REVIEW

High-density lipoproteins, inflammation and oxidative stress

Fatiha TABET* and Kerry-Anne RYE*†‡

*Lipid Research Group, Heart Research Institute, Camperdown, NSW 2050, Australia, †Faculty of Medicine, University of Sydney, Sydney, NSW 2006, Australia, and ‡Department of Medicine, University of Melbourne, Melbourne, VIC 3065, Australia

ABSTRACT

Plasma levels of HDL (high-density lipoprotein)-cholesterol are strongly and inversely correlated with atherosclerotic cardiovascular disease. Both clinical and epidemiological studies have reported an inverse and independent association between serum HDL-cholesterol levels and CHD (coronary heart disease) risk. The cardioprotective effects of HDLs have been attributed to several mechanisms, including their involvement in the reverse cholesterol transport pathway. HDLs also have antioxidant, anti-inflammatory and antithrombotic properties and promote endothelial repair, all of which are likely to contribute to their ability to prevent CHD. The first part of this review summarizes what is known about the origins and metabolism of HDL. We then focus on the anti-inflammatory and antioxidant properties of HDL and discuss why these characteristics are cardioprotective.

HDLs (HIGH-DENSITY LIPOPROTEINS)

Origins of HDLs

Most of the HDLs in human plasma are spherical particles that contain a hydrophobic core of cholesteryl esters and a small amount of triacylglycerol (triglyceride) surrounded by a monolayer of phospholipids (mainly phosphatidylcholine), apolipoproteins and unesterified cholesterol (Figure 1A). ApoA-I (apolipoprotein A-I), the most abundant HDL apolipoprotein, is synthesized mainly in the liver and is secreted into the plasma in a lipid-free or lipid-poor form (Figure 1D) [1]. As lipid-free/lipid-poor apoA-I enters the circulation it acquires phospholipids and unesterified cholesterol from cell membranes and other lipoproteins and is converted into discoidal HDL [2]. Discoidal HDLs consist of phospholipid bilayers that contain a small amount of unesterified cholesterol. The phospholipid acyl chains in these particles are shielded from the aqueous environment by an annulus of apolipoproteins (Figure 1A) [2–4]. Discoidal HDLs are excellent substrates for LCAT (lecithin:cholesterol transferase), the enzyme that generates almost all of the cholesteryl esters in plasma [2,5]. As cholesteryl esters are hydrophobic, they move into the centre of the HDL particles as soon as they are formed. This process is responsible for the conversion of

Key words: antioxidant, cholesterol, coronary heart disease, high-density lipoprotein (HDL), inflammation.

Abbreviations: ABC transporter, ATP-binding-cassette transporter; apoA etc., apolipoprotein A etc.; CETP, cholesteryl ester transfer protein; EL, endothelial lipase; eNOS, endothelial NO synthase; HDL, high-density lipoprotein; A-I HDL, HDL containing apoA-I without apoA-II; A-I/A-II HDL, HDL containing apoA-I and apoA-II; HL, hepatic lipase; HOCI, hypochlorous acid; HUVEC, human umbilical vein endothelial cell; ICAM-1, intercellular adhesion molecule-1; IL-1, interleukin-1; IVUS, intravascular ultrasound; LCAT, lecithin:cholesterol transferase; LDL, low-density lipoprotein; LDLR, LDL receptor; LPO, lipoxygenase; LPS, lipopolysaccharide; MCP-1, monocyte chemotractant protein-1; M-CSF, macrophage colony-stimulating factor; MPO, myeloperoxidase; MRI, magnetic resonance imaging; NF-κB, nuclear factor κB; O2−, superoxide anion; ONOO−, peroxynitrite; PAF-AH, platelet-activating factor acetylhydrolase; PLTP, phospholipid transfer protein; PMN, polymorphonuclear neutrophil; PON, paraoxonase; rHDL, reconstituted HDL; ROS, reactive oxygen species; TNF-α, tumour necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; VLDL, very-LDL; VSMC, vascular smooth muscle cell.

