Triacylglycerol-rich lipoproteins and the generation of small, dense low-density lipoprotein

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Abstract

LDL (low-density lipoprotein) is the major carrier of cholesterol in human plasma, and as such is intimately involved in the process of atherosclerosis. The lipoprotein class comprises a number of distinct subfractions, and is commonly divided into large, intermediate and small sized particles. Small, dense LDLs are held to be particularly atherogenic, since these particles are retained preferentially by the artery wall, are readily oxidized and carry an enzyme believed to have an important role in atherosclerosis, i.e. lipoprotein-associated phospholipase A_2. Generation of small, dense LDL occurs by intravascular lipoprotein remodelling as a result of disturbances such as Type II diabetes, metabolic syndrome, renal disease and pre-eclampsia. The key predisposing factor is the development of hypertriglyceridaemia, in particular elevation in the plasma concentration of large, triacylglycerol-rich VLDL (very-low-density lipoprotein). This leads to the formation of slowly metabolized LDL particles (5-day residence time), which are subject to exchange processes that remove cholesteryl ester from the particle core and replace it with triacylglycerol. LDL, so altered, is a potential substrate for hepatic lipase; if the activity of the enzyme is high enough, lipolysis will generate smaller, denser particles. Correction of the dyslipidaemia associated with small, dense LDL is possible using fibrates and statins, and this may contribute to the clinical benefits seen with these drugs. Fibrates act to lower plasma triacylglycerol (VLDL) levels, and so correct the underlying metabolic disturbance. Statins remove VLDL particles via receptor-mediated pathways and reduce the residence time (and hence limit the potential for remodelling) of LDL in the circulation.

Background

LDL (low-density lipoprotein) is the main cholesterol-carrying lipoprotein in the circulation. Essentially, it comprises a solubilized 'cargo' of cholesteryl ester surrounded by a coat of phospholipid and protein. The protein component is almost exclusively apoB100 (apolipoprotein B100), a 4536-amino-acid polypeptide that is critical for lipid transport. LDL was originally thought to be monodisperse, i.e. composed of a distribution of particles differing slightly in size from one another; the mean particle size was about 250 Å in diameter and the peak density was 1.035 g/ml. It was noted first in patients with hypertriglyceridaemia that fractions of LDL with differing size and density existed, and the seminal work of Krauss and Burke [1] using high-resolution gradient gel electrophoresis uncovered the fact that LDL comprises a number of discrete species in most individuals. Several schemes have been developed based on gradient gel electrophoresis or density gradient centrifugation, which separate LDL into subfractions (usually between three and five) [2,3]. For convenience, most workers use the terminology large, intermediate and small LDL to denote the three major subdivisions. The plasma concentrations of these subfractions can be quantified by the measurement of constituent components (protein, phospholipid, cholesterol and triacylglycerol) following isolation by density gradient centrifugation [3].

Examination of the properties of LDL subfractions has led to the belief that small, dense LDL is a particularly atherogenic form of the lipoprotein. This subfraction binds less well to the LDL receptor in comparison with its larger counterparts [4], which has the consequence of prolonging its lifetime in the circulation. Conversely, the particle appears to interact more strongly with arterial wall proteoglycans [5]. In the 'response to retention' hypothesis of atherosclerosis, this property will increase the time that lipoprotein spends trapped in the subendothelial space of the artery wall, and hence increase the opportunity to promote atherogenic changes. It is likely that these altered functions are the result of apoB in small, dense LDL adopting a conformation different from that in larger LDL species, which favours proteoglycan binding but inhibits receptor-mediated catabolism. A specific domain on apoB appears to dictate these properties of the particle [6]. In addition, it has been shown repeatedly that small, dense LDL is the most readily oxidized subfraction in the lipoprotein class [7], again increasing its atherogenic potential.

Key words: atherosclerosis, fibrate, hepatic lipase, statin, very-low-density lipoprotein.
Abbreviations used: apoB (etc.), apolipoprotein B (etc.); CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; HDL, high-density lipoprotein; HL, hepatic lipase; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.
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Epidemiology of small, dense LDL

The original observation by Krauss and co-workers (reviewed in [8]) that the LDL of survivors of myocardial infarction was abnormally small (LDL subclass pattern B) led to numerous studies that established its risk factor status for CHD (coronary heart disease). A preponderance of small, dense LDL is associated with a 3–7-fold increase in CHD risk, independent of LDL cholesterol concentration [3,8]. Small, dense LDL was also found to be elevated in patients with diabetes, renal disease and other disorders such as pre-eclampsia [9–11]. It has been added to the list of cardinal features of the 'insulin resistance' or 'metabolic' syndrome [12].

