

# Pleiotropic Functions of HDL Lead to Protection from Atherosclerosis and Other Diseases

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## 1. Introduction

High density lipoprotein (HDL) is a macromolecular complex of proteins and lipids that is produced primarily by the liver through a complex pathway that requires initially the functions of apolipoprotein A-I (apoA-I), ATP binding cassette transporter A1 (ABCA1) and lecithin:cholesterol acetyl transferase (LCAT) (Zannis et al., 2006b). Following synthesis, HDL affects the functions of the arterial wall cells through signaling mechanisms mediated by scavenger receptor class B type-I (SR-BI) and other cell surface proteins. The impetus for studying HDL has been the inverse correlation that exists between plasma HDL levels and the risk for coronary artery disease (CAD) (Gordon et al., 1989). HDL promotes cholesterol efflux (Gu et al., 2000; Nakamura et al., 2004), prevents oxidation of low density lipoprotein (LDL) (Navab et al., 2000a; Navab et al., 2000b), inhibits expression of proinflammatory cytokines by macrophages (Okura et al., 2010) as well as expression of adhesion molecules by endothelial cells (Cockerill et al., 1995; Nicholls et al., 2005b). HDL inhibits cell apoptosis (Nofer et al., 2001) and promotes endothelial cell proliferation and migration (Seetharam et al., 2006). HDL stimulates release of nitric oxide (NO) from endothelial cells thus promoting vasodilation (Mineo et al., 2003). HDL also inhibits platelet aggregation and thrombosis (Dole et al., 2008) and has antibacterial, antiparasitic and antiviral activities (Parker et al., 1995; Singh et al., 1999; Vanhollebeke and Pays, 2010). Due to these properties HDL is thought to protect the endothelium and inhibit several steps in the cascade of events that lead to the pathogenesis of atherosclerosis and various other human diseases.

This review focuses on two important aspects of contemporary HDL research. The first part considers briefly the structure of apoA-I and HDL and the key proteins that participate in the pathway of the biogenesis of HDL as well as clinical phenotypes associated with HDL abnormalities. The second part considers various physiological functions of HDL and apoA-I and the protective role of HDL against atherosclerosis and other diseases.



amino acid repeats which are organized in amphipathic  $\alpha$ -helices (Nolte and Atkinson, 1992). Based on the crystal structure of apoA-I in solution (Borhani et al., 1997) a belt model was proposed to explain the structure of apoA-I on discoidal HDL particles. In this model, two antiparallel molecules of apoA-I are wrapped like a belt around a discoidal bilayer containing 160 phospholipid molecules and shields the hydrophobic fatty acid chains of the phospholipids. Analysis of the 93 Å spherical HDL in solution by small angle neutron scattering (SANS) showed that three molecules of apoA-I fold around a central lipid core that has 88.4 Å x 62.8 Å dimensions to form a spheroidal HDL (sHDL) particle (Wu et al., 2011).

### 3.2 Interactions of ApoA-I with ABCA1 are the first step in the biogenesis of HDL

ABCA1 is a ubiquitous protein that belongs to the ABC family of transporters and is expressed abundantly in the liver, macrophages, brain and various other tissues (Kielar et al., 2001). ABCA1 was shown to promote the efflux of cellular phospholipids and cholesterol to lipid free or minimally lipidated apoA-I and other apolipoproteins and amphipathic peptides, but it does not promote efflux to spherical HDL particles (Remaley et al., 2001; Wang et al., 2000). The functional interactions between apoA-I and ABCA-1 are important for the biogenesis of HDL. In the absence of either apoA-I (Matsunaga et al., 1991) or ABCA-1 (Brunham et al., 2006) HDL is not formed. Adenovirus mediated gene transfer of apoA-I mutants in apoA-I<sup>-/-</sup> mice showed that deletion of the C-terminal region of apoA-I prevented the formation of HDL (Chroni et al., 2007). The ability of ABCA1 to promote cholesterol efflux from macrophages is very important for the prevention of formation of foam cells in the atherosclerotic lesions (Van Eck et al., 2002). Mutations resulting in inactivation of ABCA1 are present in patients with Tangier disease (Brunham et al., 2006). The deficiency is associated with very low levels of total plasma and HDL cholesterol and abnormal lipid deposition in various tissues (Christiansen-Weber et al., 2000; McNeish et al., 2000). The ABCA1 deficiency in humans or experimental animals may contribute to accelerated atherosclerosis (Joyce et al., 2002; Singaraja et al., 2003). Inactivation of the ABCA1 gene in macrophages increases the susceptibility to atherosclerosis (Van Eck et al., 2002; Van Eck et al., 2006). Specific amino acid substitutions found in the Danish general population were predictors of ischemic heart disease and reduced life expectancy (Frikke-Schmidt et al., 2008).

### 3.3 Interactions of lipid-bound ApoA-I with LCAT stabilize the nascent HDL

Plasma LCAT is a 416 amino acid long enzyme that is synthesized and secreted by the liver and esterifies the free cholesterol of HDL and LDL. ApoA-I is a potent activator of LCAT (Fielding et al., 1972). Following esterification, the cholesteryl esters formed become part of the lipid core and the discoidal HDL is converted to mature spherical HDL (Chroni et al., 2005a).

Mutations in LCAT are associated with two phenotypes in humans. The familiar LCAT deficiency (FLD) is characterized by the inability of the mutant LCAT to esterify cholesterol on HDL and LDL and causes accumulation of discoidal HDL in the plasma. The fish eye disease (FED) is characterized by the inability of mutant LCAT to esterify cholesterol on HDL only. Both diseases are characterized by low HDL levels due to the inability of LCAT to convert the nascent immature pre- $\beta$  and discoidal particles to mature spherical HDL (Santamarina-Fojo et al., 2001).