Correspondence: Professor Kerry-Anne Rye (email ryek@hri.org.au or karye@ozemail.com.au).
Heterogeneity of HDL subpopulations

(A) HDLs enter the circulation as discoidal particles that are rapidly converted into large spherical HDLs by LCAT. (B) Spherical HDLs are classified on the basis of their apolipoprotein composition into A-I HDL and A-I/A-II HDL. (C) Spherical HDLs are classified on the basis of size into five subpopulations of particles with diameters ranging from 10.6 to 7.6 nm. (D) Lipid-free/lipid-poor apoA-I that dissociates from spherical HDLs duringremodelling by plasma factors such as LCAT, HL, CETP and PLTP is converted into new HDL particles.

Discoidal HDLs are the smallest and densest of all the plasma lipoproteins. One of the most striking characteristics of HDLs is that they are extremely heterogeneous and consist of a number of discrete subpopulations of particles that vary widely in density, size, shape, composition and surface charge. HDLs are classified on the basis of density into two main subfractions: HDL2 (1.063 < d < 1.125 g/ml) and HDL3 (1.125 < d < 1.21 g/ml), and on the basis of size into five discrete subpopulations ranging from 10.6 to 7.6 nm in diameter (Figure 1C) [9].

The most abundant apolipoproteins in human HDLs are apoA-I and apoA-II. HDLs are classified on the basis of their apolipoprotein composition into two main subpopulations: those that contain apoA-I without apoA-II (A-I HDL), and those that contain both apoA-I and apoA-II (A-I/A-II HDL) (Figure 1B) [10]. A-I/A-II HDLs tend to be smaller and more dense than A-I HDLs, and prevail in the HDL3 subfraction. A-I HDLs are mostly found in the HDL2 subfraction. Other apolipoproteins that are associated with HDLs include apoA-IV (the third most abundant HDL apolipoprotein after apoA-I and apoA-II), the C-apolipoproteins (apoC-I, C-II and C-III), apoD, apoE, apoJ, apoL and apoM [10–14]. HDLs also transport a number of other proteins such as PON (paraoxonase), which inhibits oxidation [15], and acute-phase proteins [16]. A shotgun proteomics approach has recently been used to identify several additional proteins, including protease inhibitors, complement factors and complement regulatory proteins, that are associated with HDLs [17]. Although very little is known about the impact of most of these proteins on HDL function, this observation is worthy of further investigation.

When subjected to agarose gel electrophoresis, HDLs can be resolved on the basis of surface charge into particles with either α-, pre-β- or γ-mobility [18,19]. Spherical HDLs migrate to an α position during agarose gel electrophoresis. α-Migrating HDLs include HDL2 and HDL3, as well as A-I HDLs and A-I/A-II HDLs [20]. Lipid-free/lipid-poor apoA-I and discoidal HDLs both migrate to a pre-β position [18]. HDLs with γ-mobility are large spherical particles that contain apoE, but not apoA-I [21].

Heterogeneity of HDLs: remodelling by plasma factors

Remodelling is defined as any process that changes the size, shape, surface charge or composition of HDLs. HDLs are continuously being remodelled by plasma factors such as LCAT, HL (hepatic lipase), EL (endothelial lipase), CETP (cholesteryl ester transfer protein) and PLTP (phospholipid transfer protein) [22]. One of the most important aspects of HDL remodelling is that it generates lipid-free/lipid-poor apoA-I, which is rapidly converted into new HDL particles by acquiring phospholipids and unesterified cholesterol from cell membranes and other lipoproteins as outlined above. The recycling of apoA-I between the lipid-free and lipid-associated forms is a fundamentally important aspect of HDL metabolism [23]. It delays the clearance of lipid-free apoA-I via the kidney, and thus maintains HDL levels in the plasma.

HL and EL are both members of the triacylglycerol lipase gene family. HL is synthesized mainly in hepatocytes, where it is anchored to glycosaminoglycans on the surface of endothelial cells [24,25]. It is also present at
lower levels in macrophages [26] and steroidogenic tissues [25,27]. EL, which has a much wider tissue distribution than HL, is synthesized in the liver, lung and kidneys as well as in endothelial cells [28,29]. HL and EL are approx. 40% homologous and both interact preferentially with HDLs [28]. Despite these similarities, EL and HL differ significantly in terms of their substrate specificities. HL hydrolyses HDL phospholipids and triacylglycerols [30]. It also mediates the dissociation of lipid-free/lipid-poor apoA-I from HDLs and decreases their size [31]. EL, by contrast, has high phospholipase activity but extremely low triacylglycerol lipase activity. Although it has the capacity to mediate a modest reduction in HDL size, this is not sufficient to cause lipid-free/lipid-poor apoA-I to dissociate from the particles [28,32].

