-like structure (Fig. 1.3). In each of the mature subunits, the five cysteines that are involved in disulfide bonds are clustered in domains of about 30 amino acids located in the central part of the subunits. ApoJ contains about 30% of N-linked carbohydrate rich in sialic acid and possesses nonspecific binding activity to hydrophobic domains of various proteins. The distinct structure of apoJ allows binding of both a wide spectrum of hydrophobic molecules on the one hand and of specific cell-surface receptors on the other.

In humans, the gene is encoded on chromosome 8 and is highly conserved between species (70%–80% homology). The secretory form of apoJ is initially synthesized as a 449-residue, 60 kDa precursor protein containing a 22-residue signal peptide. The proprotein is glycosylated and proteolytically cleaved into alpha- and beta-subunits held together by disulfide bonds.
**ApoL-I.** ApoL-I, a key functional component of the trypanolytic factor of human serum, is a 371-residue protein associated with HDL [71] (Table 1.1; see also 7.5 of chapter 7). ApoL-I possesses a glycosylation site and shares structural and functional similarities with intracellular apoptosis-regulating proteins of the Bcl-2 family (Fig. 1.3). On the other hand, apoL-I is a lipid-binding protein with a high affinity for phosphatidic acid and cardiolipin [72]. ApoL-I is expressed in pancreas, lung, prostate, liver, placenta and spleen. Synthesis of apoL-I involves alternative splicing of the apoL-I gene located on chromosome 22 with the production of two isoforms of 44 and 46 kDa, and cleavage of a 27-residue signal peptide.

**ApoM.** ApoM is a novel, 25 kDa, 188-amino acid apolipoprotein that was isolated and characterized in 1999 [73]. It is mainly found in HDL, but also in VLDL and LDL [74] (Table 1.1). ApoM possesses an eight-stranded anti-parallel beta-barrel lipocalin fold and a hydrophobic pocket that ensures the binding of small hydrophobic molecules, such as retinol and retinoic acid [75] (Figs 1.2 and 1.3). ApoM reveals 19% homology with apoD, another apolipoprotein member of the lipocalin family. The 1.95 Å resolution crystal structure analysis reveals that recombinant human apoM is complexed with fatty acids containing 14, 16 or 18 carbon atoms which can be replaced by sphingosine 1-phosphate (S1P) [30]. The gene for apoM is located on chromosome 6 and is synthesized in the liver and kidney. Expression of the apoM gene in the liver is regulated by transcription factors that control key stages in hepatic lipid and glucose metabolism [76]. Interestingly, apoM is exclusively associated with lipoproteins despite lacking an external amphipathic motif. The binding of apoM to lipoproteins is ensured by its hydrophobic N-terminal signal peptide which is retained on secreted apoM, a phenomenon atypical for plasma apolipoproteins. The retained signal peptide functions to anchor apoM to the lipoprotein surface and prevents rapid loss by filtration in the kidney [77–79].

**Other Apolipoproteins.** Minor protein components isolated within the density range of HDL are exemplified by apoB and apo(a), which reflect the presence of Lp(a) and result from overlap in the hydrated densities and physicochemical properties of large, light HDL2 and Lp(a) [10]. ApoO, another minor HDL component, is a 198-amino acid protein that contains a 23-amino acid signal peptide and is expressed in several human tissues [80]. ApoO belongs to the proteoglycan family, is present in HDL, LDL and VLDL and contains chondroitin sulfate chains, a unique feature distinguishing it from other apolipoproteins. At present, the physiologic function of apoO remains unknown [81].

**OPEN QUESTION**

By what type of interactions are the multiple protein components of HDL associated with its surface?
Enzymes

Plasma HDL particles equally carry enzymes involved in lipid metabolism and redox reactions (Table 1.1), including LCAT, paraoxonase 1 (PON1), PON3, platelet-activating factor-acetyl hydrolase (PAF-AH, also called lipoprotein-associated phospholipase A\(_2\) [LpPLA\(_2\)]) and glutathione selenoperoxidase 3 (GSPx-3) [82].

**LCAT.** Discovered by John Glomset in 1962 [83], LCAT (EC 2.3.1.43) catalyzes the esterification of cholesterol to cholesteryl esters in plasma lipoproteins, primarily in HDL, but also in apoB-containing particles (Table 1.1; see also 4.1 of chapter 4). Approximately 75% of plasma LCAT activity is associated with HDL. In plasma, LCAT is closely associated with apoD which frequently co-purify [84].

The LCAT gene, located on chromosome 16, is expressed primarily in the liver and, to a lesser extent, in the brain and testes. The primary amino acid sequence of LCAT is highly conserved between species. Mature LCAT protein is synthesized from a 440-residue precursor following cleavage of a 24-residue signal peptide. The mature protein contains 416 amino acids and is heavily N-glycosylated. As a result, its molecular mass of 63 kDa is higher than predicted. The tertiary structure of LCAT is maintained by two disulfide bridges and resembles that of lipases and other proteins of the alpha/beta hydrolase fold family [85] (Fig. 1.3). The structure involves an active site formed by serine (Ser)181, aspartate (Asp)345 and histidine (His)377 residues and a lid formed by residues 53–71 which covers the active site [86]. In addition, LCAT contains two free cysteine residues at positions 31 and 184.

