LDL as a Cause of Atherosclerosis

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Abstract: The low density lipoprotein particle is the major transporter of cholesterol around the body and has been shown to be a strong independent risk factor for atherosclerotic events. This review discusses the normal and abnormal metabolism of low density lipoprotein (LDL). Mechanisms by which LDL causes atherosclerosis are discussed with particular reference to the importance of alteration in LDL composition including attachment of other proteins to the circulating LDL particle.

Keywords: Low density lipoprotein (LDL), Oxidised LDL, Atherosclerosis, Cholesterol, Proprotein convertase subtilisin kexin type 9 (PCSK9) Niemann Pick C1L1, Apolipoprotein B.

INTRODUCTION

There has been a huge increase in our understanding of the atherosclerotic process but there also has been a huge increase in both patients with the condition and those who potentially will in the future have the condition. The rising tide of obesity, diabetes and hypertension together with our reluctance to exercise and desire for fast unhealthy food, has made atherosclerosis of major importance economically, politically, scientifically and medically. Treatment of the condition with procedures such as by pass operations, insertion of stents and pharmaceutical agents to treat hypertension, diabetes and dyslipidaemia, pose an enormous burden on society.

The atherosclerotic plaque is cholesterol and fatty acid laden and research efforts have been successful in unraveling some of the mechanisms involved in the formation and rupture of these plaques. The lipoproteins are the major transporters of both cholesterol and fatty acids. Low density lipoproteins (LDL) is the best known lipoprotein not only because Brown and Goldstein [1] discovered the receptor for LDL and demonstrated the importance of the receptor in maintaining cholesterol homeostasis but also because statins, which inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme for cholesterol synthesis, were shown to up regulate the LDL receptor, lower cholesterol in the blood stream and reduce cardiovascular events by about 30% [2]. Indeed the popularity of the statin drugs has been such that it has been proposed that they should be given to every one in middle age. However, this is probably not cost effective [3].

CHOLESTEROL ABSORPTION AND TRANSPORT

Cholesterol may be synthesised, the majority in the liver and about 25 % in the intestine, may be absorbed from the diet or reabsorbed from bile. Cholesterol from the intestine is delivered to the liver by the intestinally-derived, apolipo-protein apo B 48 containing lipoprotein the chylomicron, a large lipid rich particle with a short half life of circa 90 min. The chylomicron acquires apo E, another apolipoprotein which is transferred to the chylomicron from high density lipoprotein (HDL) in the circulation, and is partially delipidated by lipoprotein lipase before being taken up by the B/E receptor in the liver [1]. The cholesterol released is repackaged, together with de novo synthesized and hepatically derived, cholesterol, triglyceride and phospholipids, solubilised by apo B 100 and excreted as very low density lipoprotein (VLDL) into the circulation. The VLDL particles are delipidated by lipoprotein lipase attached to the capillary wall which removes most of the triglyceride and the particle becomes a VLDL remnant. This may be further degraded to form LDL. Apo E is involved in clearance of most of the delipidated VLDL *via* the LDL B/E receptor in the liver. Apo E is then transferred back to HDL. Under normal circumstances LDL catabolism depends on the particle uptake by the LDL receptor which is present in almost all the cells of the body [1]. Thus, LDL is a vehicle to supply cholesterol all over the body in order to maintain cell viability and to provide cholesterol for the synthesis of the steroid hormones. HDL plays a part in reverse cholesterol transport and also protects LDL from oxidation [4] (Fig. **1**).

The regulation of LDL is finely tuned. Most cells have the ability to synthesise cholesterol through the HMGCoA reductase pathway, a safety net when cholesterol absorption is diminished. This is well demonstrated by the effect of the statins which inhibit HMGCoA reductase. Reduction in endogenous cholesterol synthesis up-regulates the LDL receptor and stimulates LDL clearance [5]. However it also stimulates cellular cholesterol absorption from the intestine [6]. The mechanism by which cholesterol absorption is regulated was discovered when it was found that ezetimibe, a compound that was known to reduce serum cholesterol, has been shown to bind to the brush borders of the enterocyte and to NPC1L1 and reduce cholesterol transport [7]. Cholesterol absorption is further regulated by ATP binding cassette proteins (ABC) G5 and G8 in the intestine [8]. These proteins work in tandem to re-excrete plant sterols virtually completely and cholesterol to a lesser extent [8]. The genes were also found to be expressed in the liver where