LCAT has phospholipase as well as acyltransferase activities. These combined activities are responsible for generating most of the cholesteryl esters in plasma. Discoidal HDLs that contain apoA-I are the primary substrates of LCAT [5]. As LCAT interacts with the unesterified cholesterol in discoidal HDLs, the resulting cholesteryl esters are sequestered into the centre of the particles and the discs are converted into spherical HDLs. This depletes the HDL surface of cholesterol and establishes a concentration gradient down which additional unesterified cholesterol moves from cell membranes and other lipoproteins into the HDL fraction. LCAT also esterifies cholesterol in discoidal HDLs that contain other apolipoproteins, such as apoA-IV and apoE. However, the rate of cholesterol esterification in these particles is much slower than in apoA-I-containing discoidal HDLs [33,34]. It should also be noted that LCAT does not esterify cholesterol in discoidal HDLs that contains apoA-II [35].

CETP is a hydrophobic glycoprotein that transfers cholesteryl esters and triacylglycerols between different lipoprotein fractions. CETP can, for example, mediate bi-directional equimolar exchanges of cholesteryl esters between HDLs and LDLs (low-density lipoproteins) [36]. CETP remodels HDLs by transferring cholesteryl esters from HDLs to VLDLs (very-LDLs) in exchange for triacylglycerols, which it transfers in the reverse direction, from VLDLs into HDLs [37]. When the concentration of VLDLs is high and the cholesteryl ester transfers from HDLs to VLDLs exceed the transfers of triacylglycerol from VLDLs to HDLs, the HDLs become depleted of core lipids in a process that decreases their size and mediates the dissociation of lipid-free/lipid-poor apoA-I from the particles [38]. This decrease in HDL size, and the accompanying dissociation of lipid-free/lipid-poor apoA-I, is enhanced by HL, which rapidly hydrolyses the triacylglycerols that are transferred into HDLs by CETP [31,39].

PLTP is a member of the same LPS (lipopolysaccharide)-binding/lipid transfer protein family as CETP [40]. PLTP transfers phospholipids between HDL and other plasma lipoproteins, as well as between different HDL particles [41]. PLTP remodels HDLs into large and small particles by a process that involves particle fusion and the dissociation of lipid-free/lipid-poor apoA-I [42]. These events are enhanced in HDLs that are enriched with triacylglycerols [42,43]. The ability of lipid-free/lipid-poor apoA-I to dissociate from HDLs is a key determinant of the mechanism by which PLTP remodels HDLs. Thus, when PLTP interacts with HDLs that contain apoE, which does not exist in a lipid-free/lipid-poor form [34], the HDLs are remodelled into large and small particles via a complex cascade of particle fusions and structural re-organizations that are not accompanied by the dissociation of apoE [44].

**HDLs and reverse cholesterol transport**

The reverse cholesterol transport pathway is one of the better understood mechanisms by which HDLs protect against atherosclerosis [45]. This pathway involves the removal of cholesterol from cell membranes, including macrophages in the artery wall, and its transport to the liver, where it is excreted in the bile [46]. Lipid-free/lipid-poor apoA-I is a key acceptor of the cholesterol that is exported from cells in the first step of the reverse cholesterol transport pathway. The efflux of cholesterol from cell membranes to apoA-I is driven by the ATP-binding-membrane-cassette transporter ABCA1 [47]. Although studies in mice with specific inactivation of hepatic ABCA1 have identified the liver as a major source of the lipids that are exported to apoA-I [48–50], extrahepatic ABCA1 can also make a significant contribution to this process [51]. As apoA-I acquires lipids via its interaction with ABCA1, it is converted into particles that can accept additional cholesterol, and possibly phospholipids, from cell membranes via two other ATP cassette transporters, ABCG1 and ABCG4 [52,53]. ABCG1 also exports cholesterol from cell membranes to large spherical HDLs [54]. The sequential lipidation of apoA-I by ABCA1 and ABCG1/ABCG4 is particularly important for generating new spherical HDL particles, the cholesteryl esters of which are either selectively taken up by the liver via SR-B1 (scavenger receptor B1) [55] or transferred by CETP to LDL. The cholesteryl esters that are transferred from HDL to LDL are removed from the circulation by hepatic LDLRs (LDL receptors) [56]. This represents one of the key pathways for the disposal of cholesterol in humans, as evidenced by the hypercholesterolaemia and premature atherosclerosis in individuals with LDLR loss-of-function mutations [57].