The trait of an LDL distribution characterized by the predominance of small, dense LDL has been linked to various genetic loci. Its heritability is estimated at 35–45% [8]. The prevalence of the trait is low in young men and pre-menopausal women, and increases with age. The differences between men and women of the same age in the appearance of small, dense LDL is remarkable, and offers a clue as to potential regulatory factors. Interestingly, women who become pregnant exhibit a temporary redistribution of LDL towards smaller sized particles [13]. Low-fat, high-carbohydrate diets have been reported to predispose subjects to the generation of small, dense LDL [8].

Key to understanding the metabolic conditions that lead to the generation of small-sized LDL particles is the observation that they appear in individuals who are hypertriglyceridaemic and have a low concentration of HDL (high-density lipoprotein) cholesterol. Indeed, the pattern of raised triacylglycerols [i.e. elevated VLDL (very-low-density lipoprotein)], low HDL cholesterol and a predominance of small, dense LDL has been recognized as a distinct dyslipidaemia, and is termed the atherogenic lipoprotein phenotype [14]. It is the characteristic lipoprotein abnormality of insulin resistance, metabolic syndrome and Type II diabetes mellitus.

Generation of small, dense LDL – steady-state studies

The conditions under which small, dense LDL appears give strong clues as to how it is generated in humans. It is an almost universal finding that a pattern B LDL subclass distribution is not seen until plasma triacylglycerol levels exceed 1.5 mmol/l (approx. 120 mg/dl) [14,15]. In studies from our and other laboratories, approx. 50% of the variation in LDL size is determined by plasma triacylglycerol levels [8,15]. As plasma triacylglycerol levels rise in individuals, it is large VLDL ($VLDL_1$) that accumulates (Figure 1). The presence of an excess of this lipoprotein species seems to be the trigger that promotes the generation of small, dense LDL. A further key factor is the activity of the enzyme HL (hepatic lipase). The concentration of small, dense LDL is correlated positively with the activity of this liver-situated lipase, which is responsible for hydrolysis of triacylglycerol in LDL and HDL [16]. In formulating a metabolic model for the appearance of an atherogenic lipoprotein phenotype, we postulated that formation of small, dense LDL was favoured when HL activity was high, as in men [16]. Women have half the HL levels of age-matched male counterparts due to the inhibitory effects of oestrogen on enzyme expression. This may explain the sex difference in the prevalence of LDL pattern B.

Studies have suggested that CETP (cholesteryl ester transfer protein) may also play a regulatory role [17]. This protein facilitates the inter-particle exchange of hydrophobic lipids found in the core of lipoproteins. The degree of hetero-exhange, i.e. of triacylglycerol for cholesteryl ester, between particles is governed principally by the availability of large triacylglycerol-rich VLDL [18]. Individuals with high CETP levels exhibit accelerated transfer of triacylglycerol into LDL, thus making the lipoprotein a better substrate for HL activity.