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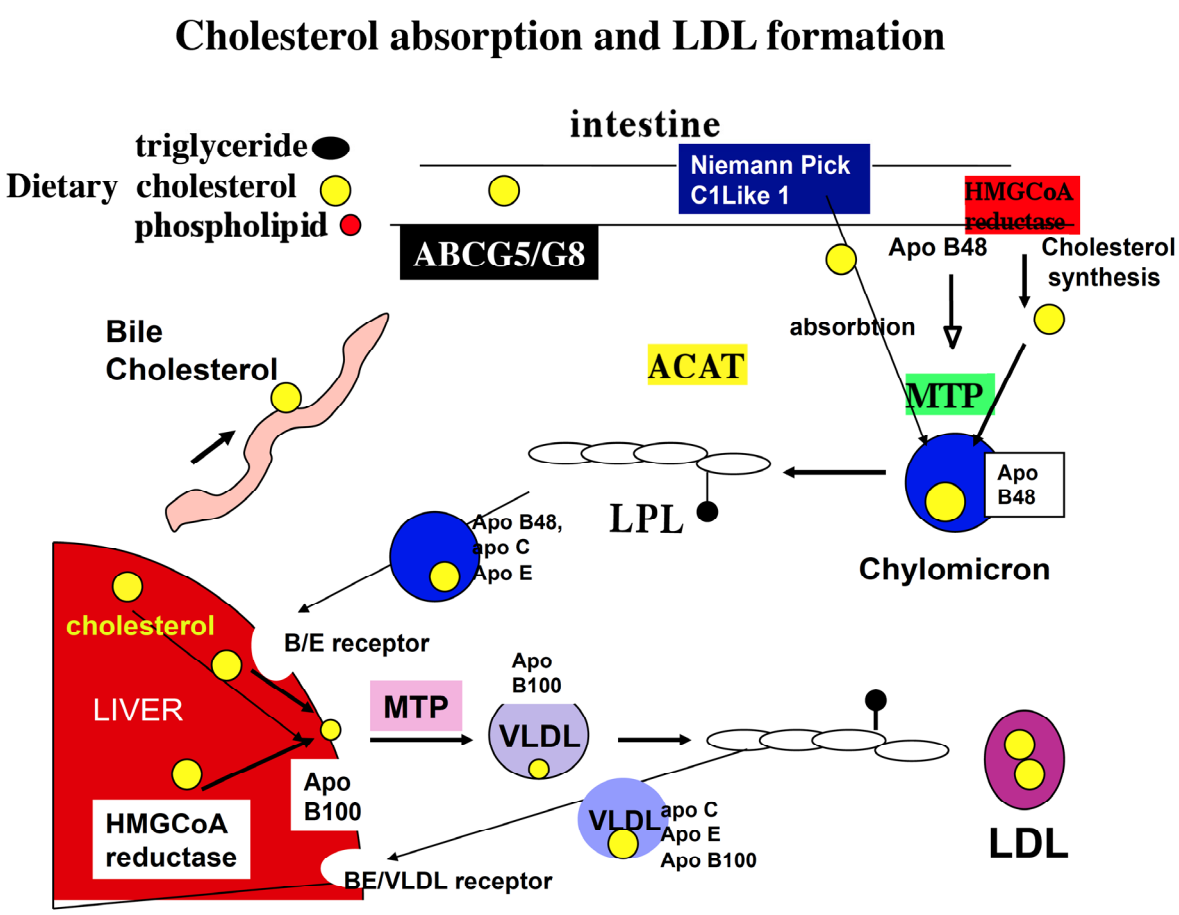

Fig. (1). Dietary cholesterol, biliary cholesterol and cholesterol synthesized in the intestine for which HMGCoA is the rate limiting enzyme, is transported across the cell membrane by NPC1L1 and, together with triglyceride, phospholipid, and the intestinally derived apoB48 protein, is assembled, under the influence of MTP into the triglyceride-rich chylomicron. Some of the absorbed cholesterol is excreted back into the lumen of the intestine under the influence of ABCG5/G8. The chylomicron is partially hydrolysed in the circulation by lipoprotein lipase and acquires apo CIII and apo E. The resulting chylomicron remnant is taken up by the B/E receptor in the liver. The cholesterol and triglyceride released are re-assembled with hepatically synthesised cholesterol and apo B100 to form VLDL. Lipoprotein lipase in the artery wall releases the triglyceride from VLDL and it acquires apo CIII and apo E. Some of the VLDL is taken up again by the liver and the rest is further hydrolised and looses apo CIII and E to become IDL and then LDL.

they are responsible for controlling cholesterol re-excretion into the bile [8-10].