**HDLs AND INFLAMMATION**

**HDLs and atherosclerotic plaque development**

Atherosclerosis, once regarded simply as excessive accumulation of lipids in the artery wall, is now considered to
be a chronic inflammatory disorder that is characterized by the presence of macrophages and other inflammatory cells in the arterial intima [58]. There is mounting evidence that HDLs inhibit the inflammation that is associated with atherosclerotic plaque development, including the initial step, where circulating leucocytes become tethered to the endothelial surface and migrate into the artery wall [59]. Under normal circumstances the endothelium is quiescent and resistant to leucocyte adhesion. However, pro-inflammatory stimuli, such as a diet high in saturated fat, hyperglycaemia, hypercholesterolaemia, obesity, insulin resistance and hypertension, activate the endothelium by increasing levels of circulating cytokines, such as IL-1 (interleukin-1) and TNF-α (tumour necrosis factor-α) [60–63]. This up-regulates endothelial expression of adhesion molecules such as ICAM-1 (intercellular adhesion molecule-1), VCAM-1 (vascular cell adhesion molecule-1), P-selectin and E-selectin and facilitates the capture, rolling and firm tethering of leucocytes, including monocytes, to the endothelial surface (Figure 2) [59]. Monocytes that have adhered firmly to the activated endothelium transmigrate into the intima in a process that is dependent on MCP-1 (monocyte chemoattractant protein-1) [64]. They then differentiate into macrophages by a process that involves MCSF (macrophage colony-stimulating factor) (Figure 2) [65]. In vitro and in vivo studies have demonstrated that HDLs are highly effective at inhibiting endothelial expression of adhesion molecules and preventing monocyte recruitment into the artery wall (Figure 2) [66,67].

LDLs also play an important role in atherosclerotic lesion development. When LDLs are recruited from the circulation into the artery wall they become oxidized and are converted into ligands for scavenger receptors that are expressed on the macrophage surface (Figure 2) [68]. The unregulated uptake of oxidized LDLs into macrophages generates foam cells, one of the hallmark cell types of atherosclerotic lesions [69]. HDLs inhibit these processes by preventing oxidized LDL formation (Figure 2) [70]. HDLs also have potent antithrombotic properties and can enhance plaque stability, thus reducing the likelihood of plaque rupture [71].

**Anti-inflammatory properties of HDLs: in vitro**

Numerous studies have shown that HDLs are potent anti-inflammatory agents. Cockerill et al. [66] were the first to report that HDLs inhibit inflammation in vitro. In this and subsequent studies, physiologically relevant concentrations of plasma HDLs and discoidal rHDLs (reconstituted HDLs) containing apoA-I complexed to phosphatidylcholine inhibited VCAM-1, ICAM-1 and E-selectin expression in activated HUVECs (human umbilical vein endothelial cells) in a time- and

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**Figure 2  Anti-inflammatory properties of HDLs**

HDLs inhibit inflammation by decreasing the expression of ICAM-1, VCAM-1, P-selectin and E-selectin, and inhibiting the tethering and firm adhesion of monocytes to the endothelial surface. HDLs also inhibit the expression of MCP-1, which prevents the transmigration of monocytes across the endothelium. HDLs also inhibit the oxidation of LDLs in the artery wall.
concentration-dependent manner [66,72]. This inhibition was apparent at the mRNA as well as the protein level [66]. These observations have since been confirmed by a number of other investigators [73,74]. It should be noted that this result is not specific for apoA-I, as rHDLs containing phosphatidylcholine and either apoA-II or a naturally occurring disulfide-linked dimeric form of apoA-I, apoA-I_{SII}, also inhibit VCAM-1 and ICAM-1 expression in stimulated cultured HUVECs [75,76].