### 3.4 Interactions of lipid-bound ApoA-I with SR-BI

SR-BI is an 82 kDa membrane glycoprotein primarily expressed in the liver, steroidogenic tissues and endothelial cells but is also found in other tissues. The most important property of SR-BI is considered to be its ability to act as the HDL receptor (Acton et al., 1996). SR-BI mediates both selective uptake of cholesteryl esters and other lipids from HDL to cells (Acton et al., 1996; Stangl et al., 1999; Thuahnai et al., 2001), as well as efflux of unesterified cholesterol (Gu et al., 2000). Transgenic mice expressing SR-BI in the liver had greatly decreased apoA-I and HDL levels as well as increased clearance of VLDL and LDL (Ueda et al., 1999) and were protected from atherosclerosis (Arai et al., 1999). SR-BI deficient mice had decreased HDL cholesterol clearance (Out et al., 2004), two fold increased plasma cholesterol and presence of large size abnormal apolipoprotein E (apoE) enriched particles that were distributed in the HDL/IDL/LDL region (Rigotti et al., 1997). The SR-BI deficiency in the background of LDL receptor (LDLr) deficient or apoE deficient mice accelerated dramatically the development of atherosclerosis (Huszar et al., 2000; Trigatti et al., 1999). The double deficient mice for apoE and SR-BI developed occlusive coronary atherosclerosis, cardiac hypertrophy, myocardial infarctions, cardiac dysfunction and died prematurely (mean age of death ~6 weeks) (Braun et al., 2002; Trigatti et al., 1999). The SR-BI deficiency reduced greatly cholesteryl ester levels in the steroidogenic tissues that utilize HDL cholesterol for synthesis of steroid hormones (Ji et al., 1999). It also decreased secretion of biliary cholesterol by approximately 50% (Mardones et al., 2001; Rigotti et al., 1997). The SR-BI deficiency also caused defective maturation of oocytes and red blood cells due to accumulation of cholesterol in the plasma membrane of progenitor cells (Holm et al., 2002; Trigatti et al., 1999) and caused infertility in the female but not the male mice (Trigatti et al., 1999; Yesilaltay et al., 2006). Interactions of HDL with SR-BI in endothelial cells triggers signaling mechanisms discussed below that involve activation of endothelial nitric oxide synthase (eNOS) and release of NO that causes vasodilation (Mineo et al., 2003; Yuhanna et al., 2001). Human subjects have been identified with a P297S substitution in SR-BI. Heterozygote carriers for this mutation had increased HDL levels, decreased adrenal steroidogenesis and dysfunctional platelets but did not develop atherosclerosis (Vergeer et al., 2011).

A [Gly2Ser]SR-BI substitution in humans is associated with decreased follicular progesterone levels in Caucasian women and non viable fetuses 42 days post embryo transfer. Another single nucleotide polymorphism (SNP) (rs10846744) was associated with gestational sacs and fetal heart beats and with poor fetal viability in African-American women (Yates et al., 2011).

### 3.5 Interactions of HDL with ABCG1

ABCG1 is a 67 kDa protein which is a member of ABC family of half transporters. ABCG1 is expressed in the spleen, thymus, lung, brain, endothelial cells and other tissues (Savary et al., 1996) and promotes cholesterol efflux from cells to HDL but not to lipid free apoA-I (Nakamura et al., 2004; Vaughan and Oram, 2005). The absence of ABCG1 in mice causes cholesterol accumulation in various tissues (Kennedy et al., 2005) and selective deletion of both ABCA1 and ABCG1 genes in macrophages further increases cholesterol accumulation and results in severe atherosclerosis (Out et al., 2008; Yvan-Charvet et al., 2007).

## 4. Phenotypes of humans and experimental animals having apoA-I mutations

### 4.1 Natural apoA-I mutations

Several apoA-I mutations have been described in the general population that are associated with low plasma HDL levels. Most of the mutations affect the interaction of apoA-I with LCAT. Eight mutations between residues 26 and 107 and one on residue 173, have been associated with amyloidosis and low HDL levels (Sorci-Thomas and Thomas, 2002; Zannis et al., 1993) and one mutation on residue 164 is associated with increased risk for ischemic heart disease and reduced life expectancy (Haase et al., 2011). The *in vivo* interactions of representative naturally occurring apoA-I mutants with LCAT were studied by adenovirus-mediated gene transfer in apoA-I deficient mice. The mutants apoA-I(Leu141Arg)<sub>Pisa</sub> and apoA-I(Leu159Arg)<sub>FIN</sub> produced only small amounts of HDL that formed mostly pre $\beta$ 1 and small size  $\alpha$ 4 HDL particles. The apoA-I(Arg151Cys)<sub>Paris</sub> and apoA-I(arg160Leu)<sub>Oslo</sub> formed discoidal HDL particles. These studies indicated that apoA-I(Leu141Arg)<sub>Pisa</sub> and apoA-I(Leu159Arg)<sub>FIN</sub> mutation may inhibit an early step in the biogenesis of HDL due to insufficient esterification of the cholesterol of the pre $\beta$ 1-HDL particles by the endogenous LCAT. The LCAT insufficiency appears to result from depletion of the plasma LCAT mass (Koukos et al., 2007). A remarkable finding of these studies was that all the aberrant phenotypes were corrected by treatment with exogenous LCAT. This indicates that LCAT administration could be a potential therapeutic intervention to correct low-HDL conditions in humans that are caused by these and other unidentified mutations (Amar et al., 2009).

### 4.2 Specific bioengineered mutations in ApoA-I may cause dyslipidemia

Four bioengineered mutations in apoA-I have been studied by adenovirus-mediated gene transfer in apoA-I deficient mice. Mutants apoA-I[ $\Delta$ (62-78)], apoA-I [Glu110Ala/Glu111Ala] and apoA-I[Asp89Ala/Glu90Ala/Glu92Ala], caused combined hyperlipidemia, characterized by elevated plasma cholesterol and severe hypertriglyceridemia (Chroni et al., 2004; Chroni et al., 2005b; Kateifides et al., 2011). An apoA-I[ $\Delta$ 89-99] mutant induced high plasma cholesterol, but did not affect plasma triglyceride levels (Chroni et al., 2005b).

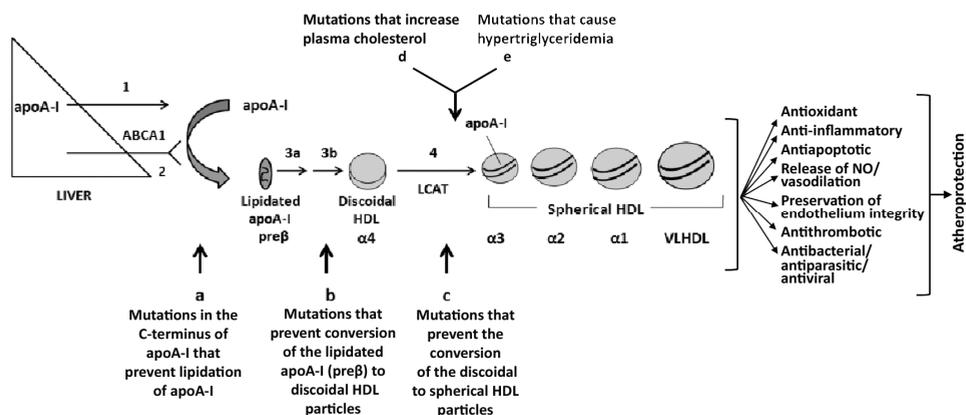


Fig. 2. The pathway of HDL biogenesis and functions of HDL. a-e represent sites of possible disruption of the HDL biogenesis pathway. Different subpopulations of HDL that have been generated due to these defects may have different functions.