LDL COMPOSITION

Patients in the coronary care unit frequently do not have raised LDL cholesterol but the quality of LDL may be altered. In 1995 Austin and Krauss [11] described the association between small dense LDL and atherosclerosis. The LDL particle is a cholesterol-rich, triglyceride-poor particle. LDL is composed of a hydrophilic surface layer of phospholipid, free cholesterol and hepatically-derived apo B100 to package the particle and add stability. The core of the particle includes esterified cholesterol and triglyceride together with the fatty acid tails of the phospholipid. As with most proteins in the circulation, the particle may act as a carrier for other insoluble particles such as free fatty acids which may be loosely attached [12]. Perhaps more importantly lipoprotein lipase also attaches to the particle and this facilitates attachment of the particle to the endothelial surface. We have previously shown that lipoprotein lipase was increased on diabetic LDL [12]. LDL can be sub-divided into sizes by gradient gel electrophoresis and separated into a pattern A and a pattern B, pattern B being termed small dense LDL [13]. This pattern has been associated with an increase in atherosclerosis but it has been difficult to define changes in composition of the LDL that create the increased atherogenicity. The usual way to separate the different sizes of apo B containing lipoproteins is by ultracentrifugation but the correlation between the denser particles on ultracentrifugation and electrophoresis is uncertain. The most recent addition to the methods to investigate lipoproteins is magnetic resonance (MR) spectroscopy which can sort particle size in large numbers of samples over very short time but this technique still does not define small dense LDL [14]. Some years ago a subfraction of LDL with oxidized characteristics was described and was named electronegative LDL (LDL^-) based on its properties of electrical mobility [15] and later re-named minimally oxidized LDL. More heavily oxidized LDL is more electronegative than LDL^- and is identified as LDL^{--}. It now appears that electronegative LDL may also be produced by phospholipase A2. Rosenson *et al.,* in the PLASMA11 Trial [16] have shown that an inhibitor of PLA2 reduced LDL by 7% and small dense LDL by 11%. Enrichment of LDL with

apo CIII contributes to the electronegativity [17]. Anti LDL-monoclonal antibody had a protective effect against atherosclerosis in LDL receptor knock-out mice [18]. It has been suggested that LDL- is a potential stress biomarker present in health and disease [19]. Small dense LDL isolation by various methods has been compared by Cheung [20]. The suggestion is that LDLs atherogenicity resides in its large amount of cholesterol being packaged in a relatively small volume hence the surface area of the particle is relatively large making it more easily amenable to modification and therefore more avidly taken up by scavenger receptors. Small dense LDL is also more susceptible to glycation even in non-diabetic people [21]. The association between small dense LDL and VLDL has been investigated, not least because of the difficulty of demonstrating hypertriglyceridaemia as an independent risk factor for atherosclerosis. VLDL, like LDL comes in many sizes depending on its triglyceride load and the Scottish and Finnish groups [22, 23] many years ago demonstrated the relationship between large triglyceride-rich VLDL and small dense LDL.

The reason why small dense LDL is associated with atherogenic risk has been debated for many years and familial hypercholesterolaemia, the cause of early atherosclerosis is associated with larger more buoyant LDL rather than small dense LDL [24, 25]. It has been proposed that the increasing surface area of the particle makes it more amenable to oxidation and glycation, both modifications being associated with antibody formation. The result is an increase in scavenger receptor/fc receptor uptake by the macrophage. Oxidation of the LDL particle depends on oxidation of the protein and/or fatty acids. Polyunsaturated but not monounsaturated fatty acids are amenable to oxidation hence a particle rich in linoleic acid is more susceptible to oxidation than one rich in oleic acid. An increase of free radical production occurs in hyperglycaemia of diabetes, one reason for the increased LDL oxidation that occurs in poorly controlled diabetes. The increase in free fatty acids in poorly controlled diabetes is associated with an increase in fatty acids attached to the LDL particle, a further potential cause for increased oxidation of the particle [12, 20] (Fig. **2**).

LIPOPROTEIN (A)

Lp(a) is a variant of LDL that is covalently linked to apo B. Concentrations vary widely through different populations and more than 90% of this variant is determined by inherited DNA sequence variation [26]. Evidence that Lp(a) is a cause rather than a consequence of CAD has been strengthened by a report by Robert Clarke *et al.,* [27] who found that two Lp(a) SNPs explain 36% of the variation in Lp(a) in their population. Both SNPs were associated with coronary disease and, after adjustment for the plasma Lp(a) lipoprotein level, the association between LPA genotypes and CAD are abolished. The mechanism whereby Lp(a) may be particularly atherogenic is through its binding and transportation of phospholipids [28]. However, due to the particle homology with plasminogen a pro-enzyme related to fibrinolysis [29, 30]. It is suggested that the particle also plays a part in thrombosis. Combining a pro-atherogenic factor with an antifibrinolytic factor makes Lp(a) an interesting candidate for a link between plaque and stenosis and a likely risk factor for thrombotic events, including atherosclerotic occlusion [31]. More recently Lp(a) has been shown to interfere with Annexin A5 binding to the pro coagulant phosphatidyl serine. Annexin 5 is involved in anticoagulation on the endothelial surface and thus another mechanism whereby Lp(a) might promote thrombosis [32].

