



Oxidative Modification of Lipoproteins: A Potential Role of Oxidized Small dense LDL in Enhanced Atherogenicity

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Author's contribution

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ABSTRACT

Atherosclerosis (AS) is a multifaceted inflammatory syndrome of the arterial wall to which number of mediators have been implicated in lesion progression. Triglyceride (TG)-rich lipoproteins consist of the large diversity of lipoprotein particles that fluctuate in density, size, and apolipoprotein composition. Two foremost phenotypes, on basis of size, chemical configuration, and density, of low-density-lipoprotein (LDL) have been recognized i.e., pattern A, having LDL diameter greater than 25.5nm (large buoyant LDL or lb-LDL) and pattern B, having LDL diameter less than or equal to 25.5nm (small-dense LDL or sd-LDL). Small-dense low-density-lipoprotein (sd-LDL) particles are produced by potential intravascular hydrolysis of TG-rich VLDL particles via lipoprotein lipases (LPLs), hepatic lipases (HLs) and cholesterol ester transfer protein (CETP). sd-LDL is more atherogenic due to its smaller size, increased penetration into the arterial wall, extended plasma half-life, lesser binding affinity for LDL receptors (LDL-R) as well as lower resistance to oxidative stress when equated with lb-LDL. The higher atherogenic potential of sd-LDL is due to its enhanced susceptibility to oxidation, owing to high polyunsaturated fatty acids (PUFA), low cholesterol and Apoprotein B (ApoB) content. An enhanced understanding of sd-LDL metabolism at the molecular level, transport and clearance may result in the development of sd-LDL as an independent predictive marker for AS events and may be used to maintain cholesterol homeostasis and prevent the succession of AS.

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ABBREVIATIONS

•OH:	Hydroxyl radicals
ABCA1:	ATP-binding carrier transporter
ABCG1:	ATP-binding cassette sub-family G member 1
apo C-III:	Apolipoproteins C-III
ApoB:	Apoprotein B
apoB100:	Apolipoproteins B-100
AS:	Atherosclerosis
CAD:	Coronary artery disease
CE:	Cholesteryl ester
CETP:	Cholesterol ester transfer protein
CETP:	Cholesterol ester transfer protein
CHD:	Coronary heart disease
CRP:	C-reactive protein
EC:	Endothelial cells
ED:	Endothelial dysfunction
HDL:	High-density lipoprotein
HL:	Hepatic lipases
HMG-CoA:	3-hydroxy-3-methylglutaryl-coenzyme A
HMG-R:	3-hydroxy-3-methylglutaryl-coenzyme A reductase
HSPGs:	Heparan sulphate proteoglycans
IDL:	Intermediate-density lipoproteins
IL:	Interleukin
LCFA:	Long-chain free fatty acids
LDL:	Low-density-lipoprotein
LDL-C:	Low-density-lipoprotein Cholesterol
LDL-R:	Low-density-lipoprotein receptors
LP:	Lipoproteins
LPLs:	Lipoprotein lipases
MCP-1:	Monocyte chemoattractant protein-1
MS:	Metabolic syndrome
PCSK-9:	Proprotein convertase subtilisin/kexin type 9
PMW:	Post-menopausal women
PUFA:	Polyunsaturated fatty acids
RCS:	Reactive chlorine species
RNS:	Reactive nitrogen species
ROS:	Reactive oxygen species
sd-LDL:	Small-dense low-density-lipoprotein
SMCs:	Smooth muscle cells
sPLA2:	Secretory phospholipase A2
SR-B1:	Scavenger receptor
SREBP:	Sterol regulatory element binding proteins
T2D:	Type 2 diabetes
TG:	Triglyceride
VLDL:	Very low-density lipoproteins

1. INTRODUCTION

Numerous pieces of evidence support the perception that the process of derangement between free radicals and antioxidants i.e., oxidative processes contribute to the pathogenesis of atherosclerosis (AS) and coronary heart disease (CHD). The identity of oxidants that produce high concentrations of oxidized lipid and protein, and low levels of antioxidants, are found in human atherosclerotic lesions, yet, it is unclear, for most of the species already requested [1–4]. Atherosclerosis and its associated complications are the most common causes of death in developed countries. Several risk factors contribute to the pathogenesis of AS i.e., hypertension, smoking, obesity, diabetes and dyslipidemia which is represented by increased triglycerides (TG), total and elevated low-density-lipoprotein (LDL) cholesterol levels, as well as decreased high-density lipoprotein (HDL) cholesterol [2,5,6].

Since LDL is the foremost transporter of cholesterol in human plasma, its elevated levels are associated with an increased risk of AS [7,8]. High levels of LDL cholesterol have long been established as the most potent risk factors for coronary artery disease (CAD) [1,9–11]. Both normal and hyperlipidemic individuals showed differences in LDL size and size [12–14]. Depending on the particle size, chemical composition and quantity, two major types of LDL are identified-pattern A, with a LDL width greater than 25.5nm (high LDL or lb-LDL) and pattern B, with a LDL width lesser or equal to 25.5nm (small, high-density LDL or sd-LDL). About 30% of total LDL-C in the blood contains sd-LDL in normal individuals and its rate rises significantly in subjects with CAD depending on the severity of the disease [15,16]. In addition to genetic factors, LDL heterogeneity is also determined by age, gender and diet [17,18], whereas, LDL size is associated with plasma TG levels and their metabolism [19]. LDL is secreted largely by the liver via high binding of apoprotein B (apoB) to the LDL receptor (LDL-R) followed by cellular endocytosis, lysosomal hydrolysis of lipid moiety and apoB degeneration [20]. Reports in animals mentioned that although the liver is a key organ in removing LDL especially through LDL-R, 60-70% via independent LDL-R pathways that occur in extrahepatic tissues [21,22]. Therapeutic variability of different LDL subspecies is also very

helpful in decreasing the menace of cardiovascular events [14,15].