The systematic study of the functions of apoA-I by adenovirus mediated gene transfer as well as the phenotypes of naturally occurring apoA-I mutants identified the following five steps where the pathway of biogenesis and/or catabolism of HDL can be disrupted: a) Lack of synthesis of HDL due to mutations in ABCA1 or mutations in apoA-I that affect the ABCA1/apoA-I interaction, b) Failure to convert efficiently the lipidated pre- $\beta$  HDL to discoidal HDL. This defect most likely results from fast catabolism of apoA-I following its lipidation by ABCA1, c) Accumulation of discoidal HDL. This phenotype has been generated by the mutations in the 149-160 region of apoA-I that affect LCAT activation, d) Accumulation of discoidal HDL and induction of hypercholesterolemia. This condition has been observed in the case of the apoA-I[ $\Delta$ (89-99)] mutant, e) Induction of hypertriglyceridemia. This defect has been observed in the case of apoA-I[ $\Delta$ (62-78)], apoA-I[Glu110Ala/Glu111Ala] and apoA-I[Asp89Ala/Glu90Ala/Glu92Ala] mutants (Fig. 2).

## 5. Physiological functions of ApoA-I and HDL that may be relevant to its atheroprotective properties

### 5.1 Cell signaling pathways mediated by HDL and apoA-I

Various studies have shown that increased HDL levels are associated with greater vasodilator effects in humans and this effect is impaired in patients with coronary heart disease (CHD) (Li et al., 2000; Zeiher et al., 1994). Treatment with HDL increased eNOS protein levels in cultured human aortic endothelial cells (HAECs) (Ramet et al., 2003). Other studies in endothelial cells and Chinese hamster ovary (CHO) cells that express SR-BI, showed that SR-BI-HDL interactions lead to the phosphorylation and activation of eNOS. The HDL-induced eNOS activation occurs in the caveolae. The HDL-mediated NO-dependent relaxation is lost in aortic rings of SR-BI<sup>-/-</sup> mice (Yuhanna et al., 2001). Experiments in cultures of endothelial cells and COS M6 cells transfected with eNOS and SR-BI showed that interaction of HDL with SR-BI triggered signalling mechanisms which led to phosphorylation of eNOS at Ser1179 and increased its activity. On the other hand phosphorylation of Thr 497 of eNOS attenuated its activity. The signalling cascade initially involves the nonreceptor tyrosine kinase Src which phosphorylated PI3 kinase (PI3K). Inhibition of Src by specific inhibitors prevented eNOS phosphorylation. PI3K activation led to phosphorylation of Akt and mitogen-activated protein kinase (MAPK) which independently phosphorylated eNOS. Inhibitors of MAPK did not affect HDL-mediated Akt activation and a dominant negative Akt did not affect HDL-mediated MAPK activation and eNOS phosphorylation (Mineo et al., 2003) (figure 3A).

The mechanism of the SR-BI mediated activation of eNOS was studied in detail (Assanasen et al., 2005). HDL and cholesterol-free reconstituted HDL (rHDL) particles containing apoA-I and phosphatidylcholine (Lp2A-I) as well as cyclodextrin stimulated eNOS activity whereas rHDL particles that contain cholesterol did not. Blocking of cholesterol efflux with a monoclonal antibody to SR-BI abolished the activation of eNOS. Experiments were performed using SR-BII, a splice variant of SR-BI as well as a SR-BI mutant that lacks the carboxyterminal amino acid 509 [SR-BI( $\Delta$ 509)] and chimeric receptors where the transmembrane and the C-terminal domains of SR-BI were replaced by the corresponding domains of CD36. These studies established that the C-terminal cytoplasmic PDZ-interacting domain and the C-terminal transmembrane domain of SR-BI were both required for eNOS activation (Assanasen et al., 2005). The cytoplasmic PDZK1 interacting domain of SR-BI binds adaptor proteins such as PDZK1 that may participate in cell signalling (Kocher

et al., 2003). A photoactive derivative of cholesterol binds in the transmembrane region of SR-BI indicating that this region serves as a cholesterol sensor on the plasma membrane (Assanasen et al., 2005). HDL and lysophospholipids that are components of HDL including sphingosylphosphorylcholine, sphingosine-1-phosphate (S1P) and lysosulphatide cause eNOS dependent relaxation of mouse aortic rings via intracellular Ca<sup>2+</sup> mobilization and eNOS phosphorylation mediated by Akt (Nofer et al., 2004). Another study however, indicated that interactions of HDL with SR-BI stimulate eNOS by increasing intracellular ceramide levels without affecting intracellular calcium levels and Akt phosphorylation (Li et al., 2002). The proposed role of HDL-associated estradiol in the stimulation of eNOS activity is unclear (Gong et al., 2003). 5' AMP-activated protein kinase (AMPK) may also play a role in the HDL-mediated phosphorylation of eNOS at multiple sites (Ser116, Ser635, and Ser1179) (Drew et al., 2004). It was suggested that activation by AMPK may involve physical interactions between the apoA-I component of HDL and eNOS. Such interactions may be possible following SR-BI mediated endocytosis of HDL (Silver et al., 2001). HDL also affected the signaling in endothelial cells by the bone morphogenetic protein 4 (BMP4) and increased expression of the activin-like kinase receptor 1 and 2 (Yao et al., 2008). This resulted in increased expression of vascular endothelial growth factor (VEGF) and matrix gla protein (MGP). VEGF promotes endothelial cell survival and MGP prevents vascular calcification and thus contribute to the maintenance, the integrity and the preservation of the functions of the endothelium (Yao et al., 2008).

