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Remnant lipoprotein size distribution profiling via dynamic light scattering analysis

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ABSTRACT

Background: Remnant lipoproteins (RLP) are a metabolically derived subpopulation of triglyceride-rich lipoproteins (TRL) in human blood that are involved in the metabolism of dietary fats or triglycerides. RLP, the smaller and denser variants of TRL particles, are strongly correlated with cardiovascular disease (CVD) and were listed as an emerging atherogenic risk factor by the AHA in 2001.
Methods: Varying analytical techniques used in clinical studies in the size determination of RLP contribute to conflicting hypotheses in regard to whether larger or smaller RLP particles contribute to CVD progression, though multiple pathways may exist.
Results: We demonstrated a unique combinatorial bioanalytical approach involving the preparative immunoseparation of RLP, and dynamic light scattering for size distribution analysis.
Conclusions: This is a new facile and robust methodology for the size distribution analysis of RLP that in conjunction with clinical studies may reveal the mechanisms by which RLP cause CVD progression.

1. Introduction

Cardiovascular disease (CVD) is one of the leading causes of mortality in the U.S. According to the 2014 American Heart Association (AHA) statistical update, even though the rate of mortality attributed to CVD declined in the last decade, the burden of this multidimensional disease remains high [1]. Scientific efforts focus on LDL (low density lipoproteins, d = 1.019–1.063 g/ml) and clinical quantification of LDL cholesterol remains as a mainstay of CVD prediction and diagnosis [2]. The American Heart Association specifically lists triglyceride evaluation (hypertriglyceridemia) as a novel risk factor [1], and in cases where triglyceride values are >200 mg/dl, the National Cholesterol Education Program Adult Treatment Panel III recommends measurement of non-HDL-cholesterol specifically as remnant lipoproteins (RLP) as a potential CVD causal agent [3]. Hypertriglyceridemia causes CVD progression independent of other risk factors including LDL-cholesterol and HDL-cholesterol; however mild hypertriglyceridemia without other risk factors present is not correlated with CVD. The mechanism by which hypertriglyceridemia causes CVD incidence are complex, and methods to distinguish cases where there is a causal relationship are needed [4]. We addressed the need for triglyceride evaluation through a new and unique vantage point of size distribution analysis of proatherogenic lipoprotein particles intrinsic to triglyceride metabolism.

Triglyceride-rich lipoproteins (TRL) are the population of lipoproteins in human blood that are involved in the metabolism of dietary fats or triglycerides. As illustrated in Fig. 1, TRL particles include 4 main populations: chylomicrons (CM), VLDL (very low density lipoprotein), and the remnant particles of chylomicrons and VLDL (CMR and rVLDL respectively). The remnant lipoproteins, CMR and rVLDL are formed through the interaction of CM and VLDL with lipoprotein lipase on the capillary walls of muscle and adipose tissue. All four populations comprise the TRL class; the RLP subclass is bracketed.

Chylomicrons (CM) range in size from 75 to 3000 nm in diameter, and their size decreases metabolically as they are converted to CMR upon lipolysis by lipoprotein lipase [4]. VLDL range in size from 18 to 50 nm, with a median diameter of 26 nm, and an average diameter of 27 ± 6.4 nm (±SD) when measured by cryo-electron microscopy [5]. Remnant lipoproteins (RLP), the triglyceride-depleted and smaller metabolic products of TRL particles, are strongly correlated with type III dyslipidemia, diabetes mellitus, and cardiovascular disease and were listed as an emerging atherogenic risk factor by the AHA in 2001 [3,6,7]. Recent studies suggest that smaller RLP, as a catabolized product of triglyceride metabolism, may be the vehicle for disease progression in the coronary arteries [4].

Abbreviations: DLS, dynamic light scattering.
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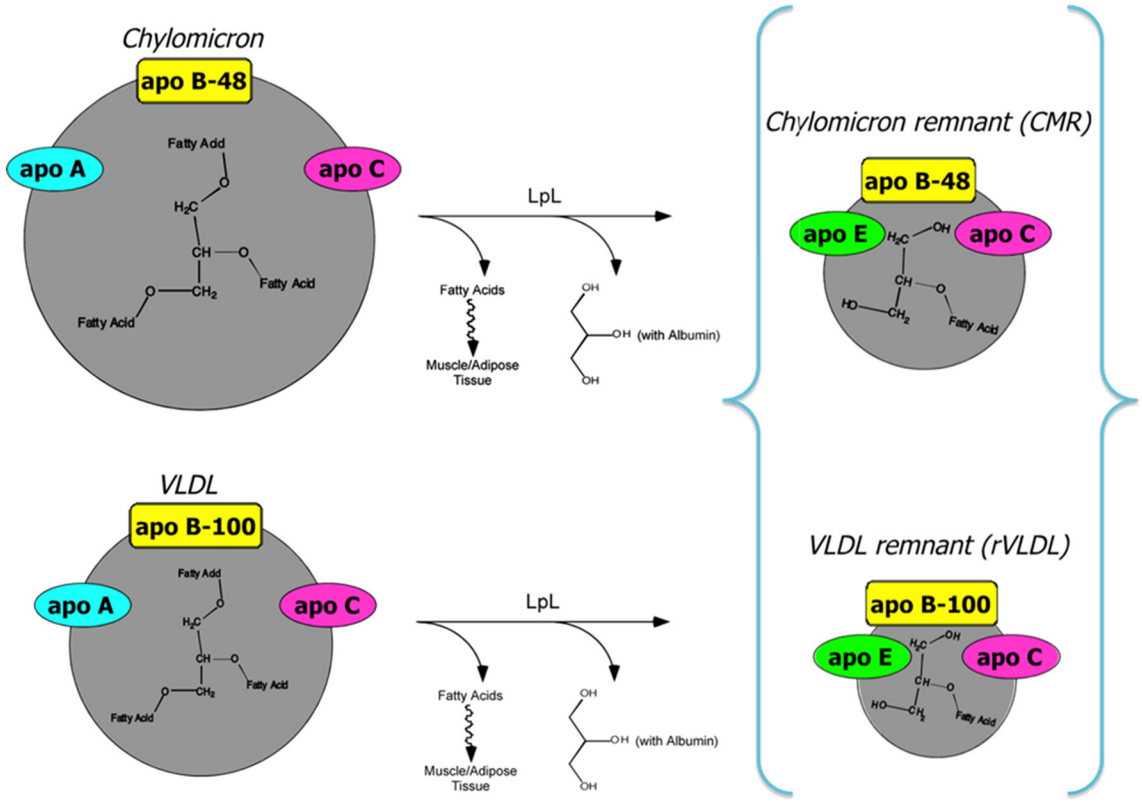