2. LIPOPROTEIN CLASSES AND THEIR ATHEROGENICITY

Triglyceride containing lipoproteins (LPs) have a wide variety of LP particles that vary in size, density, and lipid as well as apolipoprotein configuration. Chylomicrons and large very low-density lipoproteins (VLDLs) may not be able to invade the arterial wall, whereas small VLDLs and Intermediate-density lipoproteins (IDLs) can easily invade the blood vessel which concludes that some rich LPs play an important role in atherogenicity. Increased hepatic secretion of VLDL particles leads to higher concentrations of plasma TG. Consequently, TGs are substituted with the cholesteryl ester (CE) for cholesterol ester transfer protein (CETP) activity. This further results in TG-enriched HDL particles which are catabolized at a faster rate than CE-enriched VLDL particles that are finally converted into sd-LDL particles [23].

Extensive evidence suggests that oxidative modification of VLDL, IDL, LDL and sd-LDL is independently associated with AS. In addition, an opposite association between HDL levels and premature heart disease has been observed in many large prospective studies [24] as well as in animal studies [25]. HDL also prevents LDL oxidation and subsequent complications of CVD [26].

Researchers affirmed that plasma LP is distributed into seven classes according to their size, lipid composition, and apolipoproteins. Chylomicrons are large particles of rich TG produced by the intestines, mainly associated in the transport of dietary TGs and cholesterol to the bordering and liver tissues as well as have apolipoproteins A-I, A-II, A-IV, A-V, B-48, C-II, C-III, and E. The process of chylomicron formation is very complex, and the transport of moderate and long-chain fatty acids are done by chylomicrons. High-fat diets upsurge the expression of apo-AIV which acts as a surface element of apoB48 particles [27]. The net transfer of membrane TGs to luminal particles may be promoted by Apo-A IV due to increased levels of microsomal triglyceride transfer protein (MTP) at the pre-translational level [28]. These chylomicron particles are found in many sizes; therefore, more fat/cholesterol load can be borne by increasing the number of chylomicron particles and/or size. Chylomicron assemblage can involve 3 major processes: the synthesis of

large LPs, the formation of lipid droplets, and basic expansion [29–31]. Further, the chylomicron can also be thought of as an oil transporter from the intestinal lumen to the liver. The removal of TGs from large chylomicrons by LPL activity in muscle and adipose tissues results in chylomicron remnants.

VLDL is a rich TG cell produced by the liver. They contain apoB100, CI, C-II, C-III, and E. VLDL has apoB100 as its regular-forming protein with a single molecule of apoB100 per VLDL. Alike chylomicrons, the amount of TG regulated in VLDL determines the size of the VLDL particles. VLDL particles are highly secreted due to increased TG production in the liver. Heparan sulfate proteoglycans (HSPGs) facilitate the first step in activating chylomicron/VLDL through LPL-mediated lipolysis of TGs. HSPGs are mainly expressed by the cell of the capillaries in the heart, adipose tissue and skeletal muscle. The freshly formed VLDL particles may be modified by the action of hepatic lipase (HL) and CETP into LDL particles or otherwise directly removed from the liver [32]. Excessive elimination of TGs from VLDL causes the formation of cholesterol containing IDL/VLDL residues that contain apoB100 and E. Intermediate-density lipoproteins belong to the family of LP particles and enable fats and cholesterol structures to move within the aqueous solution from the blood. Excessive TG hydrolysis of VLDL and IDL leads to the formation of LDL particles and is significantly enhanced by the cholesterol that carries most of the cholesterol present in circulation.

ApoB100 is the most important and primary LDL protein with a single molecule of apoB100 per LDL particle. In general, LDL is composed of neutral lipids with hydrophobic core, CEs and TGs, encircled by a monolayer of hydrophilic phospholipids, mainly sphingomyelin and phosphatidylcholine, presenting N-trimethyl head group to the environment (aqueous). LDL has many different particles that differ in size and density. Among them, sd-LDL are strongly associated with hypertriglyceridemia, obesity, low HDL levels, type 2 diabetes (T2D) in patients with metabolic syndrome (MS) and infectious and inflammatory diseases [33]. Many factors that make sd-LDL more pro-atherogenic than larger LDL particles. sd-LDL particles have spent a lot of time in circulation on account of their reduced affinity for LDL-R [34].

High-density lipoprotein particles are responsible for the reverse transfer of cholesterol from

peripheral tissues to the liver which is the probable way for HDL to be anti-atherogenic. The apolipoproteins related to HDL particles are A-I, A-II, A-IV, CI, C-II, C-III, and E. apoA-I is a built-in HDL protein and each HDL molecule can contain many apoA-I molecules. Further, LP (a) is an LDL particle-containing apolipoprotein (a) attached to apoB100 via disulphide (-S-S-) bond and have pro-atherogenic properties with unknown activity [35]. The crucial protective role of HDL is attributed to its antioxidant property through paraoxonase-1 (PON-1) which showed its modulatory effects in CVD as well as a diabetic complication [36,37].

3. OXIDATIVE MODIFICATION OF LDL

The oxidative modification of LP constitutes a key episode in inflammation and atherosclerotic progression [38]. LDL undergoes various chemical modifications after it is synthesized in either plasma or in the intimal layer of the artery. Modification mainly affects either the protein or the lipid constituent of this LP particle. Oxidative modification of LPs and increased LDL levels in serum represent a major and independent risk factor for AS. Ox-LDL molecules play an important role in initiating and sustaining atherosclerotic lesion growth and platelet formation. Both enzymatic and non-enzymatic mechanisms in vivo may proceed to LDL oxidation. LDL oxidation in vivo is likely to be influenced by local physiochemical and environmental factors like pH and availability of oxidants in order to oxidize LDL and its subpopulations. Factors like antioxidant content, particle size and fatty acid (FA) composition are also important determinant of LDL oxidation [39–42].

Mechanisms of LDL oxidation in vivo involve intensive modification by oxidants/free radicals produced by cells of the arterial wall, for example reactive nitrogen species (RNS), reactive oxygen species (ROS), hydroxyl radicals (\bullet OH), reactive chlorine species (RCS) and lipid-soluble free radicals [43]. Plasma LDL-C is preferentially attacked by these free radicals and converted into ox-LDL forms. Ox-LDL leads to the increased attraction of blood monocytes beneath the endothelium to clear these atherogenic molecules. Ox-LDL is extremely atherogenic and stimulates the enhanced accumulation of cholesterol in macrophages leading to the development of foam cells [Fig. 1]. These events produce cytotoxic effects on the arterial wall and give rise to increased inflammation and thrombotic processes [44]. Lipid peroxidation is

the major step in LDL oxidation, which involves the rapid conversion of the LDL polyunsaturated fatty acids (PUFA) into aldehydic lipid peroxidation products [45]. The immediate targets of ROS in the cytosolic compartment are long-chain free fatty acids (LCFA) and membrane-bound lipids. After to attack on LCFA and membrane-bound lipids, ROS lead to the formation of lipid peroxides. LDL is now recognized as a critical contributor to the pathogenesis and progression of AS in humans.