HIGH DENSITY LIPOPROTEIN (HDL)

There is a strong inverse correlation between triglycerides and HDL which has been known for many years. Low HDL is known to be an independent and powerful predictor of atherosclerosis and the functions of HDL have been

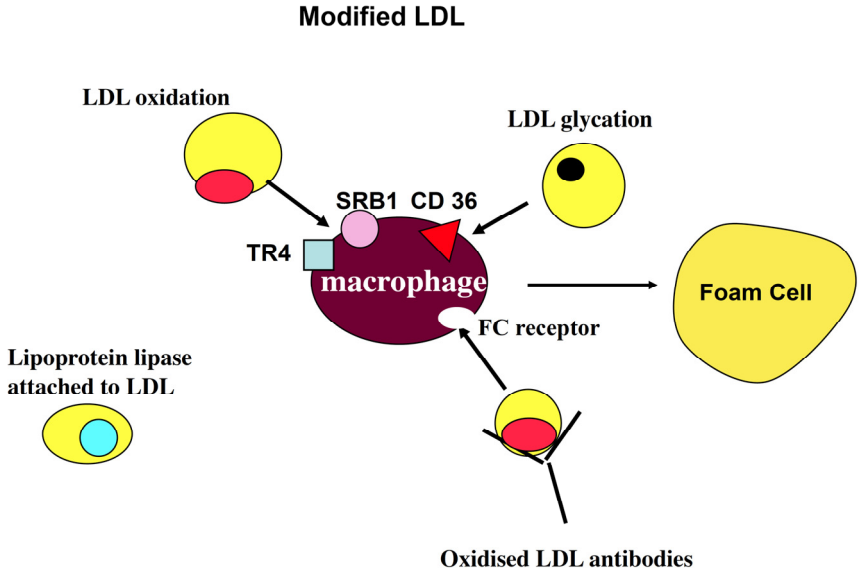

Fig. (2). LDL may be modified by oxidation or glycation, and it may have increased lipoprotein lipase attached. Oxidised and glycated LDL are taken up by the macrophage scavenger receptors such as SRB1, CD36 and TR 4. Oxidised LDL may also form oxidized LDL-antibody complexes which can be taken up by the macrophage FC receptor. Scavenger receptor uptake is not regulated and leads to macrophage cholesterol accumulation and foam cell formation.

intensively investigated. HDL's major function may be reverse cholesterol transport but it would seem that an important function relates to its ability of Paraoxinase 1 (PON 1) to protect LDL from oxidation. Thus, low HDL and, perhaps more importantly dysfunctional HDL such as is found in polymorphisms of PON 1, have a bearing on the atherogenicity of LDL [33].

CAUSES OF HYPERCHOLESTEROLAEMIA

Having discussed alterations in the particle that promote atherogenesis there is very good evidence to demonstrate a close relationship between the amount of cholesterol and the risk for example, of myocardial infarction. It is difficult to know how best to summarise the numerous studies and difficult to know which studies to select for discussion in this review. In the UK two fairly recent studies are worth mentioning. The Heart Protection Study [34] in which 20,536 people were non diabetic and 5,000 were diabetic and the CARDS Study [35] where all the 2838 patients were diabetic. These studies and many other studies have shown the benefits of reduction in cholesterol on cardiovascular end points. Although most patients with high levels of LDL cholesterol do not have a secondary cause such as hypothyroidism or nephrotic syndrome, dietary intervention leads to only a very moderate lowering of cholesterol i.e. in the Whitehall Study [36] in which 4469 patients completed the study, the patients who improved diet and lifestyle lowered cholesterol by only about 0.9 mmol/l over 9 years compared with 2.7 mmol/l for lipid lowering drug treatment. High LDL is often genetic in origin and, as with most common conditions there probably are multiple genes involved in the majority of patients. There are however rare monogenic causes of high LDL cholesterol [37].

Familial hypercholesterolaemia (FH) is caused by a defect in the LDL receptor gene. The story of the discovery of the LDL receptor has been written by Goldstein and Brown [1]. The story starts with Karl Muller [38] who described hypercholesterolaemia as an inborn error of metabolism and Goldstein the Nobel prize, came about by examination of homozygous patients. Their research led to the conclusion that a high affinity receptor existed with a feed back mechanism to suppress cholesterol synthesis through suppression of HMGCoA reductase. It is now known that this mechanism is mediated through sterol regulated binding protein (SREBP) [1]. Patients with FH lacked the high affinity receptor activity. Many different mutations of the LDL receptor gene have now been found in FH patients. LDL receptor gene transfer as a possible cure for FH is discussed in the recent review by Van Craeyveld *et al.,* [39] (Fig. **3**).

Proprotein convertase subtilisin kexin type 9 (PCSK9) is the gene which regulates the breakdown of the receptor [40]. PCSK9 is a regulator of liver LDL receptor expression. It binds tightly to the LDL receptor and channels it towards the lysosomal compartment for degradation. This results in decreased LDL receptor numbers and increased plasma LDL levels. An interesting loss of function polymorphism of PCSK9 increases the number of LDL receptors and increases LDL removal from the plasma, reducing LDL levels. There is strong evidence that PCSK9 and LDL receptor transcription are both activated by cellular cholesterol depletion *via* sterol regulatory element binding protein-2 (SREBP-2) [41]. This notion is supported by human studies where plasma PCSK9 concentration is increased with statin [42]. Noguchi *et al.,* [43] have recently shown that fibrates also significantly increase circulating PCSK9 levels. Thus, an inhibitor of PCSK9 would be a useful addition to statin and fibrate therapy and Chan *et al.,* [44] have recently described dramatic LDL lowering in non human primates with the use of monoclonal antibodies (mAB) against PCSK9 (Fig. **4**).