Fig. 1. Triglyceride-rich lipoprotein class including the intestinally derived chylomicrons and their remnants (chylomicron remnant, CMR), hepatically derived VLDL and their remnants (VLDL remnant, rVLDL).

Characterizations of the RLP subclass include RLP-cholesterol, RLP-triglycerides, RLP protein, and RLP density determinations [4,8–12]. Evidence in animal and in vitro studies of RLP accumulation in the arterial intima points to the decreased size of RLP in comparison to their TRL precursors as a potential mechanism for RLP atherogenicity [13]. Specifically, atherosclerotic development in the form of fatty streaks is thought to occur by an entry of lipoproteins in circulation into the vascular wall or arterial intima [4]. This is akin to the hypothesis proposed for smaller oxidized LDL [14]. Chylomicrons and VLDL are considered too large to enter the arterial intima; however, RLP are hypothesized to be in a size range that is compatible with this mechanism. Also, in animal studies, once there is injury to the arterial wall, even larger remnant lipoproteins may enter the intima and accumulate to cause atherosclerotic plaque development [4]. In contrast, current consensus to the mechanisms by which chylomicron remnants cause CVD, points to pro-inflammatory processes involving formation of macrophage foam cells and monocyte dysfunction [15]. Since RLP consist of both intestinally and hepatically derived particles, they are a heterogeneous population of particles, which makes determination of more atherogenic RLP challenging [4]. The atherogenic component of the RLP population is also theorized to be in the size range of LDL [4] but no direct comparison between the 2 using a single, standard methodology exists. Another hypothesized mechanism for the atherogenicity of RLP pertains to an impaired clearance from circulation of larger RLP, as defined by a higher triglyceride to cholesterol ratio [8]. These 2 hypotheses are in opposition regarding the relationship of size and atherogenic potential for RLP. The upshot of these different hypotheses and studies is that RLP causes atherosclerosis through multiple pathways directly involved in plaque formation and/or indirectly through impaired vasodilation and pro-inflammatory mechanisms [3]. Clinical studies involving size distribution analysis of RLP are paramount to a better understanding of the mechanisms behind CVD progression and RLP prevalence.

Variation in lipoprotein size correlates highly with CVD incidence [16] and has been measured by a range of analytical techniques including biochemical assays, proton nuclear magnetic resonance (NMR), gradient gel electrophoresis, high performance liquid chromatography (with gel permeation columns), ion-mobility, cryo-electron microscopy and dynamic light scattering [5,8,17–24]. Biochemical measurements of RLP size depends on the RLP-triglyceride to RLP-cholesterol ratio, which is a metabolic indicator of a decrease in particle size [8,21]. Diffusion-ordered NMR uses the methyl peak of lipoproteins to calculate diffusion coefficient in order to estimate size [22]. Gel electrophoresis separates particles based on molecular weight as a measure of their size [19]. High performance liquid chromatography involves differential elution of lipoproteins which is used as an indicator of particle size differences among lipoprotein fractions [20,22]. Cryo-electron microscopy is used to determine the size and ultrastructure of lipoproteins in the lipoprotein's native environment by first isolating via separation by ultracentrifugation, ultra-rapid freezing, and then analysis by an electron microscope [5,18].

Dynamic light scattering (DLS) is a method commonly used in material sciences to determine the hydrodynamic particle size of colloidal nanoparticles in solution [25,26]. Principle and mathematical background of the DLS technique is well known [27,28]. Incoming laser light from a DLS instrument is backscattered by particles in the solution; a detector coupled with a time - correlated photon counter inside the DLS instrument measures the backscattered light intensity repeatedly over a short time interval. The instrument accumulates the data into one measurement result, recording the rate at which the intensity of the backscattered light fluctuates during the given time interval. Those intensity fluctuations occur since particles in a liquid medium (0.05 mmol/l tris-HCl, pH 7.4) are diffusing due to random Brownian motion. This diffusion results in fluctuations in the local concentration of particles and therefore in a continuous change of the interference