Oxidized-LDL particles are found to have high levels of the oxysterol derivatives [39,42] may induce cell injury by both apoptotic and necrotic pathways [46]. Instead, raised levels of LP can be more susceptible to glycation that happens both on the LDL apoB as well as on its phospholipid components. LDL glycation leads to both active changes in the LDL clearance pathway and its increased tendency to oxidative changes. These chemically modified LDL are then taken up by receptors or scavenger receptors expressed differentially on the surfaces of monocytes/macrophages, endothelial cells (EC), circulating inflammatory cells and kupffer cells. Ox-LDL represents the most important atherogenic molecules among various chemically modified LDL whereas glycosylated LDL exhibits enhanced susceptibility to oxidation when compared to normal LDL [47–49]. Some other studies demonstrated that depletion of LDL endogenous lipophilic antioxidants, such as, β -carotene (lycopene) and vitamin E, results in initiation of LDL oxidation [8,50–52]. Several studies make a robust indication that LDL undergoes oxidation in vivo and this ox-LDL is associated with the progression and development of the early and late atherosclerotic consequences [1,34,53].

4. sd-LDL: BIOCHEMISTRY AND METABOLISM

Low-density lipoproteins are usually circular particles with a non-polar CE and TG intermediate spine. In addition, free cholesterol inserts between chains of phospholipid fatty acid providing an amount of firmness in the phospholipid monolayer and that outer layer of LDL that will interact with plasma. LDL has the apoB100 exposed on its surface making it easier to detect LDL-R [54]. The different components of LDL have been demonstrated in different studies using different methods i.e., density gradient, Polyacrylamide gel electrophoresis (PAGE), ultracentrifugation, NMR etc. [16,55–

58]. Depending on their size, many different types of LDL have been identified that differ in their pattern of behaviour and disease roles. sd-LDL particles are highly formed in response to elevated levels of TGs. The sd-LDL particles are produced by a probable intravascular makeover of TG-rich VLDL particles where they interact mainly with lipoprotein lipases (LPLs), HL and CETP. CETP facilitated the exchange of CE/TG between VLDL and LDL together with HL-mediated hydrolysis results in the conversion of LDL to sd-LDL [59].

The subfractions of LDL share many common features. CE (38.3-42.8%), the main lipid, and free cholesterol (8.5-11.6%) inclines to decrease as the density increases. TGs are a small constituent (3-5%) whereas; apoB-100 is a major protein in all sub-LDL substances that leads to an increase in LDL strength as the protein content increases. Further, apoE forms 0.1-1.3% and 0.2-1.9% of LDL proteins in low and high-density subfractions, respectively. It's worth mentioning that the ratio of apoE to apoB fluctuates from 1:60 to an extreme of 1: 8 in denser subfractions that might account for causing differences in the binding affinities for LDL-R. Moreover, apoC-III is existing in subfractions with a density greater than 1.0358 g/ml. The calculation of the quantity of each chemical component per subspecies of LDL

showed the presence of a single apoB molecule in association with a reduced number of CE, free cholesterol and phospholipids [60]. The range of human LDL particles corresponds well to the phospholipid/apoB level in LDL but not with the molar ratio of cholesterol/apoB or TG/apoB indicating that the phospholipid content also has a strong determination of LDL size [57,61].

5. sd-LDL MEDIATED COMPLICATIONS AND ITS ATHEROGENICITY

In addition to their ability to be taken up efficiently and quickly by macrophages to generate foam cells, ox-LDL has several potential mechanisms which promote atherogenesis. Ox-LDL acts as a chemoattractant for monocytes present in blood stream [48,62], both directly and also via stimulation of monocyte chemoattractant protein-1 (MCP-1) [48]. A Quebec Cardiovascular Study [63] demonstrated the fact that men with higher sd-LDL cholesterol possess a marked increased threat of CHD on follow-up. Another report [64], also showed that the CHD patients with a minor LDL peak particle size, a raised fraction of sd-LDL and an augmented plasma level of sd-LDL cholesterol than healthy controls. Harchaoui [65] also depicted a significant correlation between the number of sd-LDL particles and the occurrence of CAD, rather than among serum LDL-C and CAD.

