Lipids and the endothelium: an update

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In addition to being a major cause of morbidity, cardiovascular disease remains the dominant cause of all deaths in industrialized countries. Atherosclerosis is the principal underlying etiology for cardiovascular events and dyslipidemia, the main pathology leading to atherosclerosis. While lipid-lowering therapies, in particular the statins, have proven highly successful, monotherapy with low-density lipoprotein-cholesterol-lowering drugs appears to have reached its limit in terms of impeding cardiovascular events. Thus, other lipid-related risk factors, namely low levels of high-density lipoprotein (HDL)-cholesterol, are currently targeted. The most well-defined role of HDL is cholesterol efflux, the transportation of cholesterol from the periphery to the liver where it is subsequently removed. In addition, there are many other effects of HDL which are cardioprotective, including its antioxidative effects, its inhibition of lipid oxidation and, thus, foam cell formation, and its anti-inflammatory effects, decreasing the expression of adhesion molecules, in addition to enhancing nitric oxide production. The latter two effects are particularly pertinent to the endothelium and endothelial function. This review explores these cellular and biological relationships with a focus on the emerging strategies surrounding the potential utility of HDL in the clinical management of patients with dyslipidemia.

Cardiovascular disease remains the dominant cause of death globally, and while it is recognized as a multifactorial disease with multiple sources of risk, atherosclerosis is its pre-eminent precursor and the major pathology contributing to end-stage heart disease. Dyslipidemia, or the imbalance of plasma lipid levels, together with disturbances of intracellular lipid metabolism, is the underlying risk factor in atherosclerosis and plaque development. Such dyslipidemias include increases in both total plasma and low-density lipoprotein (LDL)-cholesterol, leading to LDL modification and increased accumulation of modified LDL in the intima of the vasculature. This cascade of events leading to plaque formation is usually accompanied, if not preceded, by a condition commonly termed endothelial dysfunction.

A strong predictor of cardiovascular disease, a dysfunctional endothelium, is characterized by a decrease in production, activity or bioavailability of endothelial-derived nitric oxide (NO). Another feature of endothelial dysfunction is the upregulated expression of adhesive molecules, a key factor in the inflammatory component of atherosclerosis [1]. The therapeutic relevance of these well-researched phenomena in the context of lipids has yet to be demonstrated, although statins, administered routinely for lipid management, can affect both NO production and inflammation in atherosclerosis [2], either or both of which may well contribute to the benefits seen with this class of compounds. However, it is clear that the effect of LDL and high-density lipoprotein (HDL) is not confined to their traditional roles in forward and reverse cholesterol transport but that they also profoundly affect NO function and vascular inflammation, and these broader roles may well yield new insights for superior therapeutic indications. This perspective explores these cellular and biological relationships with a focus on the emerging strategies surrounding the potential utility of HDL in the clinical management of patients with dyslipidemia.

Nitric oxide

NO-dependent vasodilation:
NO production & activity

As an antiatherogenic protective layer of cells, the endothelium has many functions. One of the more well known functions is to synthesize, house and release factors that regulate vessel contractation and relaxation. These factors include prostaglandins, endothelin and possibly endothelium-derived angiotensin II [3-6]. However, the most important of these is arguably NO since it is released not only upon activation but also basally, giving it a significant role for normal, healthy vessel function.

NO is a well-characterized vasodilator. It is the end product of the nitric oxide synthase (NOS) pathway where it is synthesized from...
the guanidino terminal of the substrate L-arginine. In endothelial cells, this reaction is catalyzed by the enzyme endothelial nitric oxide synthase (eNOS or NOS III) yielding the by-product L-citrulline [7,8]. NO is an extremely labile compound with a biological half-life of seconds. Produced as an intracellular gaseous molecule, it is unique in that it diffuses across the cell membrane and induces signaling events. The most well known of these is the stimulation of smooth muscle cells causing guanylate cyclase to produce increased amounts of cGMP which, in turn, decreases intracellular calcium levels with the end result of vascular dilatation.