**PON1 and PON3.** Human paraoxonases are calcium-dependent lactonases and consist of three members: PON1, PON2 and PON3 [87]. The three PON genes are aligned on the long arm of chromosome 7 (Table 1.1).

The name “PON” reflects the ability of PON1, the first enzyme of the family (discovered in 1946 [88]), to hydrolyze the organophosphate substrate paraoxon (PON activity, EC 3.1.8.1). PON1 is capable of hydrolyzing a broad spectrum of organophosphate substrates and aromatic carboxylic acid esters, thereby representing the main means of protection of the nervous system against organophosphate toxicity. As the latter compounds do not normally occur \textit{in vivo}, subsequent studies have identified lactonase activity as the probable physiologic function of PON1 [89]; hydrolysis of homocysteine thiolactone has been proposed to represent a major step in this pathway [89] (see 7.2 and 7.3 of chapter 7). Catalytic activities of the enzyme require the binding of two calcium ions and involve reversible binding to the substrate as the first step of hydrolytic cleavage.

PON1 is structurally organized as a six-bladed beta-propeller, with each blade consisting of four beta-sheets [90] (Fig. 1.3). Two calcium atoms needed for the stabilization of the structure and catalytic activity are located in the central tunnel of the enzyme. Three helices, located at the top of the propeller, are involved in the anchoring of PON1 to HDL. The enzyme is N-glycosylated and may contain a disulfide bond.
Human PON1 is largely synthesized in the liver, but also in the kidney and colon [91], as a 355-amino acid protein and secreted into the blood after removal of the N-terminal Met residue. Circulating PON1 is, therefore, a 354-residue protein with a molecular mass of 43 kDa. In the circulation, PON1 is almost exclusively associated with HDL; such association is mediated by HDL surface phospholipids and requires the hydrophobic leader sequence retained in the secreted PON1. Interestingly, PON1 protein is much more widely distributed than its mRNA, possibly indicating the delivery of PON1 by HDL to various tissues [91].

PON3 is a 354-residue protein with potent lactonase, limited arylesterase and no PON activities. PON3 displays properties similar to those of PON1, such as the requirement for calcium, N-glycosylation, secretion in the circulation with retained signal peptide and association with HDL. Similar to PON1, PON3 is expressed in the liver but also, to a lesser extent, in the kidney. Interestingly, PON3 is specifically enriched in human follicular fluid [92].

Another member of the PON family, PON2 is a 354-residue intracellular enzyme not detectable in serum despite its expression in many tissues, including brain, liver, kidney and testis. As for other members of the family, PON2 hydrolyzes organophosphate substrates and aromatic carboxylic acid esters.

PAF-AH (LpPLA2). Equally termed LpPLA2, PAF-AH (EC 3.1.1.47) is a calcium-independent hydrolytic enzyme that degrades platelet-activating factor (PAF) by hydrolyzing the sn-2 ester bond to yield biologically inactive lyso-PAF [93] (Table 1.1). Such activity allows PAF-AH to regulate the biologic actions of PAF. The enzyme cleaves phospholipid substrates with a short residue at the sn-2 position and can, therefore, hydrolyze pro-inflammatory oxidized short-chain phospholipids in addition to PAF; however, it is inactive against long-chain non-oxidized phospholipids (see 7.2 and 7.3 of chapter 7).

The mammalian PAF-AH family consists of four enzymes, two of which belong to the phospholipase A2 subfamily designated Group VII; the other two have been classified as Group VIII phospholipases. Plasma PAF-AH belongs to the Group VII enzymes and is also known as PLA2G7; another enzyme of this group is liver type II PAF-AH (PAFAH2). PAF-AH is synthesized throughout the brain, white adipose tissue and placenta as a 441-amino acid precursor secreted in the circulation following the cleavage of a 21-residue signal peptide. The mature, 53 kDa enzyme contains 420 amino acid residues and is N-glycosylated. Macrophages secrete the largest amount of PAF-AH and represent the most important source of the circulating enzyme [94]. Plasma PAF-AH circulates in association with LDL and HDL particles, with the majority of the enzyme bound to particularly atherogenic, small, dense LDL and to Lp(a) [95]. The lipoprotein location of PAF-AH may affect both the catalytic efficiency and the function of the enzyme in vivo. The PAF-AH gene is located on chromosome 6.

The crystal structure of this enzyme, characterized by x-ray diffraction at a resolution of 1.5 Å, reveals a typical lipase alpha/beta-hydrolase fold and a catalytic triad of Ser273, His351 and Asp296 [96] (Fig. 1.3). The active site is close to the lipoprotein surface and, at the same time, accessible to the aqueous phase.
Two clusters of hydrophobic residues build a lipid-binding domain ensuring association with lipoproteins. C-terminal residues are essential for the association of human PAF-AH with HDL [97]. On the other hand, an acidic domain containing 10 carboxylate residues and a neighboring basic domain of 3 amino acid residues are probably involved in the enzyme partitioning between LDL and HDL [96].

**GSPx-3.** Also called glutathione peroxidase 3 (EC 1.11.1.9), plasma GSPx-3 was first purified in 1987 [98] (Table 1.1). The enzyme is distinct from two other members of the GSPx family, GSPx-1 and GSPx-2, which represent erythrocyte and liver cytosolic enzymes. All GSPx enzymes protect biomolecules from oxidative damage by catalyzing the reduction of hydrogen peroxide, lipid peroxides and organic hydroperoxide in a reaction involving glutathione.