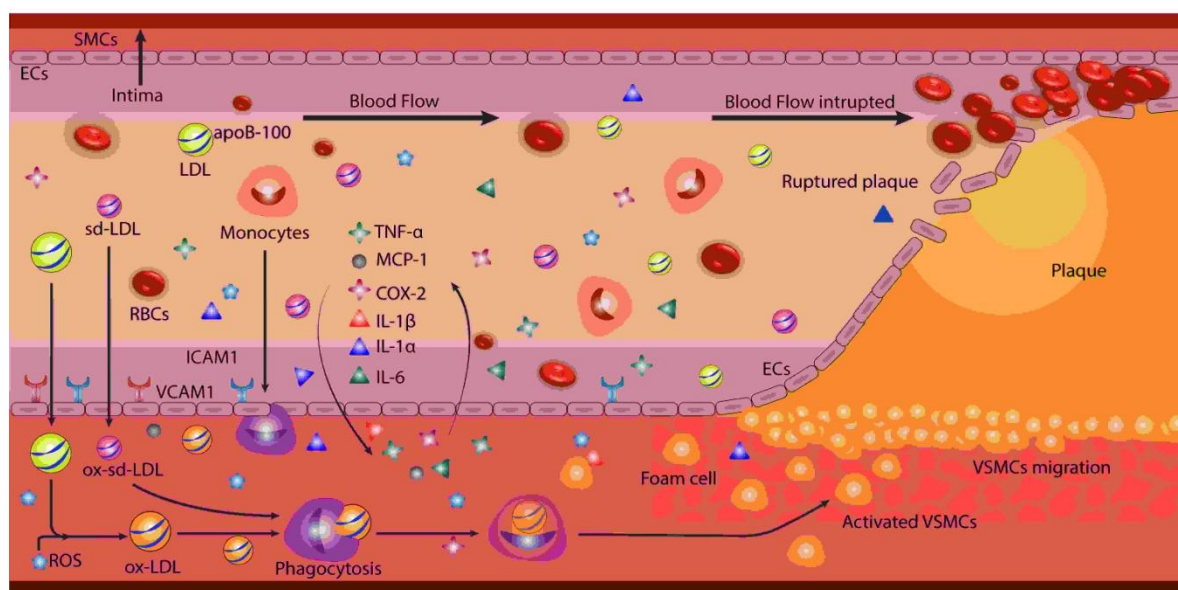


Fig. 1. Schematic demonstration of sd-LDL and LDL infiltration into endothelial bed followed by plaque formation via macrophages, T lymphocytes, various chemo-attractants (cytokines i.e., TNF- α , NF- κ B, IL-1 and IL-6) and foam cell accumulation

Additional studies also validated that sd-LDL is more atherogenic due to its higher degree of penetration of the arterial wall, long plasma half-life, lesser binding affinity for LDL-R and reduced resistance to oxidative stress when related with large buoyant LDL [59,36]. Since, sd-LDL particles have low antioxidant content and cholesterol level, and increased PUFA and apoB content, they are known to be more prone to lipoxidative modification [66,67]. Toft-Petersen et al. [68] demonstrated that the quantity of sd-LDL was a robust univariate analyst of substantial coronary artery stenosis evaluated by both coronary angiography and non-invasive CT scanning methods.

5.1 Impact of LDL/sd-LDL on SMC and Trans Endothelial Filtration

It is well reckoned that the cholesterol is capable to pass through the vascular endothelium into the intima of the artery. The metabolism provides LPs for its transport and the sd-LDL due to their size and density can through it below vascular sub endothelium. There is an association between the size of LP and endothelial damage. Smaller and denser is the LDL molecule, bigger is the atherogenic effect. The decrease in the size and density of the LDL facilitates its infiltration into the intima and increases its atherogenic effect [1,20,69]. During early atherogenesis, there is increased recruitment of monocytes into the arterial sub-endothelium [1].

Oxidized sd-LDL (ox-sd-LDL) should play a key role in early atherogenesis as it is well taken up by macrophages through macrophage scavenger receptors, which lead to the formation of foam cells, lipid-laden macrophages, a characteristic feature of primary AS [48,70] [Fig. 1]. Arterial wall cells, such as ECs, smooth muscle cells (SMCs), and macrophages, can in vitro ox-LDL particles in the presence of catalytic transition iron ions [1,48].

Previous researchers have shown that ECs and SMCs [48,62] in cultures produce MCP-1 acting on blood monocytes and not neutrophils. The reports confirmed that the activity of chemotactic monocyte in supernatants from multiple tumour cell lines was inhibited by an antibody made against the monkey SMC chemotactic factor (anti-SMCF) [71,72]. In addition, it also demonstrated a robust concordance with immuno-precipitation of proteins having 14.4 kDa molecular weight. The human glioma cell line, 1OSMG, clearly reveals the activity of the

chemotactic monocyte. The protein involved in this chemotactic monocyte activity has been identified, sequenced and named for MCP-1 [73].

Minimally modified LDL (mm-LDL) induces EC to produce colony-stimulating substances, including monocyte colony-stimulating factor, expressed as a potent monocyte chemoattractant [74]. After exposure to ox-LDL human EC and SMC cultured independently/mutually produced more monocyte chemotactic activity when related with unexposed control cells or cells exposed to unmodified LDL. This increase in chemotactic monocyte activity was compared with an increase in MCP-1 mRNA levels produced by the glioma cell line U 105MG [75].

5.2 sd-LDL Leads Atherogenic Consequences in Post-menopausal Women (PMW)

It is extensively acknowledged that in post-menopausal women (PMW) the risk of CAD progressively increases, mostly coupled through a drop in estrogens level and raise in circulatory LDL [76–78]. Other researchers also demonstrated that the sd-LDL percentage was more in PMW than in PreMW [79,80].

Higher levels of this atherogenic subfraction lead to improved access to these particles by endothelium allowing their binding to proteoglycans, as sd-LDL has a greater affinity than lb-LDL [59]. Data from another study from women with the clinical and subclinical disease showed that sd-LDL was linked with a greater frequency of CHD among older women [81–83]. As mentioned earlier, PMW showed high levels of HLs, possibly due to a decrease in circulatory estrogen and an increase in insulin value [84].

Surprisingly, the sd-LDL percentage showed a substantial positive correlation with HL activity, even after extensive regression analysis, demonstrating that augmented HL would improve sd-LDL production [85]. Furthermore, no relationship was found between CETP activity and sd-LDL, the role of HL on sd-LDL generation should be emphasized. High percentages of sd-LDL are part of the atherogenic LP profile attributed to the related insulin resistance state and diabetic consequences. There is an association between sd-LDL and intima-media thickness regardless of age and apoB. Being a strong marker of early carotid AS, the measurement of sd-LDL could be of great

importance in the risk assessment for progressive AS in PMW [86].

5.3 LDL/sd-LDL in Glycation Assisted Impairments and Insulin Resistance

High circulatory TG, abnormal HDL and raised sd-LDL particles are frequent lipid deficiencies in persons with insulin resistance and non-insulin-dependent diabetes mellitus (NIDDM) [87]. Thus, it is clear that the same factors which are linked with an increased risk for insulin resistance syndrome are also associated with the CVD risk factor profile of persons with sd-LDL. There are number of biological characteristics that might be a reason those sd-LDL particles to be more atherogenic than large LDL particles. Such as, these smaller LDL particles have fewer phospholipids and unesterified cholesterol present in surface monolayers as matched to lb-LDL's. This variance in lipid content might be responsible for changes in apoB100 conformation that leads to the further display of proteoglycan-binding regions which can be one of the purposes for high-affinity binding of sd-LDL to arterial proteoglycans.