The majority of eNOS is housed in caveolae [9]. The localization of eNOS in caveolae appears to be driven by irreversible co-translational myristylation of eNOS at the N-terminal glycine residues and post-translational palmitoylation of cysteine residues at positions 15 and 26, respectively [10–12]. Its location has been found to be essential for its receptor-mediated stimulation by agonists such as acetylcholine and bradykinin [13–16]. This is a particularly important finding, establishing that eNOS is negatively regulated directly by caveolin-1 in such a way that the inhibitory interaction between eNOS and caveolin-1 must be removed for activation of eNOS to occur [9].

Anti-inflammatory effects of NO

The regulation of vascular tone (as outlined above) is a well-defined role of NO; however, it also plays an important part in vascular homeostasis [17]. NO has been demonstrated to inhibit nuclear factor-κB activation in endothelial cells, thus attenuating cytokine-induced expression of adhesion molecules, including selectins and the cell adhesion molecule (CAM) family [18], and preventing the attraction of leukocytes and platelets. It is also implicated in vascular smooth muscle cell regulation since it is capable of inhibiting smooth muscle cell migration [19], proliferation [20] and extracellular matrix deposition [21,22]. In addition to these other properties, NO also hinders thrombus formation mainly due to its autocrine/paracrine role in platelets [23,24]. These findings indicate that NO can prevent platelet activation and aggregation by inhibiting surface expression of glycoproteins and P-selectin [23,25]. Thus, NO has multiple roles, including the regulation of vascular tone and inflammation in addition to being a powerful antiatherothrombotic agent.

Interactions between lipids & NO function

LDL-induced impairment of NO function

Reactive oxygen species (ROS) production in the vascular wall is stimulated in many cardiovascular disease states, including hypercholesterolemia. Under certain conditions, for example during depleted L-arginine states, this can occur due to eNOS uncoupling resulting in increased production of the superoxide radical O₂. Superoxide and NO combine to form toxic peroxynitrite [26]. One of the many outcomes of oxidative stress within the vessel wall includes facilitation of LDL oxidation [27]. The uncontrolled entry of these modified lipoproteins into the cell contributes significantly to the formation of atherosclerotic plaques by promoting cholesterol accumulation and cellular inflammation. In addition, the ability of oxidized (Ox)LDL to impair NO production is well established, although the exact mechanism by which this occurs is still unknown. However, it would appear that the depletion of intracellular levels of NO via the formation of peroxynitrite is a downstream event from the interaction between OxLDL and its receptor, lectin-like OxLDL receptor (LOX)-1 [28]. A positive feedback loop then occurs where the generation of ROS facilitates further oxidation of native LDL as well as further upregulation of LOX-1 expression, effectively amplifying the production of ROS and, in particular, the superoxide radical O₂ [29]. This leads ultimately to inactivation of NO via the formation of peroxynitrite and has been demonstrated by direct measurement of intracellular NO in the presence and absence of OxLDL [30].

Another mechanism by which OxLDL is thought to impair NO production is through the class B scavenger receptor, CD36. This interaction causes the eNOS–caveolin complex to be translocated from the caveolae and internalized into temporary holding vesicles where it cannot be stimulated. Interestingly, when OxLDL is removed, the complex can return to the caveolae, thus restoring eNOS function [14]. While OxLDL can cause inhibition of eNOS function, it has no effect on eNOS expression, palmitoylation, myristoylation or phosphorylation [14]. Much of these data are substantiated further by functional experiments confirming that the ability of OxLDL to impair endothelium-dependant NO-mediated dilation of vessels concurs with the premise that OxLDL is linked to a decrease in intracellular NO levels [31].
HDL & NO function

The primary mechanism responsible for the antiatherogenic properties of HDL is its participation in reverse cholesterol transport. In recent years, however, other atheroprotective properties of HDL have been explored. These include the involvement of HDL in regulating vascular tone and homeostasis [32].

The involvement of HDL in vascular tone regulation can, in part, be attributed to two factors: the independent stimulatory effect of HDL on eNOS [32,33] and the ability of HDL to remove the inhibitory effects of OxLDL [34]. The effects of HDL on eNOS include enhancing eNOS production [35], inhibiting eNOS degradation [36] and activating existing eNOS [6,33,37].