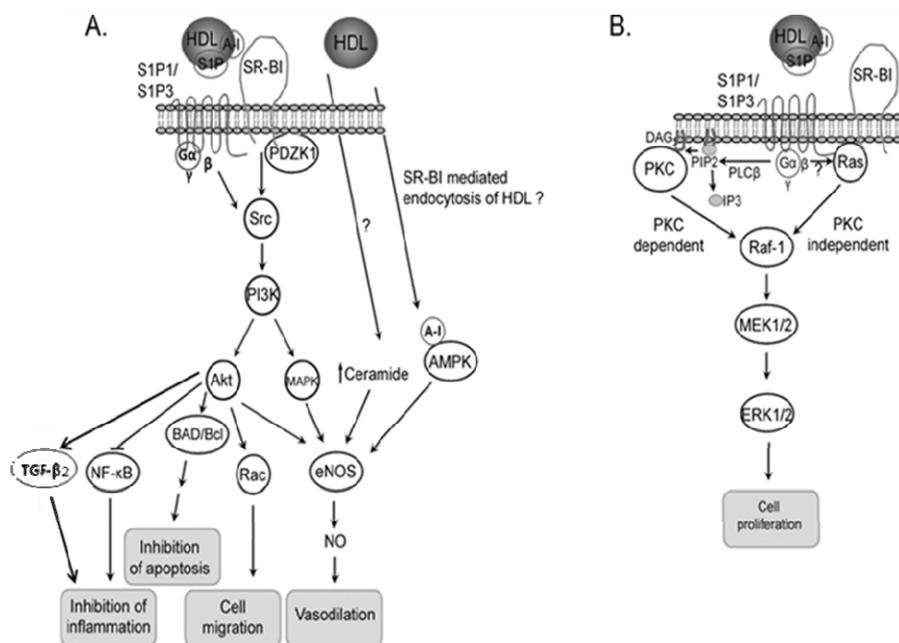


Fig. 3. A) Schematic representation of SR-BI signaling that can lead to vasodilation, cell migration and inhibition of inflammation and apoptosis. B) Schematic representation of PKC-dependent SR-BI-dependent signalling pathway that promote cell proliferation (Drew et al., 2004; Grewal et al., 2003; Kimura et al., 2003; Mineo et al., 2003).

## 5.2 Effect of HDL and apoA-I on inflammation

An initial step in the pathogenesis of atherosclerosis is the association of the monocytes to adhesion molecules of the endothelial cells that facilitates their entry in the sub-endothelial space (Zannis et al., 2004b). Induction of adhesion molecules is promoted by pro-inflammatory stimuli (Cybulsky and Gimbrone, Jr., 1991). Recruitment and migration of monocytes into sub-endothelial space is promoted by the monocyte chemoattractant factor (MCP-1) as well as by oxidized LDL (Peters and Charo, 2001). HDL has anti-oxidant properties and can prevent the oxidation of LDL (Navab et al., 2000a; Navab et al., 2000b). Interactions of HDL or apoA-I with cells of the vascular wall were shown to prevent the expression of pro-inflammatory cytokines and chemokines that induce the expression of adhesion molecules (Bursill et al., 2010; Cockerill et al., 1995; Nobecourt et al., 2010). The anti-inflammatory functions of HDL were manifested in several ways. HDL binds via its apoA-I moiety to progranulin produced by macrophages. This prevents conversion of progranulin to inflammatory granulins which were shown to induce expression of tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin (IL) 1 $\beta$  in monocyte macrophages (Okura et al., 2010). HDL and rHDL were shown to inhibit the cytokine induced expression of vascular cell adhesion molecule-1 (VCAM-1) and inter-cellular adhesion molecule-1 (ICAM-1) by endothelial cells (Cockerill et al., 1995). In addition, HDL promoted the expression of anti-inflammatory cytokines in endothelial cells. Thus treatment of endothelial cells (HUVEC) with HDL and lysosphingolipids present in HDL, increased the expression of TGF- $\beta$ <sub>2</sub> through mechanisms that involve the activation of Akt and extracellular signal regulated kinases (ERK) 1/2. Consistent with cell culture studies, the expression of transforming growth factor- $\beta$ <sub>2</sub> (TGF- $\beta$ <sub>2</sub>) and the phosphorylation of ERK1/2, Akt and Smad2/3 were increased in apoA-I transgenic mice and diminished in apoA-I deficient mice (Norata et al., 2005). In vivo studies also showed that infusion of rHDL inhibited the pro-oxidant and pro-inflammatory events that occurred following implantation of non occlusive periarteral collars in rabbits that induce acute arterial inflammation. In these studies the rHDL inhibited neutrophil infiltration, production of reactive oxygen species (ROS) and the expression of VCAM-1, ICAM-1, MCP-1 and E-selectin (Nicholls et al., 2005b). The carotid vascular inflammation and neutrophil infiltration could be inhibited by rHDL containing normal apoA-I but not by apoA-I obtained from diabetic patients (Nobecourt et al., 2010). The beneficial effects of rHDL containing apoA-I in vivo and in cell cultures could be duplicated using synthetic apoA-I mimetics such as 5A, L37pA and D37pA (Gomaschi et al., 2008; Tabet et al., 2010). The 5A/PLPC complexes reduced the vascular inflammation that is associated with collar insertion in a rabbit model by reducing the ICAM-1 and VCAM-1 expression, the infiltration of neutrophils and the Nox4 activity. In cultures of human carotid endothelial cells (HCEC) 5A/PLPC and rHDL containing apoA-I inhibited the TNF- $\alpha$  induced VCAM-1 and ICAM-1 expression as well as the activation of nuclear factor  $\kappa$ B (NF- $\kappa$ B) pathway and these effects were abolished by ABCA1 silencing (Tablet et al., 2010). Complexes of L37pA or D37pA with  $\beta$ -oleoyl- $\gamma$ -palmitoyl-L- $\alpha$ -phosphatidylcholine reduced the postischemic cardiac contractile dysfunction in a rat heart model of ischemia/reperfusion. They also reduced TNF- $\alpha$  levels and increased prostacyclin levels in the perfusate and inhibited the TNF- $\alpha$  mediated VCAM-1 expression in endothelial cell cultures (Gomaschi et al., 2008). In monocyte and endothelial cell cultures HDL also suppressed expression of chemokines CCL2, CCL5 and CX3CL1 and chemokine receptors CCR2, CCR5 and CX3CR1 (Bursill et al., 2010). This effect was mediated by inhibition of I $\kappa$ -