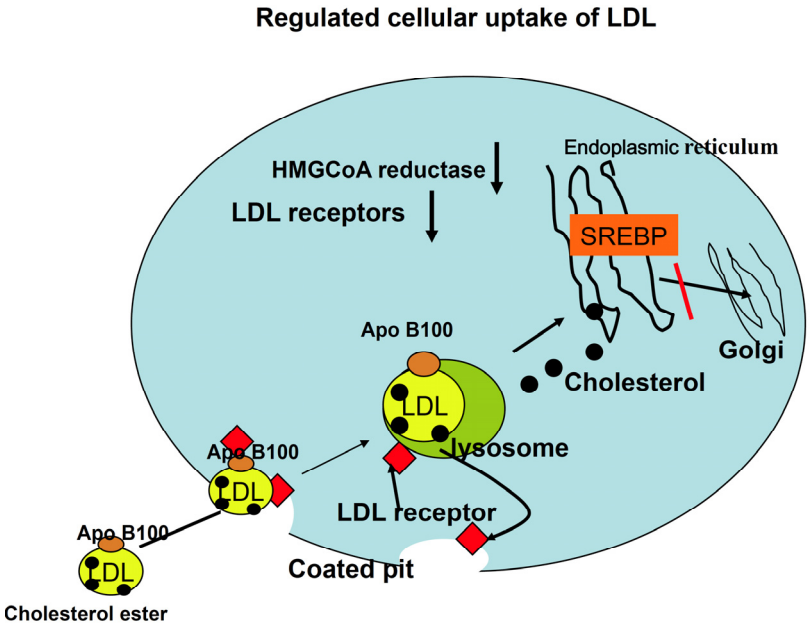

Fig. (3). Cholesterol taken into the cell through the LDL receptor pathway is released in the lysosome and taken up by the endoplasmic reticulum. This blocks the transport of SREBP to the Golgi complex preventing transcription of HMGCoA reductase thus reducing de novo cholesterol synthesis and also blocking LDL receptor synthesis, preventing further LDL uptake.

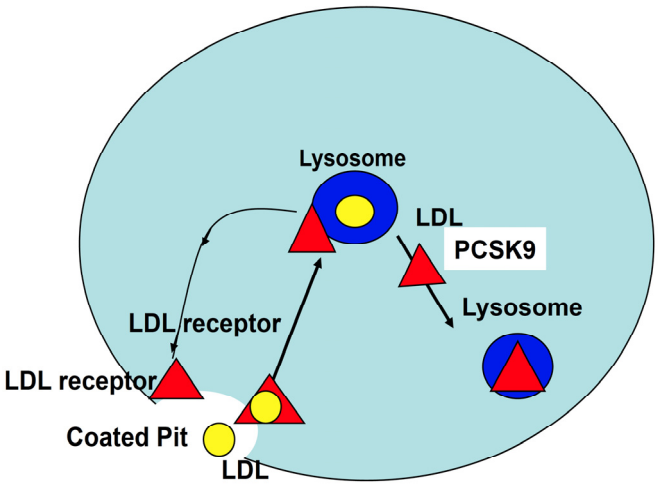

Fig. (4). PCSK9 is a regulator of liver LDL receptor expression. Normally the LDL receptor, once it has delivered LDL to the lysosome re-circulates to the coated pit on the cell surface. PCSK9 binds tightly to the LDL receptor and instead channels it towards the lysosomal compartment for degradation resulting in decreased LDL receptor numbers and increased plasma LDL cholesterol.

Apo B gene abnormalities which lead to abnormal apo B which is unable to bind effectively to the receptor is another monogenic cause of dyslipidaemia. These genetic defects can lead to either hyper- or hypocholesterolaemia. Mis-sense mutations in the LDL receptor domain of Apo B are characterised by premature atherosclerosis and coronary artery disease. Familial defective apolipoprotein B-100 (FDB) is a dominantly inherited disorder caused by the substitution of glutamine for arginine at position 3500 in apo B-100 [45]. The presence of mutant apo B-100 in low-density lipoproteins (LDL) markedly reduces its affinity for the LDL receptor, leading to hypercholesterolaemia and increased proneness to coronary artery disease. Other mutations in apo B can cause hypobetalipoprotinaemia characterised by hypocholesterolaemia and resistance to atherosclerosis. Here the defect in apo B results in defective cholesterol binding and LDL formation. [46- 49]

The genome-wide search looking for DNA variants that might impact on risk of coronary artery disease noted a DNA variant on chromosome 1p13 that increased the relative risk of coronary artery disease by 29% per allele [50]. The story as related in "clinical implications of basic research" [51, 52] describes the next milestone which was another genome-wide association which found the above genetic variant was related to increased serum levels of LDL cholesterol [53]. In 2010 Musunuru *et al.,* [54] discovered that the genes on 1p13 were associated with a transcription factor which led them to SORT-1 encoding sortilin 1 protein. Over expression of SORT1 in mice led to an increase in LDL cholesterol and knock down of SORT1 had the opposite effect explaining why the DNA variant on 1p13 might increase the risk of coronary artery disease. It appears that sortilin 1 decreases particles containing apo B in the liver and over expression increases these particles. Even more interesting, the over expression of sortilin was shown to enhance the endocytosis of LDL particles *in vitro* [51, 52]. Reduction of sortlin 1 expression regulates both VLDL and LDL and, as Linsel-Nitschke [52] suggest, perhaps these new findings could ultimately translate into new approaches to treatment.