The therapy with secretory phospholipase A2 (sPLA2) results in a reduction of phospholipid content in the surface monolayer LDL and forms sd-LDL with an improved affinity to interact with proteoglycans [88]. Further, sd-LDL showed greater predominance for binding with arterial proteoglycans and also holds higher penetrability through the endothelial barrier, following the formation of foam cells [89]. These modifications and conformational changes in LDL leads to reduced receptors clearance and enhanced its property of accumulation as well as binding to arterial proteoglycans, therefore making it more atherogenic [89,90]. Berneis K and co-workers [91] also demonstrated that in clinically apparent as well as non-apparent AS in T2D, determination of LDL size is the strongest marker. As previous reports have shown that sd-LDL is especially glycated in nondiabetic subjects than lb-LDL, but now, it is clear that apoB is glycated (glyc-apoB) in T2D and MS also. Further, the sd-LDL concentration could be an effective factor of plasma glyc-apoB than glycaemia. Statin persuaded modulations in sd-LDL can be imperative in diminishing glycated apoB in diabetes. This impression might clarify the influence of recovering glycaemia comparative to statin treatment to diminish the menace of atherosclerotic progression in T2D

and MS even before the beginning of diabetes [92].

5.4 Correlation between Smoking and sd-LDL

There are numeral risk factors responsible for the progression of atherosclerosis such as high cholesterol levels, elevated levels of LDL-C, cholesterol-induced oxidative stress, tobacco consumption, hypertension, diabetes and cigarette smoking [93]. Among various risk factors like homocysteine, endotoxins, hypoxia, viruses or other infectious agents, cigarette smoke-derived products seem to be potent causative in endothelial dysfunction (ED) [94–99]. Cigarette smoking can induce the oxidative stress and downregulate the antioxidant defense system which are known to be directly related to oxidized LDL [100]. Recent studies found an association between the incidence of sd-LDL and smoking. Exposure to nicotine, a component of cigarettes, reduces the secretion of insulin and affecting the normal action of this hormone. So, the insulin resistance may be an important link between the effects of smoking and atherogenic sd-LDL particles observed among smokers in addition to elevated levels of TC and TG, decreased insulin sensitivity and increased HL activity. HL is also an independent mediator of sd-LDL formation. Patients with elevated sd-LDL levels and smoking habits have coronary disease, confirming the importance of the relationship between sd-LDL, smoking and CHD [101]. Smoking may also result in the production of dysfunctional and abnormal LPs having a smaller particle size (sd-LDL) that make worse effects on senescence and atherosclerotic progression due to oxidative modification and glycation [102].

5.5 Formation of Pro-aggregatory/ Vasoconstrictor Mediators

In contrast to other LP variables, LDL particle size is concomitant with vasodilator dysfunction in patients with CAD [103]. The Framingham Heart Study also showed that augmented sd-LDL particles as a single measure significantly predicts metabolic syndromes and that the high number of sd-LDL particles could be associated with an increased CVD event rate in people with MS [104]. Sd-LDL particles activate thromboxane-A2 synthesis in vitro, beyond lb-LDL particles [105]. Thromboxane-A2 excites platelet aggregation and is an effective

vasoconstrictor that can contribute to the development of CAD. The generation of 8epi-PGF2a as an outcome of the non-enzymatic oxidation of arachidonic acid in sd-LDL may too promote the synthesis of vasoconstriction and platelet accumulation.

6. RECEPTOR MEDIATED CLEARANCE OF LDL/sd-LDL

Plasma LDL levels are estimated by homeostasis between LDL production processes and LDL clearance levels, both of them are modulated by various LDL-R in the liver. Sd-LDL is slowly removed by receptor-mediated pathways compared to lb-LDL and thus lasts longer in plasma, allowing more time to be oxidized by various processes and phagocytosed by macrophages in extra vascular spaces of blood [106]. Reduced receptor uptake has been shown to decrease sd-LDL binding to LDL-R due to the corresponding changes carried in apoB by an

upsurge in TG content or a decrease in LDL size [Fig. 2] [16]. Further, the TG components of LDL particles are related to systemic inflammation. In addition, numerous people with elevated LDL-C levels within the limits of the National Cholesterol Education Program (NCEP) may also suffer from CHD [107].

mm-LDL is not distinguishable from native LDL by LDL-R and is not detected by a scavenger receptor, as well as attracts EC to secrete high levels of monocyte chemotactic activity, while native LDL does not cause EC to release high levels of MCP-1 [74]. In addition, mm-LDL is not a ligand of scavenger receptors, it may have a life span that is very much not different from that of native LDL and can build up in plasma. Also, LDL wherein a small part of lysine ε-amino groups are secreted (but not enough to form a ligand of scavenger receptors) may have a longer half-life span than that of native LDL and, in turn, can again build up and lead to AS.