B $\alpha$  phosphorylation and NF- $\kappa$ B(p65) expression and in some cases by peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) activation. Consistent with cell culture studies, in vivo infusion of apoA-I in cholesterol fed apoE deficient mice reduced expression of chemokines and chemokine receptors (Bursill et al., 2010). HDL and its protein moiety apoA-I have been shown to inhibit the expression of CD11b of human monocytes that is induced by phorbol myristate acetate (PMA) and promote cell adhesion. Inhibition of the ABCA1-mediated and to a lesser extent SR-BI mediated cholesterol efflux by monoclonal antibodies attenuated the inhibitory effect of apoA-I and HDL respectively. Expression of CD11b was also affected by depletion of the membrane cholesterol by treatment of the cells with cyclodextrin (Murphy et al., 2008), pointing out to a potential role of cholesterol efflux in the inhibition of inflammation. Consistent with the ex vivo studies infusion of rHDL in patients with type 2 diabetes mellitus, reduced the expression of CD11b of the peripheral monocytes and reduced the adhesion of the patients' neutrophils to a fibrinogen matrix. Plasma HDL isolated 4 to 72 hours post rHDL infusion suppressed the expression of VCAM-1 in cultures of HAECs and had increased ability to promote cholesterol efflux from THP-1 macrophages (Patel et al., 2009). In cultures of smooth muscle cells, HDL downregulated the NADPH-oxidase mediated generation of reactive oxygen species (ROS) and inhibited production of MCP-1. The inhibitory effect was attenuated by antagonists of S1P<sub>1</sub> and S1P<sub>3</sub> receptors. The data showed that free S1P or S3P alone or as components of HDL could attenuate production of MCP-1. Consistent with these findings, MCP-1 production and ROS generation in the aortas of S1P<sub>3</sub><sup>-/-</sup> receptor and SR-BI<sup>-/-</sup> mice were not affected by treatment with HDL, S1P and sphingosylphosphorylcholine (SPC) (Tolle et al., 2008). ApoA-I induced the expression of the adhesion molecule CD31, and changed the morphology and size distribution of lineage negative bone marrow cells. The treatment also increased the ability of the cells to bind to fibronectin and to cultured endothelial cells. Deletion of the C-terminal helix 10 of apoA-I abolished the effects of apoA-I on bone marrow cells (Mythreya et al., 2008).

### 5.3 Effect of HDL and apoA-I on endothelial cell apoptosis

Exposure of endothelial cells to inflammatory stimuli may disturb the endothelial monolayer integrity (Dimmeler et al., 2002). Numerous factors that promote endothelial apoptosis have been described and include OxLDL (Li et al., 1998), TNF- $\alpha$  (Dimmeler et al., 1999), homocysteine (Welch and Loscalzo, 1998), and angiotensin II (Strawn and Ferrario, 2002). HDL also can reverse the TNF- $\alpha$  induced and growth deprivation induced endothelial cell apoptosis (Nofer et al., 2001; Sugano et al., 2000). OxLDL increased intracellular calcium and resulted in apoptosis that could be inhibited by HDL and apoA-I (Suc et al., 1997). ApoA-I interacts with ABCA1 and F1-ATPase (Chroni et al., 2003; Vantourout et al., 2010), whereas HDL interacts with SR-BI and ABCG1 (Liadaki et al., 2000; Wang et al., 2004) respectively and the sphingolipid components of HDL interact with the S1P receptors (Kimura et al., 2003; Okajima et al., 2009). HDL protected endothelial cells from apoptosis induced by oxLDL by preventing the generation of intracellular ROS. The anti-apoptotic activity was highest for HDL<sub>3</sub> and diminished as the size of HDL increased. It was suggested that approximately 70% of the anti-apoptotic activity of HDL was attributed to apoA-I which has the capacity to accept through its methionine residues the phospholipid hydroperoxides (PLOOH) of oxLDL (de Souza et al., 2010). The anti-apoptotic functions of small size HDL<sub>3</sub> was reduced by 35% in subjects with metabolic syndrome and this

reduction was correlated with the clinical phenotype of the human subjects. Compared to normal HDL the HDL<sub>3</sub> fractions of the diabetic subjects had increased total triglyceride levels and decreased cholesteryl esters/triglycerides ratio suggesting that the lipid core of HDL<sub>3</sub> was enriched with triglycerides (de Souza et al., 2008). HDL<sub>3</sub> also inhibited apoptotic cell death induced by oxLDL and preserved lysosomal integrity of an osteoblastic cell line (Brodeur et al., 2008). The anti-apoptotic effects were attributed to the increased expression of SR-BI that is mediated by HDL<sub>3</sub>, combined with the ability of HDL to compete for the binding of oxLDL to osteoblasts as well as increased selective uptake of the cholesterol of the oxLDL by these cells (Brodeur et al., 2008).

Interactions of apoA-I with cell surface F1-ATPase inhibited apoptosis of HUVEC and stimulated cell proliferation (Radojkovic et al., 2009). In the absence of apoA-I, specific inhibitors for F1-ATPase (IF<sub>1</sub>-H49K) and angiostatin or specific antibodies to F1-ATPase promoted apoptosis and inhibited cell proliferation. In the presence of apoA-I, F1-ATPase inhibitors and antibodies diminished its anti-apoptotic and anti-proliferative effects. Down-regulation of the ABCA1 by siRNA did not affect the anti-apoptotic and proliferative functions of apoA-I whereas inhibition of SR-BI by a specific antibody diminished the anti-apoptotic and proliferative functions of HDL<sub>3</sub> (Radojkovic et al., 2009). The findings suggest that interactions of lipid free apoA-I with F1-ATPase and of HDL with SR-BI contribute to their anti-apoptotic and proliferative effects on endothelial cells. The antiapoptotic effects of HDL on endothelial cells could be mimicked by the lysosphingolipid components of HDL (Nofer et al., 2001). The SR-BI mediated signalling that leads to activation of eNOS, also promotes cell growth and migration and protects cells from apoptosis (Mineo et al., 2006; Noor et al., 2007). Activation of eNOS required its localization in the caveolae, where caveolin SR-BI and CD36 are also found (Uittenbogaard et al., 2000). It has been proposed that oxLDL acting through CD36 depletes the cholesterol content of caveolae and leads to eNOS redistribution to intracellular sites thus resulting in decreased eNOS activity (Blair et al., 1999; Uittenbogaard et al., 2000). HDL acting through SR-BI maintains the concentration of caveolae-associated cholesterol, inhibits the actions of oxLDL and maintains eNOS in the caveolae (Uittenbogaard et al., 2000). This interpretation implies that strong interactions between eNOS and caveolin-1 (Cav-1) stimulate eNOS activity. Other studies provided the opposite mechanism of modulation of eNOS activity by interactions of eNOS with Cav-1 (Terasaka et al., 2010). It was shown that these interactions were enhanced by loading cells with cholesterol or oxysterols and decreased by cholesterol depletion in endothelial cells as a result of ABCG1-mediated cholesterol efflux. Studies in murine lung endothelial cells (MLEC) also showed that HDL could reverse the inhibition of eNOS activity caused by cholesterol loading in the normal but not the Cav-1 deficient cells (Terasaka et al., 2010). It was proposed that diminished interactions between eNOS and Cav-1 caused by ABCG1-mediated efflux stimulated eNOS activity. It has been shown that oxidized phospholipids uncouple eNOS activity and lead to the generation of oxygen radicals which induces the expression of sterol regulatory element binding protein (SREBP) and IL-8 (Gharavi et al., 2006; Yeh et al., 2004). ApoA-I mimetic peptides also prevent LDL from uncoupling eNOS activity to favour O<sub>2</sub><sup>-</sup> anion production as opposed to normal production of NO (Ou et al., 2003). Finally it has been shown that SR-BI via a highly conserved redox motif CXXS between residues 323-326 can promote a ligand independent apoptosis via a caspase 8 pathway and this effect could be reversed by HDL and eNOS (Li et al., 2005). It was proposed that at low HDL levels oxidative stress causes relocation of eNOS away from the