Autosomal recessive hypercholesterolaemia (ARH) is caused by another protein involved in transport of cholesterol from LDL *via* the LDL receptor. Cells from patients with ARH fail to internalise the LDL receptor because they carry two defective alleles of LDL RAP1, a gene that encodes a specific clathrin adaptor protein [55]. Polymorphisms of the gene are rare but are associated with hypercholesterolaemia [56].

FREE FATTY ACIDS

In diabetes free fatty acid (FFA) suppression is impaired. It used to be generally believed that serum albumin was the protein carrier for these fatty acids although in 1995 Chung *et al.,* [57] demonstrated that when hypertriglyceridaemic sera were lipolysed more than 80% of the FFAs were partitioned to the lipoprotein fractions, saturated FFAs having a 4.5 - 11 times greater partitioning into lipoproteins than into the albumin fraction relative to that of polyunsaturated FFAs. *In vitro* experiments by the authors showed that FFAs bound to LDL were highly cytotoxic to macrophages, whereas FFA partitioned to albumin were not. We found a10-fold increase of FFA on LDL from diabetic patients compared to controls [12]. We have demonstrated increased oxidisablity of LDL in both hypercholesterolaemic and normocholesterolaemic diabetic patients [58]. Patients with diabetes and metabolic syndrome have both a preponderance of small dense LDL and the increase in the polyunsaturated fatty acids in the particle, hence many important mechanisms that promote the atherogenicity of LDL in hyperglycaemia [59, 60].

ATHEROSCLEROSIS FORMATION

The mechanism by which LDL attaches itself to damaged intima in the arterial tree is a fascinating story. LDL carries not only apo B but other proteins including lipoprotein lipase. Lipoprotein lipase (LPL) is synthesised in tissue parenchymal cells and then translocated to functional binding sites on the luminal surfaces of endothelial cells. Heparin sulphate proteoglycans, located in lipid rafts in the endothelial cell surfaces and small capillaries are the docking stations for LPL [61]. Although LPL is mostly involved in lipolysis of triglycerides from triglyceride-rich lipoproteins, they are also important in LDL metabolism [61]. Proteoglycans are important players in the attachment of LDL particles to the vascular wall and act as a docking mechanism for the LDL particles (Fig. **5**). We have shown an increase in LPL attached to LDL in diabetes [12] which may be important in the attachment of the LDL particle to the proteoglycans on the endothelial surface [62]. Antibodies to glycose aminoglycans have recently been shown to have an anti-atherogenic effect [63]. Platelet derived growth factor (PDGF) is strongly implicated in atherosclerosis, in part perhaps because it stimulates proteoglycan synthesis. For proof of concept Ballinger ML *et al.,* [64] inhibited PDGF receptor. They demonstrated significant reduction of carotid artery lipid accumulation. The mechanism was through inhibiting glycosaminoglycan (GAG) synthesis on the proteoglycans and thus reducing LDL binding, a possible novel method for reducing atherosclerosis in the future. It has been demonstrated that hypoxia induces changes in macrophage GAG. GAG biosynthesis which lead to higher