Human GSPx-3 is a homotetrameric protein consisting of 206-residue subunits of a molecular mass of 22.5 kDa containing selenium as a selenocysteine residue at position 73. Human GSPx-3 is synthesized in the liver, kidney, heart, lung, breast and placenta from a gene located on chromosome 5. A GSPx-3 proprotein of 226-amino acid residues is processed with a cleavage of a 20-residue signal peptide before secretion in the circulation. In plasma, GSPx-3 is exclusively associated with HDL [99]. The enzyme sequence is highly conserved between species, suggesting a critical role in survival.

**METHODOLOGIC NOTE**

Prolonged ultracentrifugation in highly concentrated salt solutions can remove some proteins from HDL, whereas other methods of HDL isolation (gel filtration, immunoaffinity chromatography, precipitation) provide HDL extensively contaminated with plasma proteins or subject HDL to unphysiologic conditions capable of modifying its structure and/or composition (e.g., extreme pH and ionic strength involved in immunoaffinity separation).

**Lipid Transfer Proteins**

Lipid transfer between lipoprotein particles is an essential element in plasma lipoprotein metabolism. Two lipid transfer proteins of key importance for lipoprotein metabolism, CETP and phospholipid transfer protein (PLTP), can be associated with HDL [8, 100].

**PLTP.** PLTP is a 476-amino acid protein belonging to the bactericidal permeability-increasing protein (BPI)/lipopolysaccharide (LPS)-binding protein (LBP)/Plunc superfamily of proteins (Table 1.1). PLTP is widely distributed across different tissues, and is synthesized in placenta, pancreas, lung, kidney, heart, liver, skeletal muscle and the brain. In the circulation, PLTP is present...
as a 78 kDa protein primarily associated with HDL; part of the plasma PLTP pool may exist as inactive complexes [101]. PLTP converts HDL into larger and smaller particles and plays a role in extracellular phospholipid transport. In addition, PLTP can bind LPS (see 4.1 of chapter 4).

Synthesis of PLTP involves the production of a 493-residue precursor which is processed to a mature protein through the cleavage of a signal peptide of 17 residues. Mature PLTP contains multiple glycosylation sites and is stabilized by a disulfide bond (Fig. 1.3). The PLTP gene is located on chromosome 20. PLTP is a positive acute-phase reactant with a potential role in the innate immune system.

**CETP.** Similar to PLTP, CETP is synthesized as a 493-residue proprotein which is converted into the mature 476-residue protein by the cleavage of a signal peptide before secretion (Table 1.1). As for PLTP, CETP belongs to the BPI/LBP/Plunc superfamily and contains multiple N-glycosylation sites. In contrast, CETP is encoded on chromosome 16 and is primarily expressed by the liver and adipose tissue. In the circulation, a 74 kDa CETP protein shuttles between HDL and apoB-containing lipoproteins and facilitates the bidirectional transfer of cholesteryl esters and triglycerides between HDL and (V)LDL (see 4.1 of chapter 4). The structure of CETP, determined at 2.2 Å resolution, includes a hydrophobic tunnel filled with two cholesteryl ester molecules and is plugged by an amphiphilic phosphatidylcholine molecule at each end [102] (Fig. 1.3). Such interactions additionally endow CETP with phosphatidylcholine transfer activity. CETP may also undergo conformational changes to accommodate lipoprotein particles of different size, such as HDL, LDL and VLDL [102].

**CRITICAL CONTRIBUTION**

Detection of more than 50 proteins involved in lipid metabolism, acute phase response, protease inhibition and complement regulation in human HDL isolated by ultracentrifugation from healthy normolipidemic humans [8].

**Acute-Phase Response Proteins**

Acute-phase response proteins, whose plasma concentrations are markedly altered by acute inflammation, form the largest family of HDL-associated proteins. Surprisingly, acute-phase proteins (23 out of 48 detected) outnumber proteins implicated in lipid metabolism (22 out of 48) [5, 8], indicative of the biologic significance of their presence on HDL (Table 1.2).

**Serum Amyloid A.** Serum amyloid A (SAA) proteins are major acute-phase reactants secreted during the acute phase of the inflammatory response. This protein family is encoded by four genes found in many species, including humans. Located on chromosome 11, three of these genes are commonly expressed in humans, resulting in three protein isoforms: SAA1, SAA2 and SAA4. The high
level of conservation of SAA proteins throughout the invertebrate and vertebrate lineages suggests that they play an important physiologic role.

SAA proteins are predominantly produced by the liver. Hepatic expression of SAA1 and SAA2 genes is regulated by the pro-inflammatory cytokines interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-alpha. Expression of SAA1 and SAA2 in the liver is induced during the acute-phase reaction, resulting in an increase in their circulating levels by as much as 1000-fold from basal concentrations of about 1 to 5 mg/l [103]. In contrast, SAA4 is constitutively expressed in the liver and is, therefore, termed “constitutive SAA”.