caveolae and this results in SR-BI induced apoptosis (Li et al., 2005). The picture that emerges from these studies is that HDL promotes survival and migration of endothelial cells by signalling mechanisms that originate from the interactions of HDL with SR-BI, the interactions of S1P with S1P1 and S1P3 receptors and the interactions of lipid-free apoA-I with F1-ATPase.

#### **5.4 Effect of HDL on endothelial cell proliferation and migration**

Damage of the endothelium is associated with vascular disease which can be blunted by re-endothelialization (Werner et al., 2003). HDL promoted proliferation of HUVEC via mechanisms that increased intracellular  $Ca^{2+}$  and upregulated the production of prostacyclin (Tamagaki et al., 1996). HDL also promoted endothelial cell migration (Murugesan et al., 1994). Migration was promoted by signalling cascades mediated by interaction of S1P with S1P1 and S1P3 receptors that led to the activation of PI3 kinase, p38MAP kinase and Rho kinases (Kimura et al., 2003). Other studies showed that HDL can activate the MAPK pathway either through processes that involve protein kinase C (PKC), Raf-1, MEK and ERK1/2 or PKC independent pathways. This latter pathway leads to the activation of Ras and can be inhibited by pertussis toxin and neutralizing antibodies against SR-BI (Grewal et al., 2003). The data suggest that interactions of HDL with SR-BI activate Ras in a PKC independent manner and this leads to subsequent activation of MAPK signalling cascade (Grewal et al., 2003) (Fig.3B). Another beneficial effect of HDL is its capacity to promote capillary tube formation in vitro. This function is pertussis toxin sensitive and requires p44/42MAP kinase which is downstream of Ras (Miura et al., 2003). Other studies showed that interaction of SR-BI with HDL or rHDL, activated Src kinases and Rac GTPases and stimulated endothelial cell migration. In vivo experiments have also shown that re-endothelialization of carotid artery following injury is promoted by apoA-I expression and is inhibited in apoA-I deficient mice (Seetharam et al., 2006).

#### **5.5 Effect of HDL on thrombosis**

Increased HDL cholesterol levels are associated with decreased risk of venous thrombosis (Doggen et al., 2004). In contrast low HDL levels are associated with increased risk of venous thrombosis (Deguchi et al., 2005). The ability of HDL to inhibit endothelial cell apoptosis (Dimmeler et al., 2002; Mineo et al., 2006) prevents vessel denudation and formation of microparticles that may contribute to thrombosis (Durand et al., 2004). It has been shown that thrombogenic membrane microparticles that may originate from apoptotic endothelial cells are increased in the plasma of patients with acute coronary syndrome (ACS) (Mallat et al., 2000). Infusion of rHDL in volunteers that received low levels of endotoxin limited the prothrombotic and procoagulant effect of endotoxin (Pajkrt et al., 1997). Furthermore infusion of apoA-I<sub>MILANO</sub> in a rat model of acute arterial thrombosis increased the time of thrombus formation and decreased the weight of the thrombus (Li et al., 1999). HDL may affect thrombosis via a variety of mechanisms: Early studies showed that HDL causes increased synthesis of prostacyclin in cultured endothelial cells (Fleisher et al., 1983; Tamagaki et al., 1996). Prostacyclin in combination with NO promote smooth muscle cells relaxation, inhibit platelet activation and local smooth muscle cell proliferation (Vane and Botting, 1995). It has been reported that HDL<sub>3</sub> induced expression of cyclooxygenase-2 (Cox-2) by smooth muscle cells and promoted release of prostacyclin (PGI<sub>2</sub>) via a signalling pathway that involves p38MAP kinase and c-Jun N terminal kinase

(JNK-1) (Escudero et al., 2003; Vinals et al., 1997). PGI<sub>2</sub> synthesis was enhanced by HMGCoA reductase inhibitors (Martinez-Gonzalez et al., 2004). It has been shown that there is a positive correlation between plasma HDL levels and anticoagulant response to activated protein C (APC)/protein S *in vitro* (Griffin et al., 1999) and negative correlation with the plasma thrombin activation markers such as prothrombin fragments F1.2 and D-dimer (MacCallum et al., 2000). APC inactivates, by proteolysis, factors Va and VIIIa in plasma and thus it can downregulate thrombin formation. Administration of HDL to cholesterol-fed rabbits also increased endothelial cell thrombomodulin levels, promoted generation of APC and inhibited formation of thrombin (Nicholls et al., 2005a). Glucosylceramide and glycosphingolipids which are present in HDL are lipid cofactors for the anticoagulant activity of APC and in a significant number of patients with venous thrombosis the levels of glucosylceramides are low (Deguchi et al., 2002; Deguchi et al., 2001). Shpingosine, another molecule present in HDL, has been shown to inhibit prothrombin activation on platelets' surface by disrupting procoagulant interactions between factors Xa and Va (Deguchi et al., 2004). HDL also downregulated expression of plasminogen activator inhibitor-1 (PAI-1) and upregulated tissue plasminogen activator (t-PA) in endothelial cell cultures (Eren et al., 2002). Transgenic mice expressing the human PAI-1 developed age-dependent coronary arterial thrombosis (Eren et al., 2002). In contrast oxidized HDL<sub>3</sub> induced the expression of PAI-1 in endothelial cells through signalling mechanisms that involve activation of ERK1/2 and p38MAPK and mRNA stabilization (Norata et al., 2004).