The interaction of HDL with scavenger receptor class B type I (SR-BI; which is also located in caveolae) and signaling to eNOS may involve increases of intracellular levels of ceramide [33] or interactions between eNOS and HDL-associated estradiol [38]. It has also been suggested that the signaling cascade leading to eNOS activation is simply a response to changes in cell cholesterol content resulting from HDL-induced cholesterol efflux with SR-BI playing the role of a cholesterol-sensing gauge [39]. However, the biochemical mechanism of eNOS stimulation by HDL is achieved through intracellular signaling mechanisms involving a complex series of interplays [37].

It is widely accepted that the binding of HDL to SR-BI induces a phosphorylation cascade beginning with tyrosine kinase (src) phosphorylating phosphatidylinositol 3-kinase. This subsequently induces independent activation of both Akt and mitogen activated protein kinase (MAPK) to extracellular signal-regulated kinase (ERK) 1/2, from which Akt phosphorylates eNOS at Ser1179 [37]. The discrete involvement of MAPK is not currently understood, although phosphorylation of ERK 1/2 may play a role in amplifying the phosphorylation of eNOS directly [37].

While the involvement of SR-BI is thought to be crucial in HDL signaling to eNOS, this may not be the only method of activation [40]. Interestingly, apolipoprotein (Apo)A-I, the major protein in HDL that interacts with SR-BI, while incapable of stimulating eNOS above basal levels, is still an essential player in the overall process, since eNOS activity is abolished when ApoA-1 is blocked [37]. Accordingly, the involvement of lysophospholipids has been studied in the context of eNOS stimulation. In an elegant study by Nofer and colleagues [40], lysophospholipids via their receptor 3,5-diphenyl-1,2,4-oxadiazole sphingosine-1-phosphate-1 (S1P3) was shown to cause an increase in intracellular calcium, triggering activation of Akt and, subsequently, eNOS activity. This signaling cascade was severely reduced in S1P3-deficient mice [40].

Taken together, the above findings suggest that HDL as a whole molecule, and not its subparticles (ApoA-I or lysophospholipids), is optimal for activation of eNOS. The involvement of the two receptors, SR-BI and S1P3, yields the possibility of receptor cross-talk (Figure 1), where ApoA-I induces signaling via the SR-BI pathway, while lysophospholipids provoke a signaling cascade through S1P3, ultimately resulting in phosphorylation of Akt and eNOS to produce NO.

As mentioned previously, another effect of HDL on eNOS is the reduction of OxLDL-induced eNOS inhibition. An effect likely to be related to its ability to stabilize cellular cholesterol content since, like many other enzymes located in the caveolae, eNOS is very sensitive to caveolae-free cholesterol content. Hence, OxLDL, via scavenger receptor CD36, alters caveolae cholesterol content causing dissociation of eNOS from caveolae, thus resulting in eNOS inactivation. HDL, again through its receptor SR-BI, restores caveolae cholesterol content, eNOS association and consequently its activity [34].

Collectively, HDL clearly increases NO production and function, an effect likely to have an important role in its antiatherogenic properties. However, it is unclear if this effect is a reflection of its effect on cellular cholesterol content or is independent from its involvement in cellular lipid metabolism.

Lipids & vascular inflammation

In addition to, and potentially via, the effect of hypercholesteremia on NO function, lipoproteins also have a significant role in regulating adhesion molecule expression on endothelial cells and leukocytes. Expression of adhesion molecules is an important element of vascular inflammation and in the pathogenesis of atherosclerosis. Increased LDL cholesterol leads to the presence of localized OxLDL, which is a potent activator of endothelial cells inducing expression of membrane-bound adhesion molecules [41,42]. In endothelial cells, OxLDL increases expression of intracellular adhesion molecule (ICAM)-1 and E-selectin, a process mediated by the LOX-1 receptor [43]. Endothelial cells stimulated with
OxLDL have also been demonstrated to release a number of atherogenic molecules, including tissue factor [44], monocyte chemotactic protein-1 [45] and monocyte colony-stimulating factor [46].