SAA1, the major member of this family, is a 12 kDa acute-phase protein whose circulating levels can be induced up to 1000-fold [104] (Table 1.1; see also see 8.1 of chapter 8). HDL is a major carrier of SAA1 in human, rabbit and murine plasma [105–107]. In the circulation, SAA1 does not exist in a free form and associates with non-HDL lipoproteins in the absence of HDL [108]. SAA1 is bound to HDL via its N-terminal domain [109] and is synthesized as a 122-residue precursor which is subsequently secreted into the circulation following the removal of an 18-residue signal peptide.

Other Proteins. Other proteins involved in the acute-phase response that are associated with HDL, include LBP, fibrinogen, alpha-1-acid glycoprotein 2, alpha-2-HS-glycoprotein, alpha-1-antitrypsin, inter-alpha-trypsin inhibitor heavy chain H4, bikunin (alpha-1-microglobulin), haptoglobin-related protein (Hrp), kininogen-1, transthyretin, transferrin, hemopexin and retinol-binding protein [8] (Table 1.2). LBP is a 60-kDa acute-phase glycoprotein capable of binding the lipid A moiety of LPS of Gram-negative bacteria and facilitating LPS diffusion [110]. LBP is produced as a 481-residue protein containing a 25-residue signal peptide encoded on chromosome 20. LBP/LPS complexes appear to interact with the CD14 receptor to enhance cellular responses to LPS. In addition to LPS, LBP binds phospholipids, thereby acting as a lipid exchange protein [111]. Indeed, LBP belongs to the same BPI/LBP/Plunc protein superfamily as PLTP and CETP.

Fibrinogen is a common acute-phase protein synthesized by the liver that is converted by thrombin into fibrin during blood coagulation. Fibrinogen is a disulfide-linked heterohexamer containing two sets of three non-identical chains (alpha, beta and gamma) encoded on chromosome 4. Conversion of fibrinogen to fibrin by thrombin involves the cleavage of fibrinopeptides A and B from alpha and beta chains with the subsequent exposure of N-terminal polymerization sites. Formation of blood clot ensues via cross-linking between gamma chains and between alpha chains of different monomers catalyzed by factor XIIIa. Furthermore, factor XIIIa further stabilizes fibrin by incorporating inhibitors of fibrinolysis, such as alpha-2-antiplasmin. Fibrinogen also acts as a cofactor in platelet aggregation.

Alpha-1-acid glycoprotein 2, also termed orosomucoid-2, belongs to the calycin protein superfamily which also includes lipocalins and fatty acid-binding proteins. The protein is a positive acute-phase reactant whose synthesis increases
up to 50-fold upon inflammation under control of glucocorticoids, IL-1 and IL-6. Alpha-1-acid glycoprotein 2 appears to modulate the activity of the immune system during the acute-phase reaction. It is encoded on chromosome 9, expressed by the liver and secreted in plasma as a 183-residue protein following the cleavage of an 18-residue signal peptide. In plasma, the protein is N-glycosylated and stabilized by disulfide bonds.

**Alpha-2-HS-glycoprotein** promotes endocytosis, possesses opsonic properties and influences the mineral phase of bone. The protein shows affinity for calcium and barium ions and contains two chains, A and B, which are held together by a single disulfide bond. Alpha-2-HS-glycoprotein is encoded on chromosome 3, synthesized in the liver as a 367-residue full-length single-chain and secreted into plasma.

Alpha-1-antitrypsin, inter-alpha-trypsin inhibitor heavy chain H4, bikunin, Hrp and kininogen-1 are better known for their roles in the inhibition of proteolysis, whereas transthyretin (a thyroid hormone-binding protein), transferrin (an iron-transport protein), hemopexin (an iron-binding protein) and retinol-binding protein are highly specialized plasma proteins described below.

Importantly, plasma levels and/or activities of some major HDL proteins, such as apoA-I, apoA-IV and PON1 are equally affected by the acute-phase response [82] (see 8.1 of chapter 8). A more recently discovered example of a HDL protein involved in the acute-phase response is provided by parotid secretory protein, a salivary protein secreted from the parotid glands. In hamsters, this protein represents a positive acute-phase reactant present exclusively in HDL [112]. The C-terminus of hamster parotid secretory protein contains a region homologous to the N-termini of a family of HDL-associated proteins, including LBP, CETP and PLTP. In addition to salivary glands, parotid secretory protein is expressed in lung, testis and ovary where it is synthesized as a 235-residue precursor containing a 20-residue signal peptide [112].

**Complement Components**

HDL-associated proteins involved in complement regulation are represented by complement components 3, 4a, 4b and 9, C4b binding protein and vitronectin [8] (Table 1.2).

**Complement component 3 (C3)** plays a central role in the activation of the complement system both through the classical and alternative complement activation pathways. The full-length single-chain C3 precursor is a large glycoprotein of 1663 amino acid residues with a predicted molecular mass of 187 kDa (Table 1.1). The precursor is processed to form two chains, beta and alpha, linked by a disulfide bond. The key initiating event in complement activation is the cleavage of C3 into a small, 77-residue C3a anaphylatoxin and a large, 915-residue C3b fragment by protease C3-convertase. After activation, C3b can covalently bind via its reactive thioester to cell surface carbohydrates or immune aggregates.