### 5.6 Effects of HDL on diabetes mellitus

*In vivo* and *in vitro* studies have provided evidence that HDL may have beneficial effects on glucose metabolism (Koseki et al., 2009; Rutti et al., 2009). *Ex vivo* studies showed that HDL and delipidated apoA-I or S1P decreased IL-1 $\beta$  and glucose-mediated apoptosis and thus increased the survival of human and murine islets. HDL treatment down-regulated the expression of iNOS and its downstream target Fas which is pro-apoptotic and up-regulated the expression of FLICE-like inhibitory protein (FLIP) which is anti-apoptotic (Rutti et al., 2009). HDL also reversed the toxic effects of oxidized LDL on beta cells that are associated with apoptosis and cJNK mediated transcriptional repression of the insulin gene caused cJNK mediated (Abderrahmani et al., 2007).

Oral glucose tolerance test in a limited number of patients with Tangier disease (that lack or have dysfunctional ABCA1) showed that they had glucose intolerance as compared to controls (Koseki et al., 2009), thus implicating ABCA1, apoA-I and HDL in glucose metabolism. Cell culture studies using primary pancreatic islets cells and a pancreatic  $\beta$ -cell line (Min6), showed that lipid free apoA-I or apoA-II or reconstituted HDL increased insulin secretion up to 5-fold in a Ca<sup>2+</sup> dependant manner (Fryirs et al., 2010). The free apolipoproteins also increased insulin mRNA levels. HDL mediated insulin secretion has also been observed in cultures of mouse pancreatic  $\beta$ -cells (MIN6N8) (Drew et al., 2009). The increase in insulin secretion mediated by lipid-free apoproteins and rHDL required the functions of ABCA1 and SRBI or ABCG1 respectively. These functions may be different from those involved in cholesterol efflux. For high glucose concentrations enhanced insulin secretion required the action of K<sub>ATP</sub> channel and glucose catabolism in the pancreatic cell, but this did not occur for low glucose concentrations (Fryirs et al., 2010).

Further insight on the role of ABCA1 in diabetes was obtained by studies in mice with selective deficiency of ABCA1 in the pancreas (ABCA1<sup>-P/-P</sup>). These mice accumulated

cholesterol in their islets and were characterized by impaired acute phase insulin secretion and glucose intolerance. The ABCA1<sup>-P/-P</sup> mice exhibited normal insulin sensitivity indicating normal response of the peripheral tissues to insulin. The impairment in insulin secretion was verified in cell culture experiments using islets isolated from the ABCA1<sup>-P/-P</sup> mice. In contrast, whole ABCA1 deficient mice had normal glucose tolerance and displayed only small impairment in the islet function and did not accumulate significant amount of cholesterol in the islets (Brunham et al., 2007). Pancreatic islets isolated from apoE deficient mice had increased cholesterol content and reduced insulin secretion as compared to islets obtained from WT mice. The reduced insulin secretion in the pancreatic islets or cultures of  $\beta$ -cells could be restored by depletion of the cellular cholesterol using mevastatin or methyl- $\beta$ -cyclodextrin (M $\beta$ CD) (Hao et al., 2007). Experiments in cell lines of pancreatic  $\beta$ -cell origin indicated that cholesterol loading or cholesterol depletion affect the activity of glucokinase (GK) which is known to regulate insulin secretion (Rizzo and Piston, 2003). The experiments showed that under normal cholesterol levels GK is associated with a dimeric form of nNOS on insulin containing granules in the cytoplasm and is inactive (Rizzo and Piston, 2003). Increase in plasma cholesterol enhanced dimerization of nNOS and its association with GK whereas reduction in the cholesterol levels or increase in the extracellular glucose levels promoted monomerization of nNOS and release of active GK in the cytoplasm (Hao et al., 2007). The role of the increase in the cholesterol content of  $\beta$ -cells in insulin secretion was tested in transgenic mice expressing SREBP-2 in  $\beta$ -cells under the control of insulin promoter. These mice had normal plasma cholesterol levels but developed severe diabetes characterized by 5-fold increase in gluco-hemoglobin and defects in glucose and potassium-stimulated insulin secretion and were characterized by glucose intolerance. The islets were fewer, smaller and deformed and had increased levels of total and esterified cholesterol (Ishikawa et al., 2008). It was proposed that the loss of  $\beta$ -cell mass could be related to the down regulation of genes such as PDX-1 and BETA2 that are involved in  $\beta$ -cell differentiation.

A recent comprehensive study has measured the properties of HDL isolated from patients with type 2 diabetes mellitus and their functions on endothelial cells in vitro and in vivo. HDL isolated from patients with low HDL and type 2 diabetes mellitus contained increased levels of lipid peroxides and increased myeloperoxidase activity. In endothelial cell cultures diabetic HDL had reduced production of NO and increased NADPH oxidase activity that resulted in increased oxidant stress. Diabetic HDL had diminished endothelium dependent relaxation of aortic rings and endothelial progenitor cells obtained from diabetic subjects had diminished capacity to promote reendothelialization in vivo. A remarkable finding in this study was that extended release niacin treatment of the diabetic patients restored the properties and functions of HDL. The HDL obtained after treatment had normal levels of peroxides and normal myeloperoxidase (MPO) activity. Studies with endothelial cultures showed that following treatment of the diabetic patients their HDL could induce normal NO production and NADPH oxidase activity and could promote normal relaxation of aortic rings. Endothelial progenitor cells obtained from diabetic patients following niacin treatment had normal ability to promote reendothelialization in vivo (Sorrentino et al., 2010). In another study intravenous infusion of rHDL (80 mg/kg over 4 hours) in type 2 diabetic human subjects decreased plasma glucose level, increased plasma insulin level and increased  $\beta$ -cell functions as compared to patients receiving placebo (Drew et al., 2009). HDL and apoA-I increased glucose uptake of primary human skeletal muscle cultures established from patients with type 2 diabetes mellitus. HDL induced glucose uptake and fatty acid oxidation and increased AMPK $\alpha$ 2 activity and phosphorylation. These effects

