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Quantification of lipoprotein profiles by nuclear magnetic resonance spectroscopy and multivariate data analysis

Violetta Aru [a, 1], Chloie Lam [b, 1], Bekzod Khakimov [a], Huub C.J. Hoefsloot [b], Gooitzen Zwanenburg [d], Mads Vendelbo Lind [f], Hartmut Schäfer [c], John van Duynhoven [d, e], Doris M. Jacobs [e], Age K. Smilde [b], Søren B. Engelsen [a, *]

[a] Department of Food Science, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg, Denmark
[b] Biosystems Data Analysis Group, University of Amsterdam, 1090 GE Amsterdam, The Netherlands
[c] Bruker BioSpin GmbH, 76287 Rheinstetten, Germany
[d] Laboratory of Biophysics, Wageningen University, Wageningen 6703 HA, The Netherlands
[e] Unilever R&D Vlaardingen, Olivier van Noortlaan 120, 3130 AC Vlaardingen, The Netherlands
[f] Department of Nutrition, Exercise and Sports, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg, Denmark

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ABSTRACT

Lipoproteins and their subfraction profiles have been associated to diverse diseases including Cardio Vascular Disease (CVD). There is thus a great demand for measuring and quantifying the lipoprotein profile in an efficient and accurate manner.

Nuclear Magnetic Resonance (NMR) spectroscopy is uniquely able to measure the lipoprotein profile of a blood sample non-destructively due to its sensitivity to both lipid chemistry and lipid-micellar physics.

However, the NMR spectra must be scaled/regressed to a primary method of reference, such as ultracentrifugation, using multivariate regression methods.

This review provides an overview of the field and explains the methods at stake.

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1. Introduction

Lipoproteins (LPs) are important constituents of the lipid fraction of the human body that function as carriers for water-insoluble lipids through the aqueous bloodstream. LP vehicles provide the active mobilization of endogenous and exogenous (dietary) lipids through the aqueous compartments within the cells as well as in the blood and body tissues where lipid molecules can be either stored (i.e. adipose tissue) or used as energy source.

Based on their buoyant densities, LPs can be classified in five major groups: ChyloMicrons (CM), Very Low Density Lipoproteins (VLDL), Intermediate Density Lipoproteins (IDL), Low Density Lipoproteins (LDL), and High Density Lipoprotein (HDL) with CM being the biggest and least dense LP particles [1] (see Table 1). Lipoproteins fractionation and quantification is a matter of primary interest in the field of clinical medicine since elevated concentrations of Cholesterol (Cho) and TriGlycerides (TG), in specific LPs, have been associated with significantly increased occurrence of Cardio Vascular Diseases (CVDs) [2]. In particular, studies on lipoprotein particle distributions have shown a highly consistent and direct correlation between plasma LDL and the development of atherosclerosis [2]. Even though such epidemiological investigations have shown a positive correlation between total cholesterol concentrations in LDL and Coronary Heart Disease (CHD) mortality, total LDL cholesterol does not accurately predict the risk of CHD in many patients [2]. The LDL/HDL cholesterol ratios are nowadays considered risk indicators with greater predictive value than single parameters, such as LDL [3]. Due to the so called "reverse cholesterol transport", HDL prevents or reverses the formation of atherosclerotic plaques that may derive from LDL metabolism and thus may represent a non-casual integrative marker of CVDs [4]. Moreover, it has been proven that individuals

* Corresponding author.
E-mail address: se@food.ku.dk (S.B. Engelsen).
[1] Contributed equally to the manuscript.

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Table 1
List of the source, function and main biochemical characteristics of the major LP particles classified according to their buoyant density. The density of LPs depends on the lipids to protein ratio: the greater the lipid to protein ration, the larger the size and the lower the density. Adapted from Crook (2012) [1].

Complex	Source	Function	Density (g/ml)	Apoprotein	% Pro[a]	% TG[b]	% PL[c]	% Cho[d]
ChyloMicrons (CM)	Intestine	Transport exogenous lipids from the intestine to all cells	<0.9500	A, B, C, E	1	90	5	4
Very Low Density Lipoproteins (VLDL)	Liver	Transport endogenous lipids from the liver to the cells	0.950–1.006	B, C, E	8	55	12	25
Low Density Lipoproteins (LDL)	VLDL via IDL	Transport of cholesterol to cells	1.019–1.063	B	20	5	20	55
High Density Lipoproteins (HDL)	Intestine, liver (chylomicrons and VLDLs)	Transport of cholesterol from cells back to the liver	1.063–1.210	A, C, E	50	5	25	20

[a] Protein.
[b] Triglycerides.
[c] Phospholipids.
[d] Free Cholesterol.

with predominantly small LDL particles experience greater CHD risk than those with large-size LDL [5], making an accurate quantification of the LP subfractions an essential screening tool for CVDs prevention and diagnosis.