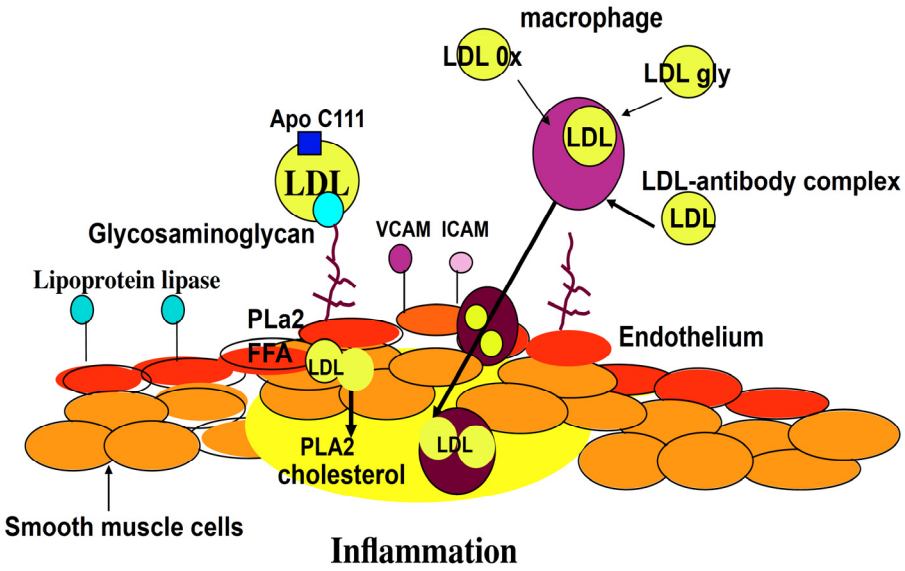

Fig. (5). The atherosclerotic plaque is composed of a lipid-rich core containing cholesterol, fatty acids and necrotic tissue, and is covered by a fibrous smooth muscle cell cap. Low density lipoprotein (LDL) is the major contributor to plaque cholesterol. LDL may attach to the endothelium through lipoprotein lipase and heparin sulphate proteoglycans (HPSG) which facilitate their uptake into the sub endothelial space. Macrophages which have accumulated large amounts of cholesterol, attach to chemotactic factors such as VCAM and ICAM on the artery wall, and slip between the endothelial cells into the intima where they are trapped, mature into foam cells and eventually disintegrate providing the cholesterol for the lipid-rich atherosclerotic core.

affinity for LDL and might contribute to the development of atherosclerosis.

Once the LDL particle has been attached to the endothelial surface further changes must occur before the cholesterol-rich particle becomes part of the atherosclerotic plaque. LDL aggregation seems very important in this process. The mechanisms whereby LDL particles fuse and coalesce into lipid droplets and thereby increase LDL in the artery wall is complex [65]. The LDL particle contains phospholipid. Phospholipase A2 (PLA2) is associated with atherosclerosis (Fig. **5**). The effects of PLA2 and its products on structural stability of human LDL has been examined recently. Jayaraman *et al.,* [66] demonstrated, in very elegant studies, that free fatty acids enhance LDL coalescence into lipid droplets. They promote the idea that lipid droplet formation contributes to the pro-atherogenic effects of FFA on LDL [66]. Most of the lipids found in atherosclerotic plaques are present in lipid droplets and LDL derived small lipid droplets are prominent in atherosclerotic lesions [67]. Modifications such as oxidation, lipolysis and proteolysis are prerequisites for droplet fusion. Enzymes from the Phospolipase A2 (PLA2) family hydrolyse phosphatidyl choline. Lipoprotein associated PLA2 preferentially hydrolyses oxidised phosphotidyl cholines in LDL and may promote fusion of LDL particles and thus contribute to the enhanced atherogenicity of the [68, 69]. Lipoprotein associated Phospholipase A2 has emerged as a causative agent of atherosclerosis and as a new therapeutic target. For review see [66]. It is suggested that the proatherogenic properties of PLA2 result from the effect of its products lysoPC and FFA, on LDL fusion and rupture, the released FFA promoting lipoprotein rupture and coalescence into lipid droplets. Interest in PLA2 has been heightened since inhibitors of PLA2 have been developed which demonstrate reduction in LDL concentration and in particular small LDL concentration suggesting that this class of drug may become an effective anti atherosclerotic agent [70].

The initial lesion leading to atherosclerosis is the fatty streak. Indeed fatty streaks occurs in human fetal aortas and are enhanced by maternal hypercholesterolaemia [71]. Analysis of the streak shows deposition of cholesterol and lipid with disruption of the endothelial surface and infiltration of macrophages and also neutrophils, typical of an inflammatory reaction. What is so interesting is that these streaks appear to repair themselves and do not lead to lasting damage. Delivery of cholesterol to the atherosclerotic plaque occurs following binding of the LDL particle to the endothelial surface *via* the lipase receptors. Endothelial dysfunction as measured by its failure to respond to nitric oxide, may be the first abnormality in the atherosclerotic process. Endothelial function impairment occurs before structural changes such as intimal hyperplasia or lipid deposition occur [72]. Endothelial dysfunction is associated with a decrease in nitric oxide production in the vasculature. Studies in the human coronary circulation have demonstrated that endothelial dysfunction is characterised by local enhancement of oxidative stress without a decrease in basal nitric oxide release [72]. Lavi *et al.,* [73] suggest that their study supports the hypothesis that local oxidative stress has a role in reduction of NO bioavailability in humans with coronary endothelial dysfunction but the repair mechanism involves monocyte/ macrophage accumulation in an effort to clear cholesterol from the plaque. The macrophage does not have an LDL receptor and can only take up modified LDL. Modifications include oxidation, glycation and antibody formation which can occur in the sub endothelial space (Fig. **2**). Once the macrophage takes up the LDL and becomes a foam cell it should by rites be allowed to remove cholesterol to the reticuloendothelial system for clearance but in the atherosclerotic process the macrophage gets trapped in the

intima and is unable to escape due to increase in size. From the above simplistic description it is apparent that the LDL species depends for its atherogenicity to a large extent on its ability to be modified. There are numerous reviews of the oxidative process but in essence the release of free radicals is enhanced from conditions such as hyperglycaemia, ischaemia, infection and would be available to oxidise the particle [74]. The major sites of oxidation include apo B and the polyunsaturated fatty acids but cholesterol and phospholipids can also be oxidised. There is one apo B molecule /particle hence apo B is considered a strong risk marker for atherosclerosis. The amount of oxidised phospholipid on LDL apo B100 appears to be a good marker of atherosclerotic progression [75].