C4 is a key component involved in the activation of the classical pathway of the complement system. The full-length, single-chain C4 precursor contains
1744 amino acid residues and has a predicted molecular mass of 193 kDa. Prior to secretion, the single-chain precursor is enzymatically cleaved to yield alpha, beta and gamma chains. As a result, C4 circulates in blood as a disulfide-linked trimer of alpha, beta and gamma chains. During activation, the alpha chain is cleaved by C1 into a small, 77-residue \textbf{C4a} anaphylatoxin and a large, 690-residue \textbf{C4b} fragment that remains associated with the beta and gamma chains. The C4b fragment is the major activation product and an essential subunit of the C3 convertase (C4b2a) and C5 convertase (C3bC4b2a) enzymes of the classical complement pathway.

\textbf{C4b binding protein} controls the classical pathway of complement activation. It binds as a cofactor to C3b/C4b inactivator, which then hydrolyzes complement fragment C4b. It also accelerates the degradation of C3 convertase by dissociating the complement fragment C2a from C4b. C4b binding protein represents a disulfide-linked complex of alpha and beta chains, with the alpha chain ensuring C4b binding. The protein is synthesized as a 597-residue precursor secreted following the cleavage of a 48-residue signal peptide.

\textbf{C9} is a pore-forming subunit of the membrane attack complex that provides an essential contribution to the innate and adaptive immune response by forming pores in the plasma membrane of target cells. C9 is secreted as a soluble monomer that subsequently oligomerizes at target membranes, forming a pre-pore and, following a conformational change, a pore. C9 is produced as a 559-residue precursor secreted as a mature protein after the cleavage of a 21-residue signal peptide.

\textbf{Vitronectin} is another HDL-associated protein involved in complement regulation. It belongs to cell-to-substrate adhesion molecules present in serum and tissues that interact with glycosaminoglycans and proteoglycans and can be recognized by certain members of the integrin family. In complement regulation, vitronectin serves as an inhibitor of the membrane-damaging effect of the terminal cytolytic pathway. The full 478-residue vitronectin proprotein is first processed with the release of a 19-residue signal peptide that can subsequently be cleaved into two chains: a 65 kDa subunit and a 10 kDa subunit, the first of which can be further cleaved with the release of somatomedin-B. The latter represents a growth hormone-dependent serum factor with protease-inhibiting activity, thereby linking complement regulation and protease inhibition pathways. The role of vitronectin in protease inhibition is further underlined by its interaction with plasminogen activator inhibitor type 1 (PAI-1). Vitronectin is largely expressed in the liver but is also expressed in visceral tissue and the adrenal glands. The presence of vitronectin in HDL indicates that certain HDL components can be derived from non-cellular sources or cells distinct from those that synthesize apoA-I in the liver and intestine [5].

The biologic role of the association of complement components with HDL presently remains unclear. The multitude of complement components found in HDL suggests that this lipoprotein may serve as a platform for the assembly of proteins involved in the innate immune response [5]. In addition, complement regulatory proteins present in HDL, such as vitronectin, may limit injury to cardiac cells and prevent activation of the coagulation cascade [7].
MAJOR CONTROVERSY

As the abundance of most of the HDL-associated proteins is below 1 mol/mol HDL (i.e., less than 1 copy per HDL particle), it remains unclear as to how they are distributed among HDL subpopulations of potentially distinct origin and function. Furthermore, the high molecular mass of some HDL-associated proteins, such as complement components C3 and C4, is comparable with the average mass of HDL particles, suggesting associations with specific HDL subpopulation(s).

Proteinase Inhibitors and Related Proteins

A family of proteins in HDL contains serine proteinase inhibitor domains [8] (Table 1.2). Serine protease inhibitors, termed serpins, are key regulators of numerous biologic pathways involved in inflammation, coagulation, angiogenesis and matrix degradation. This family is exemplified by alpha-1-antitrypsin, a potent inhibitor of serine proteinases [12] (Table 1.1). Its primary target is elastase, but it also has moderate affinity for plasmin and thrombin. Alpha-1-antitrypsin is produced from a gene located on chromosome 14 as a 418-residue propeptide with a predicted molecular mass of 47 kDa that contains a 24-residue signal peptide. In the circulation, alpha-1-antitrypsin is present exclusively in HDL as a 52 kDa protein [113].

Alpha-2-antiplasmin, a serine proteinase inhibitor that inhibits plasmin and trypsin as two major targets and also inactivates chymotrypsin, is another key proteinase inhibitor that circulates, in part, associated with HDL. Alpha-2-antiplasmin is encoded by the SERPINF2 gene on chromosome 17, is expressed by the liver and is secreted in plasma as a 452-residue mature peptide.