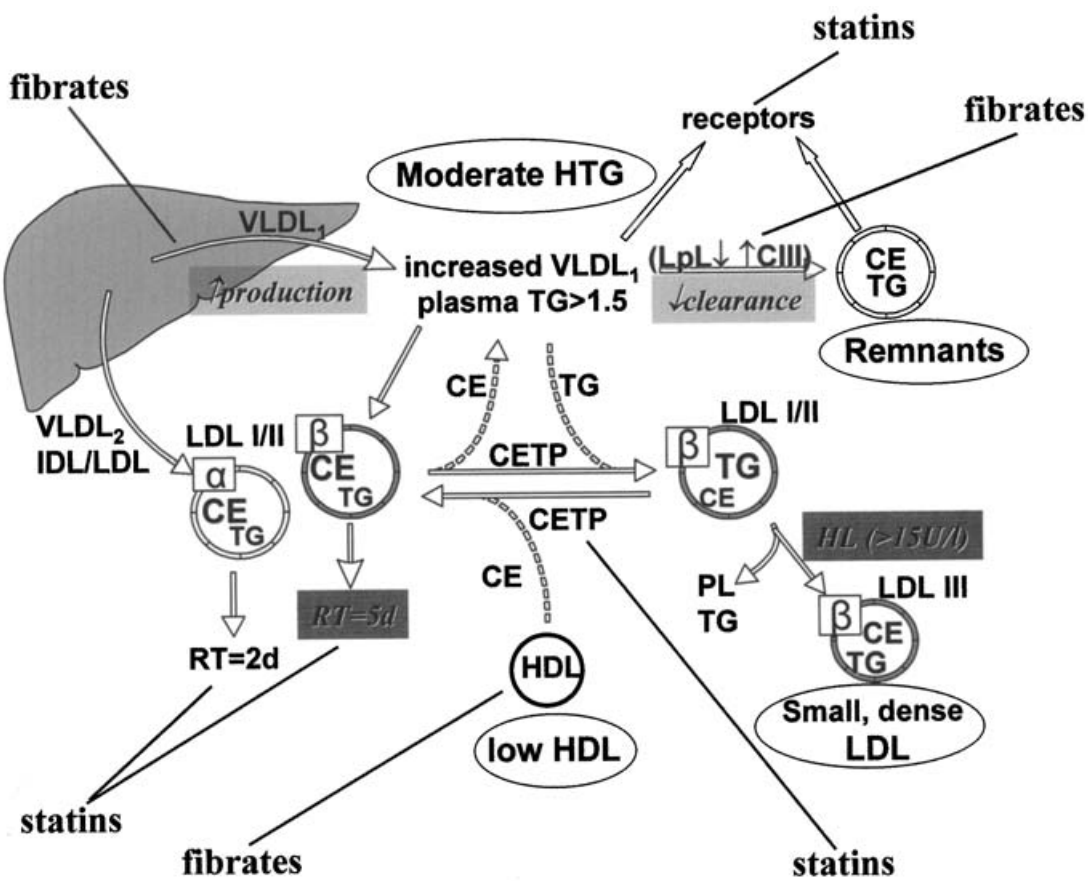
Studies of populations [15] and of the changes in lipoproteins that occur during pregnancy [13] led us to postulate that generation of small, dense LDL is a threshold phenomenon. That is, the concentration of small, dense LDL remains low as plasma triacylglycerol in the population rises from 0.5 to 1.5 mmol/l. There is a steady increase in LDL levels, but it is in intermediate-sized particles. Above a plasma triacylglycerol of 1.5 mmol/l, the total LDL concentration does not increase, but there is a fall-off in intermediate-sized LDL and a sharp rise in small, dense LDL. In pregnant women, the plasma triacylglycerol level rises during gestation from low (<1.0 mmol/l) to moderately elevated (often >2.0 mmol/l) levels. Thus in a brief period of time the level of this plasma lipid crosses the putative threshold value. We observed that small, dense LDL was virtually absent early in pregnancy, but at a particular triacylglycerol level (which differed slightly between subjects), there was abrupt formation of small LDL particles [13].

Generation of small, dense LDL – dynamic studies

The metabolic conditions that lead to the formation of small, dense LDL have been explored in a number of investigations that have employed lipoprotein tracer technology (Figure 1) [8,19]. In the 'normal' population, elevation in $VLDL_1$ (to give a plasma triacylglycerol concentration >1.5 mmol/l) was associated with decreased clearance of the lipoprotein, whereas in patients with diabetes raised plasma triacylglycerol levels were due to increased production of VLDL [20,21]. It probably does not matter what causes a high level of $VLDL_1$; elevation of the plasma concentration above the threshold creates the conditions required for the formation of small, dense LDL. Lipolysis of $VLDL_1$ has been shown to give rise to LDL particles in the density range that have a prolonged residence time (approx. 5 days) compared with LDL derived from smaller VLDL or intermediate-density lipoprotein precursors (which has a residence time of approx. 2 days). In a series of studies we were able to show that

Figure 1 | Metabolic model for the formation of small, dense LDL and the impact of lipid-lowering drugs

In this postulated scheme, the key abnormality leading to the generation of small, dense LDL is the development of mild to moderate hypertriglyceridaemia (HTG), defined as a plasma triacylglycerol (TG) concentration of >1.5 mmol/l. Under these conditions, large triacylglycerol-rich VLDL ($VLDL_1$) accumulates due to either overproduction in the liver or defective clearance from the circulation. Low lipoprotein lipase (LpL) activity or an excess of apoCIII (CIII; an inhibitor of lipoprotein lipase) can impede the efficient lipolysis of $VLDL_1$. $VLDL_1$, when lipolysed, gives rise to a population of LDL particles (denoted β) which have an altered apoB100 conformation. These particles fail to bind well to LDL receptors and so have a prolonged residence time (RT) in the circulation (d = days). Pool β LDL has therefore increased likelihood of undergoing remodelling. CETP removes cholesteryl ester (CE) and replaces it with triacylglycerol as the protein shuttles between VLDL, LDL and HDL particles. Triacylglycerol-enriched LDL is a good substrate for HL, an enzyme that removes triacylglycerol from smaller lipoprotein particles. Small, dense LDL is generated in this final lipolytic step. Fibrates improve rates of lipolysis and inhibit $VLDL_1$ production. The resultant fall in plasma triacylglycerol levels (to <1.5 mmol/l) removes the conditions for the formation of small, dense LDL. Fibrates also promote the production of HDL apoprotein AI. Statins stimulate LDL receptor activity, principally in the liver. $VLDL_1$ in patients with hypertriglyceridaemia is able to bind to these receptors, and so its clearance from plasma is enhanced. Likewise, remnant removal and LDL catabolism are promoted. CETP action is inhibited by statins, and the net effect of these changes is to lower LDL levels and, with potent statins, decrease the formation of small, dense LDL. IDL, intermediate-density lipoprotein; PL, phospholipid.