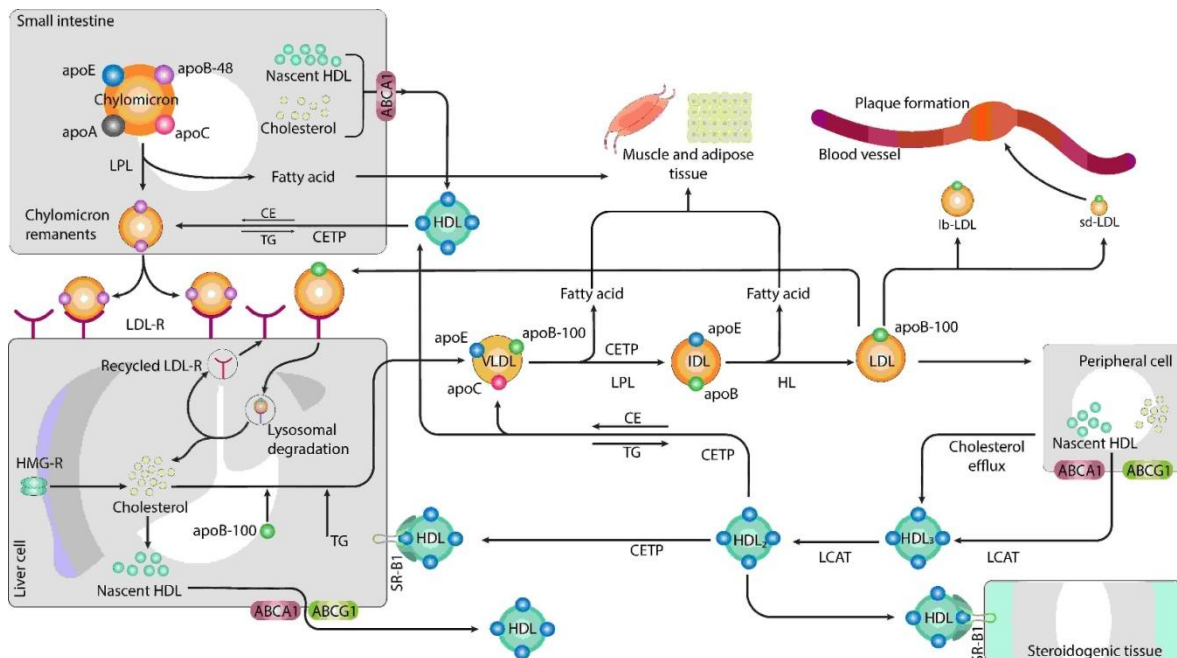


Fig 2. The TG rich LDL entry into hepatocytes, their TG hydrolysis by hepatic lipases (HL) resulting in the formation of sd-LDL. The sd-LDL with reduced LDL receptor binding affinity, incapable of receptor-mediated clearance, enters back into the circulation, thereby making itself more prone to subsequent modifications (i.e., oxidation, peroxidation, and glycation) leading to enhanced atherogenicity. In the reverse cholesterol transport mechanism, HDL production starts in the liver and intestine. Lipid poor apoA1 is secreted from the liver and intestinal cells and reaches the circulation. Some of these HDL reach to the heart vessel wall and come into contact with foam cells where lipid-poor apoA-1 protein interacts with ABCA-1 thereby developing nascent HDL particle which then interacts with transporter proteins ABCG-1 and scavenger receptor (SR-B1) to develop into mature HDL. As in contacts foam cells, HDL accumulates cholesterol becoming a cholesterol-rich ester as it matures

The cholesterol content of the cell is mainly responsible for the modulation of hepatic LDL-R expression. Further, as cellular cholesterol level decreases, non-active sterol regulatory element-binding proteins (SREBPs) are shifted from the endoplasmic reticulum (ER) to the Golgi apparatus where these are acted upon by proteases which cleave the SREBPs into active transcription factors which facilitate the expression of LDL-Rs, and other main genes concerned in the metabolism of cholesterol and fatty acid. Transcription of genes including LDL-R and 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-R), the rate-limiting enzyme in cholesterol biosynthetic pathway, are stimulated by active SREBPs when these move to the nucleus. In case cellular cholesterol level is elevated then the SREBPs persist in the ER in a sedentary form and would not trigger the synthesis of LDL-R. Moreover, oxidized cellular cholesterol and sterols trigger a nuclear hormone Liver X receptor (LXR), a transcription factor that activates E3 ubiquitin ligase transcription, inducible degrader of the LDL-R (IDOL), which mediates the ubiquitination and degradation of the LDL-R. Thus, depending upon the availability of cholesterol the cell can regulate LDL-R activity. A contrary link has been detected between the cholesterol content of the cell and activity of LDL-R in order to maintain the appropriate uptake of cholesterol in the cells, that is, LDL-R activity decreases with an exponential increase in cholesterol content of the cell which results in diminished cellular LDL uptake. Lastly, this useless LDL-R is degraded by proprotein convertase subtilisin/kexin type 9 (PCSK-9), a protein that enhances LDL-R degradation in the lysosomes when binds to the LDLR. It is known that loss of function mutations in PCSK-9 showed increased LDL-R activity and diminished LDL levels in circulation though reverse is true with a gain of function mutations in PCSK-9 [108].

7. REVERSE CHOLESTEROL TRANSPORT

High-density lipoprotein has several anti-atherogenic properties and best of all is its ability to eliminate cholesterol from macrophage-like cells in the artery wall, the first step in the reverse cholesterol transport pathway [109]. The reverse transport of cholesterol begins after the creation of small HDL by the liver and intestines. These nascent HDLs could then attain cholesterol and phospholipids released from cells by a progression modulated by ATP-binding

carrier transporter (ABCA1) leading to the formation of mature HDL [Fig. 2]. This cholesterol efflux from macrophages to HDL serves a major role in preventing the progression of AS. Further, mature HDL can receive more cholesterol from cells via ATP-binding cassette sub-family G member 1 (ABCG1), scavenger receptor (SR-B1), or inactivation. ABCA1 and ABCG1 are expressed in a variety of cell types like hepatocytes, enterocytes and macrophages and facilitate the passage of cholesterol and phospholipids from cell to HDL particles. This in turn transfers cholesterol to the liver directly by interrelating with hepatic SR-B1 or secondarily by relocating cholesterol to VLDL or LDL, a process executed by CETP.

In a direct pathway HDL bind to SR-B1 in the liver that allows cholesterol delivery. Subsequent lipid-poor HDL particles that can come out can be redistributed and repeat the process of reversing cholesterol transport. In addition to the liver, SR-B1 also expresses in adrenal glands, testes, ovaries, macrophages, and other cells and expedites the specific uptake of CE from HDL particles. Further, in the indirect pathway, cholesterol is delivered via CETP which facilitates the exchange of HDL cholesterol for TG in TG-rich apoB particles i.e., VLDL and LDL. In this case of selective commutability, HDL is supplemented with TGs and LDL with cholesterol. LDL interacts with LDL-R at hepatic cells, where LDL particles can deposit the cholesterol ester content at LDL-R.

Another important protein of LDL-R family i.e., LDL-R related protein (LRP) that demonstrated in numerous tissues comprising the liver, which recognizes apoE and facilitates the uptake of chylomicron remnants and IDL. Some transporters like ABCG5/ABCG8, showed their expression in the liver and intestine. When these are expressed in the liver, carry the cholesterol and plant sterols into the bile; whereas, when expressed in the intestine they transport the plant sterols and cholesterol exclusively from the enterocytes into the lumen of intestine to decrease their absorption. Moreover, Niemann-Pick C1-like 1 (NPC1L1), another intestinal transporter, accelerates the uptake of cholesterol and plant sterols from the intestinal lumen into the enterocytes.