There is, at present, a limited amount of information concerning the molecular mechanisms by which HDLs inhibit adhesion molecule expression. In 1999, Xia et al. [77] reported that HDLs inhibit sphingosine kinase, ERK (extracellular signal-regulated kinase) and NF-κB (nuclear factor κB) signalling cascades. There is also evidence suggesting that HDLs down-regulate VCAM-1 expression in TNF-α-activated HUVECs at the transcriptional level by blunted translocation and transactivation of NF-κB and AP-1 (activator protein-1) transcription factors [74,77].

In addition to inhibiting intracellular inflammatory signalling pathways that are involved in adhesion molecule expression, the anti-inflammatory properties of HDLs have also been linked to the inhibition of MCP-1 expression and monocyte transmigration into cells that have been stimulated with oxidized LDL [78,79]. rHDLs containing apoA-I and phosphatidylcholine also decrease the adherence of LPS-stimulated PMNs (polymorphonuclear neutrophils) to endothelial cells by binding directly to LPS and reducing its availability for interacting with the cell surface, as well as by decreasing CD11b/CD18 expression on PMNs [80,81].

Although rHDLs inhibit adhesion molecule expression in cultured endothelial cells in a reproducible manner, this is not the case for HDLs isolated from human plasma. In 1998, Ashby et al. [76] found that the ability of HDLs isolated from different human subjects to inhibit VCAM-1 expression in stimulated cultured HUVECs varied widely. The results of that study also established that the anti-inflammatory capacity of HDL₃ is superior to that of HDL₂ [76]. An insight into one of the underlying reasons for this variation comes from an in vitro study in which VCAM-1 expression in cultured HUVECs was shown to depend on the length and degree of unsaturation of rHDL phospholipid acyl chains [82]. This result suggests that differences in HDL phospholipid composition may explain, at least in part, why some investigators have found plasma HDLs from normal subjects not to be anti-inflammatory when incubated with endothelial cells [83,84].

**Anti-inflammatory properties of HDLs: in vivo**

The anti-inflammatory properties of rHDLs have also been studied in vivo in both animals and humans. Overall, the outcomes of these studies have been positive, with intravenous infusions of rHDLs inhibiting the increased adhesion molecule expression that occurs during the initial stages of atherosclerotic lesion development, and reducing the size and improving the composition of established atherosclerotic lesions.

In one of the earliest of these studies, peri-arterial cuffs were placed around the carotid arteries of cholesterol-fed apoE⁻/⁻ mice, causing an inflammatory response in the vessel wall. The animals were then treated for 3 weeks with alternate daily intravenous infusions of rHDLs containing apoA-I (40 mg/kg of body weight) complexed with phosphatidylcholine. By the end of the first week of treatment, endothelial VCAM-1 expression, monocyte/macrophage infiltration into the artery wall and oxidized LDL formation had all decreased. Neointima formation was significantly inhibited at 3 weeks [85]. These beneficial effects occurred without significant changes in plasma total cholesterol and HDL-cholesterol levels or arterial tissue cholesterol levels [85].

In a different approach, the anti-inflammatory properties of HDLs were investigated by using a mouse aortic transplantation model to study the effects of sustained elevation of circulating HDL levels on advanced atherosclerotic lesions in the thoracic aortas of apoE⁻/⁻ mice transgenic for human apoA-I [86]. Despite being maintained on a Western-type diet for 6 months, atherosclerotic lesion progression was retarded in these animals. This was due to a reduction in lesion macrophages and an increase in the smooth muscle cell content of the intima [86]. These results could not be explained in terms of a difference in the total plasma cholesterol levels of the animals. In a more recent study of apoE⁻/⁻ mice transgenic for human apoA-I, consumption of a Western-type diet reduced the area and increased the stability of the atherosclerotic lesions without affecting endothelial expression of VCAM-1, ICAM-1 and MCP-1 [87]. It should also be noted that introduction of the human apoA-I gene into apoE⁻/⁻ mice appears not to affect lipid deposition or endothelial activation during the early stages of atherosclerotic lesion development [88]. This suggests that raising endogenous apoA-I levels may be beneficial only when atherosclerotic lesions are already present.

