LDL heterogeneity: implications for atherogenicity in insulin resistance and NIDDM

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It is low density lipoprotein (LDL), the major cholesterol-rich lipoprotein, which accounts for the link between increasing plasma cholesterol and coronary heart disease risk. Although once considered to be homogeneous it has become clear that LDL comprises a spectrum of particles.

LDL subclasses

Studies employing density gradient ultracentrifugation and gel electrophoresis have demonstrated that the LDL density range is comprised of a number of discrete subclasses which vary in size, density and lipid content [1] (Fig. 1). As the density of the LDL species increases, particle diameter, flotation rate and relative content of total and polar lipids all decrease. LDL-II is the most abundant subspecies in healthy individuals. Women tend to have proportionally more LDL-I than men while men have proportionally more LDL-III.

LDL subclasses and coronary heart disease

Non-denaturing, gradient-gel electrophoresis which determines particle size is the most commonly used technique for studying LDL heterogeneity and is suitable for large-scale clinical application. Two distinct LDL subclasses have been defined; pattern A, major peak diameter over 25.5 nm, and pattern B, major peak diameter under 25.5 nm [2]. Using this technique Austin and colleagues [3] demonstrated a preponderance of LDL pattern B in myocardial infarction survivors compared to healthy control subjects. This was associated with a threefold risk of infarction independent of age, gender and body weight but dependent on plasma triglyceride and high density lipoprotein (HDL) cholesterol [3]. The term atherogenic lipoprotein profile was coined to describe the clustering of moderate hypertriglyceridaemia (> 1.7 mmol/l) circulating as large VLDL, reduced HDL cholesterol and a predominance of small dense LDL [4]. Similar findings have been described by others who have measured LDL particle size on gels and separated LDL subclasses by ultracentrifugation [5–9]. In atheroma regression studies small dense LDL has been shown to be related to reduced coronary artery diameter [10].

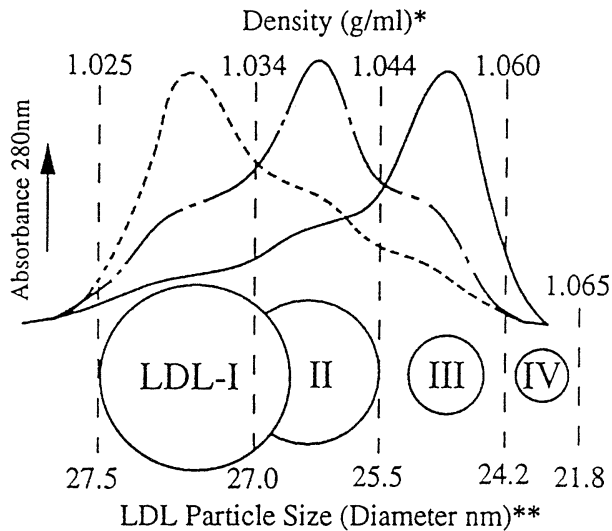## Metabolic determinants of LDL subclasses

Many factors have been shown to influence LDL subclass distribution including cholesterol ester transfer protein (CETP) activity, hepatic lipase activity, apoprotein E phenotype, gender, body weight, exercise and genetics. However, the presence of hypertriglyceridaemia appears to be a major determinant [11–13]. Plasma triglyceride is negatively correlated with LDL size and approximately 70% of the variability of LDL distribution can be explained by triglyceride and HDL cholesterol variations [14].

An attractive hypothesis to explain this relationship is the stimulation of neutral lipid exchange via CETP by the concentration of triglyceride-rich lipoproteins. Triglyceride transfers to LDL in exchange for cholesterol ester and the triglyceride-enriched particles become a substrate for hepatic lipase resulting in protein-rich, lipid-poor particles with a density

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Fig. 1. Distribution of human plasma LDL subclass density and particle size. *LDL: subclass density profiles obtained by density gradient ultracentrifugation and representative of a typical normal, healthy female (- - -), male (– • –) and CAD patient (——) ***LDL: particle size as determined by 2–16% gradient gel electrophoresis. Reproduced from reference [33]

resembling LDL-III. Kinetic studies suggest that $VLDL_1$ (Svedberg flotation units (Sf) 60–400) particles, which are present in hypertriglyceridaemia, give rise to LDL-III particles whereas the smaller $VLDL_2$ (Sf 20–60) found in normal and hyper-cholesterolaemic individuals gives rise to LDL-II particles. Enhanced postprandial lipaemia is probably also an important determinant of the production of small dense LDL [15].

LDL subclasses and atherogenicity

Whether small dense LDL are directly atherogenic remains to be fully proven. However, the literature points to several possible mechanisms whereby small dense LDL may be atherogenic including increased infiltration into the arterial wall [16]. Increased sequestration due to enhanced binding to arterial proteoglycans [17, 18] possibly related to conformational changes in apoprotein B [19] and reduced carbohydrate and sialic acid content [20] has been reported. Increased susceptibility to oxidation has also been reported for small dense LDL [21].

LDL subclass distribution in insulin resistance and non-insulin-dependent diabetes mellitus (NIDDM)

Hypertriglyceridaemia often associated with low HDL cholesterol concentration is a common finding in NIDDM and often persists despite best efforts at glycaemic control. Several studies have pointed to the

strong relationship between triglycerides and coronary heart disease risk and this relationship may be explained in part by abnormal postprandial lipaemia, the accumulation of remnant particles and a shift in the LDL distribution towards small dense particles [22].

In a group of poorly controlled, obese NIDDM patients with hypertriglyceridaemia a reduction in intermediate density LDL and a concomitant increase in small, dense LDL was observed [23]. Similarly, morbidly obese (BMI > 40 kg/m^2) and hypertriglyceridaemic NIDDM females were shown to have a preponderance of small, dense LDL particles [24]. In a further study there was a greater impact of diabetes on LDL size in women than in men [25]. LDL size and peak density in mildly hyperlipidaemic patients correlated with insulin resistance [26]. Similar results have been obtained in studies which separated LDL subclasses by ultracentrifugation; insulin resistance correlated inversely with LDL-I concentrations and positively with LDL-III and 32–33 split proinsulin correlated with LDL-III [27]. Furthermore, in non-diabetic individuals subjects with a preponderance of small dense particles have been demonstrated to be more insulin resistant [28, 29] and small dense LDL can be considered to be part of the insulin resistance syndrome [30].

Hypertriglyceridaemia has a major impact on LDL concentrations in the non-diabetic population [31] and this is also the case in NIDDM [27, 32]. In the fasting state multivariate analysis showed that fasting triglyceride concentrations, HDL cholesterol and hepatic lipase activity were the most important determinants of LDL-III (R^2 = 67%). Postprandially, chylomicron remnant clearance, hepatic lipase activity and insulin resistance were the major determinants of LDL-III (R^2 = 61%) [27].

The mechanisms explaining the link between insulin resistance and LDL heterogeneity remain to be fully elucidated. However, it is known that insulin resistance is associated with increased non-esterified fatty acid flux to the liver, increased hepatic output of large VLDL which is not suppressed postprandially, increased postprandial lipaemia, increased activity of hepatic lipase and increased CETP activity. All these factors would tend to favour the formation of small dense LDL-III particles.

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