8. AN INSIGHT TO THERAPEUTIC STRATEGIES

Since, it is well reckoned that oxidative stress, hyperlipidemia, and inflammation are the

foremost threat factors that are responsible for AS progression. So, various therapeutic agents with potent antioxidant, anti-inflammatory and hypolipidemic properties should work in the cure and controlling of risk factors facilitated AS and associated complications [110–113]. The early work on the LDL clearance and LDL-R, discussed above, led to a novel approach regarding cholesterol homeostasis and introduced 3 common concepts to biology and medicine.

The conception of selective filtration of proteins lying in the plasma membrane, that formulates the basis for receptor binding in coated pits, which is required for receptor-mediated clearance.

Concepts of receptor-facilitated endocytosis, clearance and receptor recycling that gives molecular and selective mechanism based on which cells involving macromolecules, including transporter proteins, growth factors, hormones, lysosomal enzymes, and certain viruses.

The impression of feedback regulation of receptors clarifies the effects of lowering cholesterol on statins, a class of drugs that significantly works in lowering plasma LP level, decreasing CVD events, and extending life [114].

8.1 Statins & their Discovery

Several studies established that HMG-R, the rate-limiting enzyme in the cholesterol biosynthetic pathway, plays a central role in cholesterol production. In contrary to desmosterol and other intermediaries produced in later stages of this pathway, 3-hydroxy3-methyl-glutarate is water-soluble. It can be alternatively metabolized in other pathways when HMG-R is prevented to avert the accumulation of possibly toxic precursors. So, HMG-R was a striking target. Thus, natural compounds with a potent HMG-R inhibitory property, including compactin (ML236B), that was first exposed by the Japanese microbiologist Akira Endo in the 1970s [115–118]. Statins are potent HMG-R inhibitors. Elevated levels of TC and TG are strongly allied with CVD [10,119]. The discovery of statins takes pace and as of, some statins are on the market: atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin and simvastatin [120]. Many combinatorial preparations of statins and other agents, such as ezetimibe/simvastatin, are also in the market. Statins have been known to reduce CVD events

in patients with higher risk. There is a body of evidences strongly supporting that statins are useful in the treatment of early stages of CVD and individuals at raised risk but without CVD [121].

8.2 Mechanism of Action

8.2.1 Inhibition of endogenous cholesterol synthesis

In 1976, a specific competitive HMG-R inhibitor was discovered and isolated from the fungi. This molecule was named mevastatin [122]. Another molecule was introduced in 1988, as a stronger HMG-R inhibitor, lovastatin, obtained from *Aspergillus terreus* [122]. Since, statins inhibit the conversion of HMG-CoA to mevalonate and thus dramatically control the cholesterol biosynthesis. These effects result principally in the liver, where the statins mainly distribute [122]. The major effect of these hypolipidemic agents is a marked reduction in the plasma LDL-C levels. One clinical trial with 4444 patients demonstrated that simvastatin enhanced survival in patients with CHD in addition to the reduction in the circulating cholesterol. Rosuvastatin treatment decreases plasma apoB level by enhancing apoB100 [123].

8.2.2 Reduction of specific protein prenylation

By blocking the mevalonate pathway, statins concurrently constrain the generation of cholesterol and definite prenylated proteins. This regulatory impact of statins on protein prenylation may be concerned with modulation of immune function, improvement of endothelial works and other different cardiovascular benefits of statins [124–126]. Moreover, numeral drugs that reduce LDL have not depicted the same CVD risk benefits in studies as statins, and can also exert beneficial effects in cancer reduction with statins [127]. Further, the protein prenylation inhibitory outcome could also be implicated in various undesirable side effects accompanying with statins, including muscle pain (myopathy) [128] and elevated blood sugar [129].

8.2.3 Modulation of endothelial function

The ED signifies an initial event in the progression of atherosclerotic lesions, stimulated by hypercholesterolemia. Since, nitric oxide (NO) is responsible for the anti-atherosclerotic potential of the endothelium [130]; high cholesterol level reduces the efficiency of NO

production by ECs, most perhaps owing to the lesser availability of L-arginine, which is the physiological substrate of NO synthase and regulates an augmented degradation of NO. Further, statins significantly improve the functionality of the mRNA for eNOS resulting in the enhanced generation of NO from the ECs. There is widespread agreement on the information that statins augment the expression of NO by upregulation of eNOS. The improved expression of eNOS was initially demonstrated in Liao's laboratory [131] for mevastatin and simvastatin and was established in bovine aortic ECs [132]. Further, this research was extended to lovastatin, atorvastatin [131] and fluvastatin [133] and positive results were observed to occur in human cells [131], in mice [134], and in rats [133].

8.2.4 Statin mediated modulation of inflammatory responses

Many researchers from clinical studies advocate that statin also exhibit anti-inflammatory effects apart from their hypolipidemic potential. Statin therapy reduces the levels of C-reactive protein (CRP), a liver-derived inflammatory biomarker and an autonomous risk factor for CVD [135]. Pravastatin lowered CRP levels (at both 12 and 24 weeks), independent of reductions in LDL-C levels in a pravastatin inflammation/CRP evaluation (PRINCE) study [7,136]. Another study among patients with acute coronary syndromes (ACS) treatment with pravastatin (40 mg) or atorvastatin (80 mg) concluded that the experimental effects of these statins in patients with CHD depend on declines in both LDL cholesterol and CRP [137]. In another trial, justification for the use of statins in prevention: an intervention trial evaluating rosuvastatin (JUPITER), rosuvastatin treatment showed a 65% lessening in CVD events in subjects who achieved both an LDL-C level of < 1.8 mM and a high-sensitivity CRP (hs-CRP) level of less than 2 mg/L [135]. Statin treatment also regulates the thresholds of other atherogenic cytokines to the betterment of proinflammatory processes.