Next, HDL carries inter-alpha-trypsin inhibitor heavy chain H4 and bikunin, two components of inter-alpha-trypsin inhibitors consisting of three out of four heavy chains from groups 1 to 4 and one light chain from the alpha-1-microglobulin/bikunin precursor or Kunitz-type protease inhibitor 2 groups. The full complex inhibits trypsin, plasmin and lysosomal granulocytic elastase. Inter-alpha-trypsin inhibitor heavy chain H4 is produced in the liver as a 930-amino acid proprotein secreted following the cleavage of a 28-residue signal peptide. In plasma, the protein is cleaved by kallikrein to yield 100 kDa and 35 kDa fragments; the resulting 100 kDa fragment is further converted to a 70 kDa fragment. The protein is encoded by chromosome 3 and appears to be both N- and O-glycosylated. Bikunin is a light chain of the inter-alpha-trypsin inhibitor produced via the proteolytic cleavage of the alpha-1-microglobulin/bikunin precursor protein together with alpha-1-microglobulin and trypstatin. The precursor is synthesized in the liver from a gene located on chromosome 9 as a 352-residue protein.
Other proteolysis-related proteins detected on HDL include Hrp, kininogen-1, prothrombin, angiotensinogen and procollagen C-proteinase enhancer-2 (PCPE2). Hrp contains a crippled catalytic triad residue that may allow it to act as a decoy substrate to prevent proteolysis. Hrp belongs to the peptidase S1 family and contains one peptidase S1 domain but does not possess essential catalytic residues and displays no enzymatic activity. It is synthesized as a 348-residue proprotein containing a 19-residue signal peptide from a gene located on chromosome 16.

**Kininogen-1** (alpha-2-thiol proteinase inhibitor) plays an important role in blood coagulation and inhibits the thrombin- and plasmin-induced aggregation of thrombocytes. The active peptide, bradykinin, released from high-molecular weight kininogen shows a variety of physiologic effects, including the induction of hypotension. Kininogen-1 is synthesized as a 644-amino acid proprotein that is further processed into a mature form.

**Prothrombin** is a 579-residue precursor of thrombin, a key serine protease of the coagulation pathway. Thrombin is produced by the enzymatic cleavage of two sites on prothrombin by activated factor X. The activity of factor X is greatly enhanced by binding to activated factor V, termed the prothrombinase complex. Prothrombin is produced in the liver and encoded on chromosome 11.

**Angiotensinogen** is a 452-amino acid long member of the serpin family, although it is not known to possess inhibitory activity towards enzymes. Angiotensinogen is an alpha-2-globulin constitutively produced and released into the circulation mainly by the liver. Angiotensinogen represents a substrate for renin whose action forms angiotensin I, a 10-amino acid peptide. Angiotensin I serves as a precursor to angiotensin II, an endocrine, autocrine/paracrine and intracrine hormone produced by the action of angiotensin-converting enzyme.

**PCPE2**, a 415-residue heparin-binding protein containing a cleavable 23-residue signal peptide, is another example of a HDL-associated protein involved in proteolytic regulation [114]. Indeed, PCPE2 binds to the C-terminal propeptide of type I and II procollagen and enhances the cleavage of the propeptide by bone morphogenetic protein 1 (BMP-1, also termed procollagen C-proteinase). Furthermore, PCPE2 plays a role in apoA-I production, accelerating proteolytic processing of pro-apoA-I by enhancing cleavage of the hexapeptide extension present at the N-terminus of apoA-I [114]. PCPE2 interacts with BMP-1 and pro-apoA-I to form a ternary PCPE2/BMP-1/pro-apoA-I complex. Interestingly, PCPE2 is present in mature HDL particles, indicating that it either remains associated with apoA-I after its maturation or that maturation occurs in HDL particles [115].

**Alpha-2-HS-glycoprotein** and **serpin peptidase inhibitor [clade F, member 1]** are minor HDL-associated proteins involved in the inhibition of proteolysis. The presence of multiple protease inhibitors in HDL suggests that these lipoproteins may play previously unsuspected roles in enhancing plaque stability via the protection of vascular lesions from excessive proteolysis [5], as well in intravascular lipoprotein remodeling.
Other Protein Components

HDL may also carry proteins involved in the regulation of other biologic functions (Table 1.2), such as Wnt signaling molecules which participate in cell-to-cell signaling [116]. Wnt proteins are secreted in the circulation and play a role in development and differentiation. In cultured mammalian cells, Wnt3a protein is associated with both HDL and LDL in the culture medium, whereas only HDL allows Wnt3a release from mouse fibroblasts. Palmitoylation of the protein is essential for its association with HDL [116]. Wnt3a is produced as a 352-residue proprotein in placenta, lung, spleen and prostate, with a signal peptide of 18 residues. It is a ligand for members of the frizzled family of seven transmembrane receptors which play distinct roles in cell-to-cell signaling during morphogenesis of the developing neural tube [116].

Another example of a HDL-associated regulatory protein is progranulin, a 593-amino acid, cysteine-rich protein with an estimated molecular weight of 68.5 kDa encoded by a single gene on chromosome 17 [117]. Progranulin is a precursor of seven small peptides, termed granulins, which are produced from it through proteolytic cleavage by extracellular proteases. Granulins are synthesized in epithelial cells, inflammatory cells and bone marrow, display cytokine-like activity and may play a role in inflammation, wound repair and tissue remodeling.