Both minimally and extensively, OxLDL has been demonstrated to promote adhesion of the monocytic cell line U937 to endothelial cells [47]. This adhesion is not blocked by inactivating E-selectin, ICAM-1 or vascular cell adhesion molecule (VCAM)-1. However, these studies were conducted under static conditions in the absence of blood flow and only one adhesion molecule was inhibited at any one time, therefore limiting interpretation of the findings.

Conversely, the effects of HDL are predictably anti-inflammatory [48]. When HDL was incubated with OxLDL-stimulated endothelial cells, a reduction in adhesion of U937s to basal levels was observed [47]. Additionally, HDL has been consistently demonstrated to reduce the expression of adhesion molecules in both endothelial cells and leukocytes [49-52]. This effect is not dependent on the continued presence of HDL. Preincubating endothelial cells with HDL followed by stimulation with tumor necrosis factor (TNF)-α prevents activation of endothelial cells (measured as VCAM-1, ICAM-1 and E-selectin expression). While preincubation of HDL, which was subsequently removed before stimulation with TNF-α, continued to inhibit VCAM-1 expression. The inhibition by HDL appears to be time dependent, preincubation for at least 30 min is required for an effective anti-inflammatory action.

C-reactive protein (CRP) has been demonstrated to have proinflammatory actions on both leukocytes and endothelial cells [53,54]. In another study, Wadham and colleagues [55] demonstrated the ability of HDL to prevent endothelial cell activation and expression of adhesion molecules in response to proinflammatory CRP. This effect was
independent of ApoA-I as this protein alone was unable to reduce expression of the adhesion molecules. Furthermore, the effect could be reproduced with phospholipid vesicles, suggesting that lipids may play a major role in this signaling event. The adhesion of leukocytes to the vascular endothelium is mediated not only by endothelial adhesion molecules, but also by adhesion molecules expressed on leukocytes. ApoA-I has been demonstrated to prevent the activation of neutrophils by phorbol 12-myristate 13-acetate (PMA) and N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP), although they appear to act via different mechanisms. ApoA-I can inhibit both PMA- and FMLP-stimulated neutrophil adhesion to fibronectin. However, ApoA-I can only inhibit oxidative burst by FMLP [56]. In one study, activation of peroxisome proliferator-activated receptor (PPAR) was suggested to be the mechanism responsible for the effect of HDL on leukocyte adhesion molecules [57], although other studies suggest that the role of HDL on human monocyte adhesion molecules is mediated through changes in cellular cholesterol content [58].

It is still debatable as to which constituents of HDL play the key role in inhibiting the expression of adhesion molecules. As stated above there is convincing evidence that lipid-free ApoA-I can mimic HDL's actions. However, several studies suggest that HDL-associated lysosphingolipids are capable of inhibiting adhesion molecule expression on endothelial cells [59,60]. Endothelial cells stimulated with TNF-α demonstrated a dramatic increase in E-selectin levels; this activation is significantly reduced when cells are preincubated with HDL. A similar reduction in cell activation was found when cells were treated with either sphingosylphosphorylcholine or lysosulfatide, the two lysosphingolipids associated with HDL [60], suggesting that these HDL constituents may be responsible for this effect.

In addition to the regulation of adhesion molecules, there is an emerging role for HDL in endothelial repair where HDL has been demonstrated to dose-dependently facilitate endothelial cell migration in an in vitro wound model, a process which appears to be NO dependent [61]. The importance of both HDL and SR-B1 to endothelial repair was confirmed in vivo through the use of perivascular electric injury to the carotid artery of mice. This injury model was applied to both ApoA-I- and SR-B1-null mice, respectively. Both these mice displayed a diminished ability to repair the wounded endothelium, suggesting the importance of HDL and its receptor, SR-B1, and receptor-mediated signaling. This inability to repair the denuded endothelium was restored when adenoviruses expressing either ApoA-I or SR-B1 were used, effectively reversing the defect [61]. This repair, along with endothelial cell migration, may also be attributed to the ability of HDL to stimulate the release of endothelial progenitor cells (EPC) into the circulation and to stimulate transforming growth factor (TGF)-β release [62,63]. Tso and colleagues demonstrated the beneficial role that HDL has in re-endothelialization in mice injured with lipopolysaccharide. A significant increase in the proportion of aortic endothelial cells positive for stem cell antigen-1 (sca-1), a marker of EPCs, was observed after administration of reconstituted HDL. Another response to HDL was an increase in TGF-β2 expression in endothelial cells, an effect observed with both ApoA-I and lysosphingolipids [62]. Mice expressing human ApoA-I demonstrated an approximate tenfold increase in TGF-β2 mRNA levels compared with ApoA-I null mice. This increase was attributed to the phosphorylation of ERK 1/2 and Akt. The increase in the TGF-β signaling pathway activity was supported further by the increase in phosphorylation of Smad-2/3, an intracellular effector of TGF-β.