A human blood vessel explants study has demonstrated the inhibitory potential of atorvastatin on both proinflammatory cytokine release and monocyte adhesion, even in response to greatly low levels of endotoxin [138]. Statin treatment also lowers nuclear factor kappa B (NF-KB) activation [139], CD40-CD40 ligand expression [140] and matrix metalloproteinase concentrations in humans [141]. In T-cells

isolated from healthy subjects, lovastatin treatment blocked the production of the cytokines like interleukin-2 (IL-2), IL-4, and interferon- γ by stimulated cells via down-regulation of both NF-KB expressions and activator protein-1 (AP-1) in a quantity-dependent manner [142]. Moreover, simvastatin medication reduced the expression of proinflammatory cytokines like IL-6, IL-8, and MCP-1 in peripheral blood mononuclear cells from hyperlipidemic patients [143]. All the factors summarized above are strongly correlated with plaque progression and stability.

8.3 Adverse Effects of Statins

Apart from their beneficial effects in the maintenance of cholesterol homeostasis, statins also exhibit several adverse effects. The most important among them are amplified liver enzymes concentration, muscle problems, and an augmented risk of diabetes [144,145]. In addition to these, neuropathy, cognitive loss, sexual dysfunction and pancreatic and hepatic dysfunction are other possible adverse effects [146].

8.4 Naturally Occurring Antioxidants and Modulators of HMG-CoA Reductase

Statins, well-known class of hypolipidemic drugs (HMG-R inhibitors) in the market, have host of side effects as discussed above. So, there is a great demand of time to discover some new therapeutic agents from natural sources as they are compatible to the living system to get rid of safety aspects against synthetic drugs. Several therapeutic agents have been tested for their antioxidant, anti-inflammatory and hypolipidemic potentials so far. In 2011, Khan MS et al. showed that tocomin treatment significantly decreased the cholesterol and apoB fraction of sd-LDL in LPS, turpentine or zymosan stressed hamsters [147]. We are aware that sd-LDL apoB100 is catabolized significantly at slower rate than lb-LDL apoB100 in subjects suffering from dyslipidemia on placebo or statin therapy. On the other hand, Vitamin E is also proved to be potentially beneficial in preventing circulating LDL against oxidation *ex vivo* and doses of 400–800 IU daily appear to be obligatory for maximum protection. Dietary soy protein can also reduce cardiovascular risk by declining saturated fatty acids consumption followed by lessening LDL-C [148]. A crossover randomized, double-blind study in Japanese men showed that flaxseed oil intake significantly reduces serum sd-LDL concentrations [149].

Some reports have demonstrated the potential of n-3 fatty acids on CVD risk. The maximum of health profits showed in these studies have been accredited to the seafood eicosapentaenoic acid (EPA), n-3 fatty acids and docosahexaenoic acid (DHA) [150]. In 2015, Santos et al. showed that Mipomersen, therapeutic alternative with a quite exclusive manner of action that fluctuates from that of statins, preferentially reduces sd-LDL concentration in patients with hypercholesterolemia [151]. Similarly, mipomersen which is a 20-nucleotide long, second-generation, antisense oligonucleotide specifically prevents human apoB100 formation by sequence-specific binding to its mRNA, leading to mRNA degradation via enzyme-mediated pathways or interruption in mRNA function through binding alone [152]. Since apoB100 is an essential structural component of VLDL, IDL, LDL, and lipoprotein(a), its lesser generation in the influence of mipomersen leads to decreased circulating levels of these highly atherogenic LP species [153,154]. Michael Aviram et al. again reported the consumption of pomegranate juice comprising 85% water, 10% total sugars, and 1.5% pectin, ascorbic acid and polyphenolic flavonoids markedly diminish oxidative stress, atherogenic alterations to smaller LPs and platelet accumulation in humans as well as in atherosclerotic apolipoprotein E-deficient mice [155].

Several reports suggested that natural plants, metabolites or nanoparticles prepared from them have great potentials to cure the diseases mainly via inhibiting the key regulatory enzymes [156–160]. Another way to treat the progression of atherosclerosis is to inhibit HMG-R, the rate-limiting enzyme in the cholesterol biosynthetic pathway, which catalyzes the conversion of HMG-CoA to CoA and mevalonate. As elevated plasma TG level shows a strong association with sd-LDL phenotype, TG suppressing drugs can be estimated to have a bigger effect on LDL size and density than major cholesterol-lowering remedies. The HMG-R inhibitors (statins) lower LDL-C considerably [161,162] such as atorvastatin which lowers plasma TGs more than other promoted statins at approved doses [163,164]. In addition to these predominant HMG-R inhibitors, there are several other HMG-R inhibitors that have been isolated and evaluated from natural sources, meeting the criteria for safety aspects. Some researchers are invariably researching to locate out better HMG-R inhibitor with multiple therapeutic benefits. In this context, they discovered a novel HMG-R

inhibitor, n-Octadecanyl-O- α -D-glucopyranosyl (6 \rightarrow 1)-O- α -D-glucopyranoside with potent antioxidant properties [165]. This inhibitor was not only a strong free radical quencher but also depicted significant HMG-R inhibitory activity with an IC₅₀ value of 84 \pm 2.8 ng/ml. In addition, they also showed that lycopene, a carotenoid from plant origin, exhibits strong HMG-R inhibitory potential with an IC₅₀ value of 36 ng/ml which was quite better than pravastatin (IC₅₀ = 42 ng/ml). The in vitro data also demonstrated and well supported the in-silico findings that lycopene competitively constrains HMG-R activity by binding at the hydrophobic portion of HMG-R [166].

9. CONCLUSION

In conclusion oxidative or free radical-induced modification of biological macromolecules alters their original conformations to a lesser or greater extent as they start to behave abnormally in different metabolic pathways. It is well known that ox-sd-LDL plays a key role in atherosclerotic progression, but it is still not well clear that how this oxidized LP particles recognize their receptors and cause activation of intracellular signaling pathways. Furthermore, studies of subpopulations of LDL, specifically sd-LDL, their chemistry, modification, and subsequent complications in different metabolic pathways are the demand of time in order to get rid of such lethal disorders. More scientific demonstrations in these disciplines may evaluate new treatment approaches, exclusively on sd-LDL, for the prevention of CVD.

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CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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