were modulated through a  $\text{Ca}^{2+}$  dependent pathway. Subsequent *in vitro* and *in vivo* studies showed that rHDL inhibited lipolysis in 3T3-L1 adipocytes partially via activation of AMPK pathway (Drew et al., 2011). Infusion of rHDL also inhibited fasting induced lipolysis and fatty acid oxidation but increased the circulating non essential fatty acids possibly due to the action of phospholipase on the rHDL phospholipids (Drew et al., 2011). The HDL dependent glucose uptake by the skeletal muscle cells was abrogated by inhibition of ABCA1 with a blocking antibody suggesting that ABCA1 functions not related to cholesterol efflux, may contribute to the increased glucose uptake and  $\beta$ -oxidation by skeletal muscle cells obtained from patients with type 2 diabetic mellitus (Drew et al., 2011). The effect of apoA-I on glucose metabolism was also studied in C2C12 myocytes and apoA-I deficient mice. Consistent with the studies with primary human skeletal muscle cultures, apoA-I stimulated AMPK and acetyl-CoA carboxylase (ACC) phosphorylation and glucose uptake and endocytosis into C2C12 cells. The apoA-I deficient mice had increased fat content decreased glucose tolerance and increased expression of gluconeogenic enzymes in the liver and decreased AMPK-dependent phosphorylation in skeletal muscle and the liver (Han et al., 2007).

### 5.7 Role of apoA-I and HDL in atheroprotection

Atherosclerosis is associated with lipid and lipoprotein abnormalities (Zannis et al., 2004b). Low HDL levels (Gordon et al., 1989) and decreased concentration of the largest size HDL subpopulations (Asztalos et al., 2004) are associated with increased risk of CAD. The anti-atherogenic functions of HDL and apoA-I have been, to a large extent, attributed to the beneficial effects that HDL exerts on cells of the arterial wall as well as their ability to promote efflux from macrophages and other cells of the arterial wall via ABCA1, ABCG1 and SR-BI (Wang et al., 2007). Hepatic overexpression of apoA-I gene in the background of apoE or LDL<sub>r</sub> deficient mice reduced the atherosclerosis burden of these mice following an atherogenic diet (Tangirala et al., 1999). These findings demonstrate the importance of apoA-I and HDL for atheroprotection. In contrast, double deficient mice for apoA-I and the LDL<sub>r</sub> fed an atherogenic diet developed atherosclerosis and had increased concentration of circulating auto-antibodies, increased population of T, B, dendritic cells and macrophages, as well as increased T cell proliferation and activation. The abnormal phenotype was corrected by adenovirus mediated gene expression of apoA-I (Wilhelm et al., 2010). Similarly apoA-I transgenic rabbits were resistant to diet induced atherosclerosis (Duverger et al., 1996). Two clinical trials showed that intravenous administration of 15 mg/kg of apoA-I<sub>MILANO</sub>/phospholipid complexes in five weekly doses in patients with acute coronary syndrome resulted in significant regression of atherosclerosis as it was shown by intravascular ultrasound (Nicholls et al., 2006; Nissen et al., 2003). The epidemiological studies (Gordon et al., 1989), combined with studies of experimental animals (Duverger et al., 1996; Tangirala et al., 1999) and clinical intervention studies (Barter et al., 2007; Sorrentino et al., 2010) highlight the importance of increased HDL levels for atheroprotection. However, human subjects have been identified with high HDL levels and CAD (Ansell et al., 2003). In addition, increased HDL levels in humans treated with the CETP inhibitor torcetrapib, increased cardiovascular, and non-cardiovascular deaths (Barter et al., 2007). These findings suggest that high HDL levels are not always synonymous with atheroprotection. The concept that emerges from all the studies is that the most important factor for atheroprotection is the functionality of HDL. Understanding of the structure-function and the cell signaling associated with HDL and apoA-I will provide molecular explanations for their beneficial effects for atherosclerosis and other human diseases and apoA-I.

## 6. Conclusion

Numerous prospective epidemiological studies have established an inverse correlation between HDL cholesterol levels and the risk for CAD. However, the discovery of human subjects with high HDL cholesterol levels and CAD, combined with clinical intervention studies designed to raise HDL levels and studies of animal models, led to the realization that high levels of HDL cholesterol alone are not sufficient to prevent atherosclerosis. HDL via its protein and/or lipid components participates in numerous interactions with the endothelium, other cells of the vascular wall, as well as with  $\beta$  pancreatic cells, and has a protective effect against atherothrombosis and other diseases. The key proteins that participate in the biogenesis, remodeling and signaling of HDL are of great importance for the functionality of HDL. Mutations in apoA-I, ABCA1 and LCAT affect the biogenesis of HDL and either prevent formation of HDL or generate aberrant HDL subpopulations which may have altered functions. The ability of HDL to inhibit the oxidation of LDL, prevents the induction of pro-inflammatory and pro-apoptotic pathways that are detrimental to the endothelium. HDL interacts directly with the endothelial cells via SR-BI and the ABCG1. These interactions lead to NO release and vasodilatation, promote reendothelialization and suppress expression of adhesion molecules on endothelial cells in response to pro-inflammatory cytokines. As a result of these and other interactions of the sphingolipid components of HDL with S1P receptors, HDL protects from apoptosis and inflammation and promotes endothelial cell growth and migration. Understanding the complexity and the functions of HDL may facilitate in the near future the development of new HDL-based therapies to prevent or treat atherosclerosis and other human diseases.

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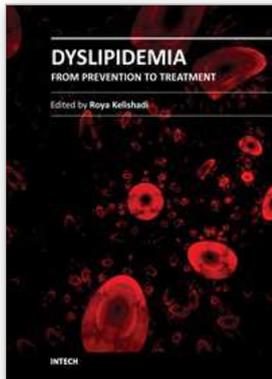
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Dyslipidemia has a complex pathophysiology consisting of various genetic, lifestyle, and environmental factors. It has many adverse health impacts, notably in the development of chronic non-communicable diseases. Significant ethnic differences exist due to the prevalence and types of lipid disorders. While elevated serum total- and LDL-cholesterol are the main concern in Western populations, in other countries hypertriglyceridemia and low HDL-cholesterol are more prevalent. The latter types of lipid disorders are considered as components of the metabolic syndrome. The escalating trend of obesity, as well as changes in lifestyle and environmental factors will make dyslipidemia a global medical and public health threat, not only for adults but for the pediatric age group as well. Several experimental and clinical studies are still being conducted regarding the underlying mechanisms and treatment of dyslipidemia. The current book is providing a general overview of dyslipidemia from diverse aspects of pathophysiology, ethnic differences, prevention, health hazards, and treatment.

### **How to reference**

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