HDLs can also inhibit acute vascular inflammation in normcholesterolaemic rabbits. In 2005, Nicholls et al. [67] reported that three infusions of small amounts of rHDLs containing apoA-I (8 mg/kg of body weight) and phosphatidylcholine inhibited the acute inflammation that occurs when non-occlusive silastic collars are placed around the carotid arteries of chow-fed normcholesterolaemic New Zealand White rabbits. These infusions, which were made 24 h prior to, at the time of and 24 h post-collar insertion, essentially abolished neutrophil infiltration into the intima-media, as well as the increase in endothelial expression of VCAM-1, ICAM-1 and MCP-1 that was caused by the collar. Comparable results were obtained in collared animals that were infused with...
lipid-free apoA-I (8 mg/kg of body weight) and, to a lesser extent, in collared animals that were infused with small unilamellar phospholipid vesicles [67]. In a recently published extension of that study, a single 8 mg/kg of body weight infusion of lipid-free apoA-I administered at the time of, or 3 h after, carotid collar insertion also inhibited the acute inflammatory response [89]. The results of the latter study also established that a single 2 mg/kg of body weight dose of apoA-I, which did not increase circulating apoA-I levels, inhibited acute vascular inflammation almost as effectively as a single 8 mg/kg of body weight dose of apoA-I [89]. These results suggest that rHDL has potential as a therapeutic agent for treating the acute vascular inflammation that occurs in acute coronary syndromes and stroke. A single infusion of rHDLs has also been reported to inhibit basal and IL-1-induced E-selectin expression by microvascular endothelial cells in a normocholesterolaemic porcine model [90].

Two reports of the effects of short-term administration of rHDLs containing phosphatidylcholine and apoA-I Milano (ETC-216) on atherosclerotic plaque size in New Zealand White rabbits have also been published recently [91,92]. In the first of these studies, MRI (magnetic resonance imaging) was used to establish that two 75 mg/kg of body weight infusions of rHDLs administered 4 days apart significantly decreased the size, increased the stability and induced favourable compositional changes in abdominal aortic lesions in animals that had been subjected to two mechanical aortic denudations and 9 months on a 0.2 % cholesterol diet [91]. In a second study, MRI and IVUS (intravascular ultrasound) were used to identify the minimum dose of ETC-216 required for reducing atherosclerotic lesion size in the carotid arteries of New Zealand White rabbits following electrical injury and consumption of a 1.5 % cholesterol-enriched diet for up to 90 days [92]. This was achieved by administering ETC-216 every 4 days at doses of apoA-I Milano ranging from 5 to 150 mg/kg of body weight. This study was conducted over 20 days. A significant reduction in atheroma volume was apparent in the animals that received ETC-216 at either 40 or 150 mg of apoA-I Milano/kg of body weight. When the animals received 150 mg of apoA-I Milano/kg of body weight, two doses were sufficient to induce significant lesion regression [92].

ApoA-I mimetics are short peptides that function the same way as wild-type apoA-I. In recent years, they have attracted considerable interest as potential therapeutic targets for preventing atherosclerosis. When administered orally to apoE−/− mice, the apoA-I mimetics D-4F and SF have been reported to inhibit atherosclerosis and LDL-induced monocyte chemotactic activity [93,94]. Other studies have indicated that D-4F increases reverse cholesterol transport and decreases the lipid hydroperoxide content of VLDL, LDL and HDL [93].

Finally, there are several reports indicating that infusion of rHDLs into humans is beneficial. A single intravenous infusion of rHDLs containing phosphatidylcholine and wild-type apoA-I (80 mg/kg of body weight) rapidly normalized endothelium-dependent vasodilation in hypercholesterolaemic men, presumably by increasing the bioavailability of NO [95]. In another study, intravenous administration of ETC-216 at either 15 or 45 mg/kg of body weight at weekly intervals for 5 weeks to subjects with acute coronary syndrome significantly reduced atherosclerotic lesion volume as determined by IVUS [96]. More recently, the results from a randomized controlled trial demonstrated that short-term infusions of rHDLs containing phosphatidylcholine and wild-type apoA-I at either 40 or 80 mg/kg of body weight significantly improved the plaque characterization index and coronary score [97]. It should be noted, however, that liver function was adversely affected at the higher dose of rHDLs in that study [97].

Although these small in vivo studies indicate that there is a potential therapeutic benefit associated with infusing HDLs into subjects with established atherosclerosis, it has yet to be established conclusively that raising endogenous HDL levels in humans reduces atherosclerotic lesion development or improves the composition of established lesions. We also do not know whether it is absolutely necessary to increase HDL levels or if altering gene expression or intracellular signalling cascades by administering much smaller amounts of apoA-I or rHDLs might be equally beneficial.