HDL has also been reported to transport distinct proteins displaying highly specialized functions. The metabolic purpose of such association is unclear; it might prolong the residence time of a protein or represent a mechanism for protein conservation in the circulation. For example, plasma retinol-binding protein, a small 21 kDa protein of 201 amino acid residues that delivers retinol from the liver stores to peripheral tissues, co-isolates with HDL3 [8]. In plasma, the complex of retinol-binding protein and retinol interacts with transthyretin, thereby preventing the loss of retinol-binding protein by filtration through the kidney glomeruli. About 40% of plasma transthyretin circulates in a tight protein-protein complex with plasma retinol-binding protein. As a corollary, transthyretin, a thyroid hormone-binding protein, is equally present on HDL [8, 10, 11]. Transthyretin is a homotetramer with four 127-residue subunits assembled around a central channel that can accommodate two ligand molecules. Each subunit possesses two stranded beta sheets and reveals an ellipsoidal shape. Transthyretin circulates in HDL through binding to apoA-I and is able to cleave the C-terminus of lipid-free apoA-I [118].

Transferrin is an iron-transport glycoprotein encoded by chromosome 3 and largely produced in the liver. Transferrin is associated with ultracentrifugally isolated HDL and represents an 80 kDa polypeptide of 679 amino acids that contains 2 specific high-affinity Fe(III) binding sites and reversibly binds iron. Hemopexin, an iron-binding protein that binds heme and transports it to the liver for breakdown and iron recovery, equally co-isolates with HDL3 [8]. The protein is encoded by chromosome 11, expressed in the liver, synthesized as a 462-amino acid precursor and is secreted in plasma as a 439-amino acid mature protein; in the circulation, hemopexin is N- and O-glycosylated.
In addition, HDL transports lysosomal proteins such as prenylcysteine oxidase, which is involved in the degradation of prenylated proteins [8]. The enzyme consists of 505 amino acids, is N-glycosylated, cleaves the thioether bond of prenyl-L-cysteines, such as farnesylcysteine and geranylgeranylcysteine, and requires flavin adenine dinucleotide as a cofactor. Other minor abundance proteins reported to be associated with HDL are: albumin, alpha-1B-glycoprotein, alpha-amylase, vitamin D-binding globulin and platelet basic protein [8, 10].

Interestingly, many proteins traditionally known to be largely, or exclusively, carried by HDL are also present in LDL and VLDL isolated by density gradient ultracentrifugation. These proteins include apoA-I, apoA-II, apoA-IV, apoD, apoF, apoJ, apoL-I, apoM, PON1 and SAA4 [119]. Furthermore, the content of some of these proteins, primarily of apoJ and apoF, is strongly (>15-fold) elevated in the electronegative LDL− subfraction [120].

In addition to proteins, HDL carries a number of small peptides of between 1 and 5 kDa in mass [11]. Indeed, more than 100 peptide components can be identified in HDL purified by density gradient ultracentrifugation or fractionated by size-exclusion chromatography. These peptides are present in HDL at low concentrations of about 1% of total HDL protein, with some representing fragments of larger proteins, such as apoB, fibrinogen and transthyretin [11]. The family of HDL-associated peptides remains poorly investigated; a 7 kDa anionic peptide factor represents a rare exception as it has been reported to contribute to the biologic activities of HDL [121, 122]. Another example of a specialized HDL-associated peptide with distinct function is provided by amyloid-beta, the principal peptide constituent of senile plaques in Alzheimer’s disease and a potent chelator of transition metal ions [123]. The association of small peptides with HDL as a vehicle may represent a pathway for peptide delivery or scavenging in order to slow renal clearance and proteolysis, and a significant reservoir of plasma peptides for diagnostic evaluation [11]. The diversity of molecules that bind to lipoprotein particles suggests that lipoproteins can serve as a versatile adsorptive surface for proteins and peptides.

Importantly, the composition of the HDL proteome strongly depends on the method of HDL isolation. Indeed, proteomic analyses of human plasma HDL obtained by ultracentrifugation in KBr solutions detect some 50 individual proteins [5, 8]. However, it is possible that the high ionic strength used for the isolation of lipoproteins with KBr alters the pattern of HDL-associated exchangeable proteins. Lipoprotein fractionation using buffers of physiologic ionic strength and pH prepared with deuterium oxide and sucrose may, therefore, improve characterization of exchangeable apolipoproteins and proteins in HDL [124]. Remarkably, proteomics of apoA-I-containing fractions isolated from human plasma by a non-denaturating approach of fast protein liquid chromatography (FPLC) reveal the presence of up to 115 individual proteins per fraction, only up to 32 of which were identified as HDL-associated proteins in ultracentrifugally-isolated HDL [125]. Co-elution with HDL of plasma proteins of matching size is inevitable in FPLC-based separation; the presence of a particular protein across
a range of HDL-containing fractions of different size would, however, suggest that such a protein is, indeed, associated with HDL [126]. Remarkably, several of the most abundant plasma proteins, including albumin, haptoglobin, transferrin and alpha-2-macroglobulin, are indeed present in all apoA-I-containing fractions [125], suggesting their partial association with HDL by a non-specific, low-affinity binding.

**CLINICAL SUMMARY**

HDL represents a protein-rich particle including multiple protein and lipid components which are potentially relevant to both the protection of the arterial wall from atherogenesis and to the regulation of innate immunity and protection from infection.

1.2 LIPIDOME

**Phospholipids**

Phospholipids constitute the major lipid class of HDL, build the surface lipid monolayer and ensure specific particle structure. Phospholipids quantitatively predominate in the HDL lipidome, accounting for approximately half of all lipids on a weight basis (Table 1.3). Typically, total plasma HDL contains 20–30% (wt/wt) phospholipids, 3–5% free cholesterol, 14–18% cholesteryl esters and 3–6% triglycerides.