Several analytical approaches can be used for accurately measuring blood LPs, such as gel electrophoresis and Gel-Permeation High Performance Liquid Chromatography (GP-HPLC), but density gradient Ultra-Centrifugation (UC) represents the "gold standard method" for lipoproteins isolation and quantification [6]. Nevertheless, LP analysis by UC is time consuming and labor intensive as it requires numerous sample handlings and specific enzymatic assays are needed to further estimate their composition (usually Cho or TGs content) [7]. High-field ^{1}H Nuclear Magnetic Resonance (^{1}H-NMR)-based lipoprotein profiling has proven to be a valuable alternative to the standard quantification methods of total lipoproteins. ^{1}H-NMR, which is normally used for structure elucidation and chemical mixture quantifications, has one more advantage, namely that it is sensitive to the size (translational and rotational diffusion) and density of macromolecules and supramolecular aggregates [8]. This makes NMR a unique platform for investigating Lipoprotein Particle Distributions (LPDs) primarily because different LP fractions and subfractions have different magnetic susceptibilities which will broadcast different signals whose amplitude reflects the particles concentration [9]. Moreover, the minimum sample pre-treatment and the possibility of gaining relevant biochemical information with a single rapid experiment make ^{1}H-NMR spectroscopy a preferable/valuable screening tool for diagnostics as well as for large scale epidemiological investigations [10]. When combined with multivariate regression, NMR spectroscopy can be used to efficiently and accurately determine LP concentrations as well as TG and Cho content in specific lipoprotein fractions. However, the NMR prediction methods still depend on calibration with reference methods such as UC, gel electrophoresis or GP-HPLC.

This review aims at providing an overview on the research conducted for developing NMR as an efficient tool for the quantification of lipoproteins and will have a special focus on studies with coherent NMR data and reference data form UC and HPLC.

2. Chemical and physical properties of lipoproteins

Lipoproteins are micelle-like particles made up of lipids and proteins whose main function is to render hydrophobic lipid molecules, such as Cho and TG, compatible with the aqueous environment of our blood. In order to facilitate their mobilization in the bloodstream, TG and Cholesteryl Esters (CE), which are the major constituents of the non-polar core of the LPs, are packed into a spherical structure with an outer shell of Free Cholesterol (FC), PhosphoLipids (PLs) (i.e. phosphatidylcholine and sphingomyelin) and polar apolipoproteins. Amongst the diverse chemical components of the LPs, apolipoproteins play an important role in regulating and controlling the metabolism of specific lipoprotein fractions [11]. Based on their size and distribution, apolipoproteins have been classified in five main groups: apoA, apoB, apoC, apoD and apoE. Several specific functions have been ascribed to these proteins. Besides mediating lipid transport and redistribution among various tissues, apolipoproteins act as cofactors for enzymes of lipid metabolism and cover an important role in the maintenance of the structure of the lipoproteins.

Plasma lipoproteins are usually classified according to their buoyant density, determined by the lipid composition itself (i.e. phospholipids have a higher density than the neutral lipids) and by the lipid to protein ratio. The classification is made into five main fractions: ChyloMicrons (CM), Very Low Density Lipoproteins (VLDL), Intermediate Density Lipoproteins (IDL), Low Density Lipoproteins (LDL) and High Density Lipoprotein (HDL). These fractions can be further refined by delicate separation procedures into subdivisions of the VLDL, LDL and HDL fractions. Each of these subfractions has distinctive apolipoprotein compositions and biological properties [1].

Even though the standard methods for lipoprotein classification are based on LPs density (i.e. UC), several methods based on lipid composition and size (diameter) have been devised [12]. This is for example the case for gel electrophoresis and GP-HPLC in which lipoproteins are classified based on size fractionation and charge. Fig. 1 shows the correlation between the relative size of plasma lipoproteins and their hydrated density. The insert in Fig. 1 shows the typical micellar structure of a lipoprotein.

3. Sample handling procedures

Lipoproteins are heterogeneous particles whose distribution in the blood depends on genotype-specific properties and reflects the dynamic response of the human body to changes in the external conditions (e.g. diet, lifestyle and environment) [13]. The multiple sample handling steps required before LPs quantification, such as sample collection, sample preparation, analysis and storage, can alter the LPs structure by destroying the natural equilibrium of the sample. For this reason, high-throughput protocols and Standard Operating Procedures (SOPs) and Quality Control (QC) criteria have been developed for minimizing as much as possible the inherent variability arising from the sample handling steps [14]. Fig. 2 shows a schematic diagram of the blood sample collection and handling for lipoprotein quantification.

Sample collection: fasting vs. non-fasting state. According to the standard protocols, fasting blood samples are required for

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