Taken together these findings prove that HDL is a potent anti-inflammatory agent, regulating cholesterol transport, NO, adhesion molecules, EPCs and growth factors. The beneficial effects of HDL, therefore, extend from plaque stability to plaque reduction, suggesting that raising HDL may affect multiple therapeutic targets.

**In vivo**

A number of studies using ApoE and LDL knockout mice have demonstrated a correlation between an increase in lesion size and impairment of endothelium-dependent relaxation [64,65]. Introduction of increased levels of ApoA-I by, for example, the creation of ApoA-I transgenic mice on both atherogenic backgrounds causes a decrease in plaque size and a restoration of vascular tone [65], whereas ApoA-I knockout mice have impaired reverse cholesterol transport, increased lesion size and increased monocyte adhesion to endothelial cells [66]. Another interesting model demonstrating the anti-inflammatory properties of HDL in rabbits was described by Nicholls and colleagues [67]. In this study, the authors used a periartrial colar to induce infiltration of leukocytes into the arterial wall, an early event in the development of the atherosclerotic plaque. Infusion of reconstituted HDL or ApoA-I inhibited both the
infiltration and the expression of VCAM-1 and ICAM-1. These studies all corroborate the many in vitro studies described above.

A separate development in the study of the antiatherogenic effect of HDL was the exciting discovery of a naturally occurring variant of ApoA-I, ApoA-I Milano, which was first described in 1980 by Franceschini [68], who identified a family from Limone sur Garda, Italy, with a lipoprotein disorder resulting in abnormally low levels of HDL cholesterol. The intriguing nature of this finding was that despite the low HDL levels, the family exhibited no atherosclerosis. ApoA-I Milano has since been well characterized to a single amino acid mutation, Arg173Cys, favoring the formation of dimers, shortened residence time and rapid catabolism of ApoA-I [69]. The structural arrangement of ApoA-I Milano increases its affinity for lipids and facilitates its easy removal [70]. In ApoE-deficient mice, recombinant ApoA-I Milano/phospholipid complex (ApoA-I Milano/PC) significantly reduced aortic atherosclerosis, lipid content and macrophage infiltration. ApoA-I Milano/PC also promoted cholesterol efflux significantly above basal levels [71]. Similar results were observed with ApoA-I Milano in Sprague-Dawley rats [72], where ApoA-I Milano-treated rats demonstrated a reduction in total and HDL cholesterol, however, platelet aggregation was reduced markedly in ApoA-I Milano rats, chemical induced thrombosis was delayed and thrombus weight significantly less compared with control rats. These results provide the basis for the therapeutic application of either ApoA-I or ApoA-I Milano. The direct effect of ApoA-I significantly reduced aortic atherosclerosis, lipid content and macrophage infiltration. The effect of ApoA-I Milano on NO function and cell-to-cell adhesion is unknown and worthy of study.

Conclusion & future perspective
Given the proatherogenic properties of LDL, particularly in its oxidized form, and the multiple antiatherogenic actions of HDL, it follows that it is therapeutically sensible to decrease the presence of the former and increase the presence of the latter. Lipid-lowering medication, particularly statins, which are highly effective and have minimal side effects, together with decade old, evidence-based data demonstrating positive outcomes after statin therapy [73], continue to be the mainstay of therapy for patients at high risk of atherosclerosis. However, there is a clear movement towards investigations into newer therapies designed to raise HDL cholesterol levels or their specific active moiety [74,75].