Apo CIII is a strong risk factor for cardiovascular disease. Apo CIII increases the binding of LDL to artery wall proteoglycans and increases accumulation of lipoproteins in the vascular wall [76, 77]. Huikka *et al.,* [78] have suggested that CIII-enriched LDL from diabetic patients might bind preferentially because of other changes in LDL since adding extra CIII to LDL did not increase binding to any great extent. The authors found that diabetic LDL with high endogenous CIII content contained less unesterified cholesterol and more triglyceride. They further found that the high apo CIII content in diabetic LDL was associated with increased esterified cholesterol, sphingomyelin, ceremide and the ceremide containing ganglioside GM1. They suggest that these changes may be associated with higher membrane fluidity and higher freedom in lateral movement thus allowing Apo B to acquire a conformation which is more favorable for proteoglycan binding. The heparin sulphate proteoglycans (HSPG) play an important role in the assembly and structure of the basement membrane [79]. There is evidence that HSPG is reduced under hyperglycaemic conditions [80]. Loss of arterial heparin sulphate correlates with the onset of atherosclerosis in a monkey model of diabetes [81]. Recently, Rao *et al.,* [79] have shown that hyperglycaemia induces heparin sulphate degradation through increase reactive oxygen production *via* increase in heparanase 1 which specifically degrades heparin sulphate proteoglycans thus a vicious circle is set up in which an increase in free radical production increases oxidation and Lp (a) aggregation and in turn stimulates inflammatory molecules such as tumor necrosis factor-α (TNFα) and interleukan6 (IL-6) and increase free radical production. Heparanase-1 is produced locally by the endothelium. TNFα, interleukin 1β (IL-1 β) and fatty acids are able to induce heparanase-1 production in endothelial cells *in vitro*. Rao *et al.,* [79] showed abundant heparanase-1 staining in endothelial cells from atherosclerotic plaques suggesting that many pathological factors in the atherosclerotic plaque can contribute to heparanase-1 production in endothelial cells. Thus increased heparanase-1 production in endothelial cells and macrophages may be responsible for decreased amounts of heparin sulphate proteoglycans in the arterial wall and may contribute to the pathogenesis of atherosclerosis.

The foam cell is perhaps the hallmark of the atherosclerotic lesion. The development of the foam cell depends on macrophage uptake of cholesterol. A novel mechanism has recently been described. The Toll-like receptor 4 (TR4) recognises minimally oxidised LDL [82, 83] (Fig. **2**). Choi *et al.,* [84] demonstrated a complex signalling pathway induced by minimally oxidised LDL led to enhanced uptake of small molecules such as minimally oxidised LDL, resulting in lipid accumulation. Thus the authors have described a novel mechanism leading to enhanced LDL uptake in macrophages that would lead to foam cell formation and atherosclerosis. In diabetes we and others have shown an increase in LDL esterified cholesterol so it is interesting that Choi *et al.,* [84] demonstrated that cholesterol ester hydroperoxides are an indigenous ligand for TR4.

THROMBUS FORMATION

Thrombus formation on atherosclerotic lesions is a late stage in the disease and leads to myocardial infarction and stroke. Tissue factor [85] is a potent pro-coagulant and matrix degrading proteinase that is involved in the rupture of the fibrous cap and promotes thrombosis. Macrophages are major source within the atherosclerotic plaque of tissue factor, a membrane bound glycoprotein receptor that triggers the thrombotic cascade. Recently Sardo *et al.,* [86] have demonstrated that low concentrations of oxidised LDL enhance tissue factor expression but higher concentrations attenuate tissue factor expression. Perhaps another reason why minimally oxidised LDL is potentially so atherogenic. Oxidised phospholipids derived from modified lipoproteins and in particular LDL, stimulate monocyte expression of urokinase and urokinase receptors which promote the secretion of matrix metalloproteinase-9 (MMP-9). Cellular exposure to MMP-9 also promotes nuclear factor kb expression in the mononuclear cell, another factor involved in destabilisation of plaque and thrombus formation [87, 88].

CONCLUSION

The evidence is overwhelming that elevated and/or modified LDL is a major risk factor for atherosclerosis. The atherosclerotic potential depends, not only on mass but also on composition. In addition HDL has a major impact on the atherogenicity of LDL. The interrelationship between all lipoprotein particles is so strong that preventative treatment should not focus on LDL alone but should encompass the whole lipoprotein family. Basic research has identified new targets for treatment that have the potential to reverse atherosclerosis to a much greater extent than is possible at present.

ACKNOWLEDGEMENT OF FUNDING

Diabetes Research Foundation Dublin Ireland.

CONFLICT OF INTEREST

None declared.

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Received: December 5, 2011 Revised: February 1, 2012 Accepted: February 4, 2012