**HDLs AND OXIDATION**

**Sources of ROS (reactive oxygen species) in atherosclerosis**

ROS, which include \( \cdot O_2^- \) (superoxide anion), \( H_2O_2 \), \( OH^- \) (hydroxyl anion) and \( ONOO^- \) (peroxynitrite), are highly reactive by-products of \( O_2 \) metabolism that play a major role in the aetiopathology of cardiovascular diseases such as atherosclerosis [98,99]. To protect against the potentially damaging effects of ROS, cells possess several antioxidant enzyme systems such as SOD (superoxide dismutase), catalase, thioredoxins and glutathione peroxidase [98]. Normally, the rate of production of ROS is balanced by the rate of elimination; however, an imbalance between oxidant production and antioxidant capacity, a phenomenon known as oxidative stress, is associated with the vascular damage observed in atherosclerosis [100]. Several lines of evidence suggest that HDLs have antioxidant properties, which may protect against oxidative damage in atherosclerosis (Figure 3). This beneficial effect has been attributed to enzymes, such as PON1 and PAF-AH [platelet-activating factor acetylhydrolase; also known as Lp-PLA2 (lipoprotein-associated phospholipase A2)], present on the HDL surface [101,102].
ROS production has been attributed to increased activation of enzymes such as MPO (myeloperoxidase), NADPH oxidase, XO (xanthine oxidase), LPO (lipoygenase) and COX (cyclo-oxygenase), as well as dysfunction of the mitochondrial respiratory chain (Figure 3) [98,103–105]. ROS are implicated in several of the processes that are involved in atherosclerotic lesion development. These include endothelial dysfunction, increased expression of adhesion molecules, increased proliferation and migration of VSMCs (vascular smooth muscle cells) and increased oxidation of LDL [106]. HDLs can potentially reduce the detrimental effects of ROS production on atherosclerotic lesion development. For example, a previous investigation into the relationship between HDLs and MPO established that MPO predicts the progression of carotid atherosclerosis in subjects with low HDL-cholesterol levels [107].

Endothelial production of ROS, especially \( \cdot O_2^- \) and its rapid reaction with NO to generate ONOO\(^-\), a potent oxidant and mediator of vascular injury, is a key mechanism in the development of vascular dysfunction in atherosclerosis [108]. It has been suggested that some of the cardioprotective effects of HDLs in the vascular wall are mediated via the increase in eNOS (endothelial NO synthase) activity and the subsequent production of NO (Figure 3) [108,109]. This can prevent atherosclerotic lesion progression by inhibiting vasoconstriction, the proliferation of VSMCs and the adhesion of monocytes to the endothelium [110].

**LDL oxidation in atherosclerosis development**

Oxidative modification of LDL plays a major role in atherosclerotic lesion progression, atherosclerotic plaque destabilization and plaque rupture [111]. Macrophages, endothelial cells and VSMCs all promote LDL oxidation *in vitro* by activating the ROS-producing systems described above (Figure 3). Phagocyte-derived MPO and its enzymatic product, HOCl (hypochlorous acid), can also oxidize LDLs [112]. HOCl-modified LDLs have also been shown to stimulate ROS production and respiratory burst activation by neutrophils and macrophages [113,114]. Previous evidence has suggested that HDLs prevent LDL oxidation by inhibiting these deleterious events (Figure 3) [115].

**Antioxidant properties of HDLs**

The antioxidant properties of HDLs are largely related to their ability to inhibit LDL oxidation (Figure 3), although the precise mechanisms of these processes are still being elucidated [67,93,116]. It should also be noted that HDLs from subjects with systemic inflammatory disorders such as systemic lupus erythematosus and rheumatoid arthritis appear not to prevent LDL oxidation [117].
There is some evidence suggesting that apolipoproteins are responsible, at least in part, for the antioxidant properties of HDLs. Both apoA-I and apoA-II can reduce cholesteryl ester hydroperoxides to cholesteryl ester hydroxides by a process that involves the concomitant oxidation of apoA-I and apoA-II methionine residues [118]. Although this reducing activity of apoA-I and apoA-II has been reported to be independent of PON [118], some investigators have found that the antioxidant properties of HDLs are impaired by the displacement of PON1 by apoA-II [119].