In addition to its defined role in cholesterol efflux, other antiatherogenic properties of HDL include their pro-NO production capabilities, anti-inflammatory and antioxidative effects. A decrease in HDL is, for example, correlated to an increase in soluble adhesion molecules, a predictor of inflammation in humans [76]. The 2003 findings of Nissen and colleagues [77] using recombinant ApoA-I Milano/PC showed HDL to be effective in decreasing atheroma volume (assessed by intravascular ultrasound) in patients with acute coronary syndromes. While confirmation with larger clinical trials with mortality and morbidity end points are clearly desirable, these data are nevertheless promising. There are several new drugs currently being tested (Phase I–III) all aimed at increasing HDL levels. These are well reviewed [74,75] and include PPAR agonists [78], cholesterylester transfer protein inhibitors [79] and liver X receptor activators, which are still in preclinical development. However, it is important to realise that while these compounds effectively raise HDL levels, whether the raised HDL levels effectively translates to increased functionality and decreased mortality and morbidity remains to be seen.

Both LDL and HDL have multifaceted roles besides their traditional roles of cholesterol accumulation and efflux. These effects include direct influence on NO function, inflammation and oxidation, all of which are key contributors to the pathogenesis of atherosclerosis. The next decade promises to be an exciting one for this area of research and current knowledge would suggest that we are in sight of new strategies for clinical lipid management.

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**Executive summary**

**Nitric oxide-dependant vasodilation: nitric oxide production & activity**

- The endothelium provides an antiatherogenic environment, housing and releasing factors that regulate vessel contraction and relaxation.
- Nitric oxide (NO) is arguably the most important factor since it is involved in maintaining normal vessel function. It is synthesized from the substrate L-arginine, a reaction catalyzed by endothelial nitric oxide synthase (eNOS).
- eNOS is found in caveolae and is negatively regulated by caveolin-1 in such a way that the inhibitory interaction between eNOS and caveolin-1 must be removed for activation of eNOS to occur.
Anti-inflammatory effects of NO

- NO inhibits activation of nuclear factor-κB in endothelial cells, thus attenuating the cytokine-induced expression of adhesion molecules.
- NO regulates vascular smooth muscle cells via inhibition of migration, proliferation and extracellular matrix deposition.
- NO also hinders thrombus formation mainly due to its autocrine/paracrine role in platelets.

Low density lipoprotein-induced impairment of NO function

- Reactive oxygen species production in vascular tissue has many outcomes including facilitation of low-density lipoprotein (LDL) oxidation.
- Oxidized (Ox)LDL depletes intracellular NO levels via the formation of peroxynitrite by binding the lectin-like OxLDL receptor-1.
- OxLDL is also thought to impair NO production via CD36. This interaction causes the eNOS–caveolin complex to be translocated from the caveolae to vesicles where it cannot be stimulated.

High-density lipoprotein & NO function

- High-density lipoprotein (HDL) can regulate vascular tone by independently stimulating eNOS and/or removing the inhibitory effects of OxLDL.
- The binding of HDL to scavenger receptor class B type I (SR-BI) induces eNOS activation via a phosphorylation cascade beginning with tyrosine kinase (src) phosphorylating phosphatidylinositol 3-kinase. This in turn induces independent activation of both Akt and mitogen-activated protein kinase to extracellular signal regulated kinase 1/2 from which Akt phosphorylates eNOS at Ser1179.
- HDL as a whole molecule is optimal for activation of eNOS. The involvement of the two receptors, SR-BI and 3,5-diphenyl-1,2,4-oxadiazole sphingosine-1-phosphate-1 (S1P3) enables the possibility of receptor cross-talk via apolipoprotein (Apo)A-I-inducing signaling through SR-BI and lysophospholipids through S1P3.

Lipids & vascular inflammation

OxLDL atherogenic properties:

- In endothelial cells, OxLDL increases expression of intracellular adhesion molecule (ICAM)-1 and E-selectin and the release of adhesion molecules including tissue factor, monocyte chemotactic protein-1 and monocyte colony-stimulating factor.
- OxLDL, both minimally and extensively, has been shown to promote adhesion of the monocyte cell line U937 to endothelial cells.