Phosphatidylcholine and sphingomyelin predominate as major molecular classes of phospholipids in HDL. In addition, HDL contains significant amounts of phosphatidylinositol, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylethanolamine- and phosphatidylethanolamine-derived plasmalogens and ceramide [16, 17, 127]. Minor HDL phospholipids are represented by phosphatidylglycerol, phosphatidylserine, phosphatidic acid and cardiolipin [18, 21, 22]. As a result, HDL is a major carrier of phosphatidylcholine, phosphatidylethanolamine, phosphatidylethanolamine-derived plasmalogens and lysophosphatidylcholine in plasma; in contrast, the largest amount of sphingomyelin is carried in the circulation by LDL [17].

Phosphatidylcholine accounts for approximately 70% of HDL phospholipid; major molecular species of phosphatidylcholine are represented by the 18:2/16:0, 18:2/18:0 and 20:4/16:0 species [16]. Compared with other lipoproteins, HDL is enriched in phosphatidylcholine containing polyunsaturated fatty acid (PUFA) moieties [17]. Phosphatidylcholine in HDL can be of both hepatic (via formation of nascent HDL) and extrahepatic (via the action of PLTP on apoB-containing lipoproteins) origin. In contrast, sphingomyelin is largely delivered to HDL from triglyceride-rich lipoproteins via PLTP-mediated transfer and only originates from nascent HDL to a minor extent [128]. Sphingomyelin content constitutes a critical factor in determining surface pressure in lipid membranes and lipoproteins,
enhancing rigidity [129, 130] (see Section 1.8.2 chapter 7). **Lysophosphatidylcholines**, products of the LCAT reaction, constitute a minor subclass of HDL phospholipids, with fatty acid moieties of predominantly 16 and 18 carbon atoms [17].

**Phosphatidylinositol, phosphatidylserine** and **phosphatidic acid** are negatively charged, minor phospholipids that may significantly impact on the net surface charge of HDL [21, 131, 132], thereby modulating the interaction of HDL with lipases, extracellular matrix and other protein components.

**Steroids**

Together with phospholipids, **steroids** are located in the surface lipid monolayer of HDL particles and regulate its fluidity. HDL steroids are dominated by cholesterol (Table 1.3), reflecting the key role of this lipoprotein in cholesterol transport through the body. Other sterols are present in HDL at much lower levels, as exemplified by minor amounts of oxysterols (27-hydroxycholesterol, 24-hydroxycholesterol, cholesterol-5,6-beta-epoxide, 7-ketocholesterol) [23] and estrogens (largely present as esters) [133].

**Cholesteryl Esters**

**Cholesteryl esters** are formed in HDL as a result of trans-esterification of phospholipids and cholesterol catalyzed by LCAT. These highly hydrophobic lipids form the lipid core of HDL. Most of HDL cholesteryl ester is accounted for by cholesteryl linoleate [16] (Table 1.3).

**Triglycerides**

HDL **triglycerides** are derived from apoB-containing triglyceride-rich lipoproteins as a result of CETP-mediated heteroexchange with cholesteryl esters originating from HDL. Similar to cholesteryl esters, triglycerides are hydrophobic and are located in the HDL lipid core. Compared with cholesteryl esters, however, triglycerides form a more fluid phase. Triglycerides are dominated by species containing oleic, palmitic and linoleic acid moieties [17].

**Minor Lipids**

Minor bioactive HDL lipids include diacylglycerides, monoacylglycerides, free fatty acids, glycosphingolipids, gangliosides, sulfatides, and lysosphingolipids [15, 17, 134–136]. Interestingly, HDL is relatively depleted in ceramides, glycosphingolipids and gangliosides compared with LDL. Significantly, **ceramides** play a key role as signaling molecules involved in cellular survival, growth and differentiation, whereas **gangliosides** determine interactions with protein receptors and signal transduction; the physiologic relevance of their presence in HDL remains indeterminate.

Among lysosphingolipids, **sphingosine 1-phosphate (S1P)** is particularly interesting as this bioactive lipid plays a key role in vascular biology and can
function as a ligand for the family of G protein-coupled S1P receptors present on endothelial and smooth muscle cells, which regulate cell proliferation, motility, apoptosis, angiogenesis, wound healing and immune response (see chapter 7). HDL is the major carrier of S1P in the circulation, ensuring its bioavailability [137]. Indeed, more than 90% of sphingoid base phosphates are found in HDL and albumin-containing fractions by liquid chromatography/mass spectrometry (LC/MS) analysis [127]. S1P is produced by phosphorylation of sphingosine by sphingosine kinases which are expressed in platelets, erythrocytes, neutrophils and mononuclear cells. Erythrocytes appear to represent the primary source of S1P in plasma followed by platelets [138, 139]; S1P release from erythrocytes can be triggered by HDL and serum albumin [141]. Other biologically active lysolipids carried by HDL are represented by sphingosylphosphorylcholine and lysosulfatide [141]. Finally, HDL carries minor amounts of lipophilic vitamins and antioxidants, such as tocopherols, carotenes and co-enzyme Q10 [142–144].

### IMPORTANT READING


### REFERENCES


REFERENCES