the amount of slowly metabolized LDL in the circulation correlated strongly with the plasma triacylglycerol level, in patients both off and on hypolipidaemic (fibrate) therapy [19]. This form of LDL has sufficient time to be remodelled via the agency of CETP (Figure 1). Thus, in the presence of elevated $VLDL_1$ levels, LDL will lose cholesteryl ester via the CETP mechanism and gain triacylglycerol. If this happens to a significant degree, then it is postulated that the next exposure of the triacylglycerol-enriched LDL to HL will lead to the removal of enough core lipid (triacylglycerol) and surface lipid (HL has phospholipase action) to promote a shift in particle size into the small, dense range. The key regulatory factors in this model are, therefore, the concentration of $VLDL_1$ and the activities of HL and CETP. The model is sufficient to explain the epidemiology of LDL pattern B subfraction distribution.

Pharmacological regulation of small, dense LDL

Correction of the hypertriglyceridaemic state is an obvious way to lower the probability of the formation of small, dense LDL. Fibrates have been shown to be highly successful agents in lowering the plasma concentration of small, dense LDL [22,23]. This action probably contributes to the efficacy of these drugs in lowering atherosclerosis and CHD risk. The compounds are agonists for specific nuclear receptors, and alter the expression of a number of key genes involved in lipoprotein metabolism. ApoCIII is decreased, while lipoprotein lipase is increased [24]. Both of these effects will accelerate the clearance of $VLDL_1$ from the circulation and, as a consequence, reduce plasma levels of small, dense LDL. Often, however, the total LDL concentration of patients on fibrates is unaltered [23]. For the LDL subfraction to shift from a preponderance of small, dense LDL (pattern B) to a more normal profile, plasma triacylglycerol levels must be reduced below the threshold of 1.5 mmol/l.

Statins act to lower LDL levels and are proven to reduce CHD risk. At first it was thought that, since they influence principally cholesterol synthesis, the drugs would not alter plasma triacylglycerol levels, and by extrapolation would not perturb the relative distribution of LDL subfractions. This was borne out in early trials of first-generation agents such as pravastatin [25]. However, more recent studies have shown that statins can have significant triacylglycerol-lowering actions in patients with hypertriglyceridaemia if the drugs are given in high dose or new, more potent agents are employed [26]. Indeed, the percentage decrease in plasma triacylglycerol equals numerically that in LDL cholesterol in these subjects. The decrease in $VLDL_1$ in hypertriglyceridaemic subjects on statin therapy occurs because these particles are recognized by the LDL receptor, probably due to the fact that they are enriched in apoE [27] (Figure 1). Indeed statins, by stimulating LDL receptor activity, lower all apoB-containing lipoproteins in patients with elevated plasma triacylglycerol levels [28]. The ability to change the LDL subfraction distribution is more evident for compounds such as atorvastatin [28] and rosuvastatin [29], which have been shown to shift the LDL subfraction distribution towards normal as well as lowering total LDL concentration. The drugs also appear to inhibit CETP action [29] and elevate HDL, thus offering an alternative to fibrates for the correction of the atherogenic lipoprotein phenotype.

Summary

A metabolic model has been developed for the formation of small, dense LDL. The key predisposing factor is an elevation in the plasma concentration of large, triacylglycerol-rich VLDL ($VLDL_1$). CHD risk arises not only from the total content of LDL in the bloodstream, but also from the qualitative nature of the lipoprotein; small, dense LDL exhibits particularly atherogenic properties. Commonly available drugs are able to correct in part the dyslipidaemic conditions that promote the generation of small, dense LDL, but more specific agents are required to control this risk factor fully.

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