HDL antiatherogenic properties:

- HDL incubated with OxLDL stimulated endothelial cells, reducing adhesion of U937s to basal levels.
- HDL has been consistently shown to reduce the expression of adhesion molecules in both endothelial cells and leukocytes.
- C-reactive protein has been demonstrated to have proinflammatory actions on both leukocytes and endothelial cells, the addition of HDL prevents endothelial cell activation and expression of adhesion molecules.
- ApoA-I has been demonstrated to prevent activation of neutrophils by phorbol myristate acetate and N-formylmethionyl-leucyl-phenylalanine.

In vivo studies

- Introduction of increased levels of ApoA-I by the creation of ApoA-I transgenic mice with atherogenic backgrounds causes a decrease in plaque size and restoration of vascular tone.
- Anti-inflammatory properties of HDL have also been demonstrated in rabbits where infusion of reconstituted HDL or ApoA-I inhibits the infiltration of leukocytes, as well as the expression of vascular cell adhesion molecule-1 and ICAM-1.
- An exciting variant of ApoA-I, ApoA-I_Milano, characterized by a single amino acid mutation (favoring formation of dimers, shortened residence time and rapid catabolism of ApoA-I) also increases its affinity for lipids and facilitates its easy removal.
- In ApoE-deficient mice, recombinant ApoA-I_Milano/phospholipid complex (PC) significantly reduces aortic atherosclerosis, lipid content and macrophage infiltration, promoting cholesterol efflux significantly above basal levels. Similar results were observed with ApoA-I_Milano in Sprague-Dawley rats where platelet aggregation was reduced markedly, chemical induced thrombosis was delayed and thrombus weight was significantly less compared with control rats.

Conclusion & future perspective

- Lipid-lowering medication, particularly the highly effective statins, continues to be the therapy of choice for patients at high risk of atherosclerosis.
- There has been progression towards investigations examining newer therapies designed to raise HDL cholesterol levels. Its defined role in cholesterol efflux, its pro-NO production capabilities, and its anti-inflammatory and antioxidative effects have clearly made it an aim for most of the therapeutics.
- The 2003 findings of Nissen and colleagues using recombinant ApoA-I_Milano/PC showed it to be effective in decreasing atheroma volume in patients with acute coronary syndromes.
- Several new drugs, including peroxisome proliferator activated receptor agonists, cholesteryl ester transfer protein inhibitors and liver X receptor activators, are currently being tested (Phase I–III) and are all aimed at increasing HDL levels.
- Research and current trials would suggest that we are in sight of exciting novel strategies for clinical lipid management.
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Papers of special note have been highlighted as either of interest (+++) or of considerable interest (+++) to readers.

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** Elegant study clearly demonstrating high-density lipoprotein's (H D L s) ability to stimulate endothelial nitric oxide synthase (e N O S) as a whole molecule and not just its major protein apolipoprotein (A po A)-1.**


Lipids and the endothelium: an update – PERSPECTIVE


• Significant paper in terms of the mechanism by which HDL signals to eNOS, implementing the involvement of Akt and mitogen-activated protein kinase directly or indirectly phosphorylating eNOS.

41. His study brings together the complete picture of the mechanism by which HDL stimulates eNOS through ApoA-I and its associated lysophospholipids.


• D demonstrates the direct inflammatory effects of oxidized low density lipoprotein (OxLDL) on the endothelium.

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• Covers HDL’s ability to reduce both endothelial cell and leukocyte activation to proinflammatory stimulants.

• Covers HDL’s ability to reduce both endothelial cell and leukocyte activation to proinflammatory stimulatory.


- Discovery of the exciting ApoA-I variant ApoA-I_Milano which may prove to have a more potent anti-atherogenic role than normal ApoA-I.


71. Shah PK, Nilsson J, Kaul S et al.: Effects of recombinant apolipoprotein A-I_Milano on atherosclerosis in atherosclerosis in ApoA-I_Milano in humans, which subsequently produced a reduction in atherosclerosis. While this study is limited it provides an insight into a promising therapeutic.


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