HDLs can accept and inactivate oxidized phospholipids from cell membranes, as well as from oxidized LDLs. These events require HDL-associated enzymes, such as PON and PAF-AH [95,120,121]. PON is an HDL-associated calcium-dependent enzyme that catalyses the hydrolyses of lipid peroxides and prevents their accumulation in LDLs [122]. This involves the interaction of a free sulfhydryl group in PON with oxidized phospholipids, oxidized cholesteryl esters and lysophosphatidylcholine in oxidized LDLs [123]. Low PON activity also appears to be associated with an increased risk of cardiovascular diseases [124]. A recent prospective study has provided the first direct evidence of a mechanistic link between the PON1 gene and PON systemic activity with multiple quantitative indices of oxidative stress and atherosclerotic heart disease development in humans [125]. PAF-AH, a calcium-independent serine esterase, protects against LDL oxidation by hydrolysing oxidized phospholipids with short sn-2 acyl chains [70].

Another mechanism by which HDLs inhibit LDL oxidation relates to their ability to remove and inactivate oxidized fatty acids, such as HPODE (hydroperoxyoctadecenoic acid) and HPETE (hydroperoxyeicosatetraenoic acid) from LDLs [116].

Lipid-free apoA-I, and apoA-I mimetic peptides also inhibit LDL oxidation in vitro [116,126] and in vivo [95,116]. In vitro studies by Navab et al. [116] have indicated that treating human coronary aortic artery cells with lipid-free apoA-I, the apoA-I mimetic peptide (37pA), HDLs or PON renders them unable to oxidize LDLs. The apoA-I mimetic peptide 5F also enhances the ability of HDLs to inhibit LDL oxidation and protect mice from diet-induced atherosclerosis [127].

Another important function of HDLs and apoA-I mimetic peptides is their ability to restore endothelial function in vitro and in vivo by increasing NO bioavailability in the vessel wall (Figure 3). For example, the apoA-I mimetic peptide L-4F preserves endothelial cell function by preventing LDLs from uncoupling eNOS activity, such that NO formation increases and $O_2^-$ generation decreases [128].

Lipid-free apoA-I also alters the potential oxidative state of LDLs in vivo. Injection of apoA-I into mice renders their LDLs resistant to oxidation for up to 24 h. This may also be the case for humans, where infusions of apoA-I render their LDLs resistant to oxidation by human artery wall cell co-cultures [129]. It should also be noted that infusions of apoA-I into mice and humans significantly increases plasma PON activity [129].

Other mechanisms by which HDLs inhibit oxidation include decreasing superoxide production and the inactivation of neutrophil NADPH oxidase, a respiratory burst enzyme which is an important source of ROS production in the vessel wall [115,130,131]. A study from Kopprasch et al. [115] revealed that both native HDLs and lipid-free apoA-I rapidly and efficiently protect resting neutrophils from respiratory burst activation by hypochlorite-oxidized LDLs. Liao et al. [130] have also shown that lipid-free apoA-I inhibits PMA- and fMLP (N-formylmethionyl-leucyl-phenylalalanine)-induced neutrophil oxidative burst production in vitro.

Another of the key antioxidant functions of HDLs relates to their ability to remove potentially cytotoxic lipid hydroperoxides from the circulation. HDLs are the major carriers of lipid hydroperoxides in human plasma [132]. These deleterious molecules are formed in LDLs and transferred to HDLs by CETP, where they are reduced to cholesteryl ester hydroxides, which are then rapidly and selectively taken up by the liver [133] in a process that may, or may not, be independent of PON activity [79,118,121,134].

CONCLUSIONS

The studies described in the present review provide compelling evidence that HDLs have the capacity to reduce atherosclerotic lesion development by multiple mechanisms. Although some of these mechanisms have been studied for many years and are well understood, this is not always the case. The fact that there is not a lot of information about some of the more recently identified anti-atherogenic properties of HDL, such as their ability to influence endothelial function and the mechanisms that are responsible for their anti-inflammatory properties, reflects the difficulties associated with working with such a complex and functionally heterogeneous group of particles. Despite this, HDLs and their constituents are likely to become key therapeutic targets for preventing atherosclerotic lesion formation and promoting the regression of established atherosclerotic lesions. The challenges are going to be identifying which HDLs will be most useful and developing strategies for maximizing their effects.

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