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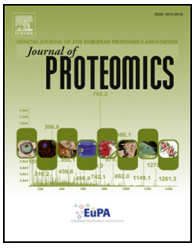

Absolute quantification of apolipoproteins and associated proteins on human plasma lipoproteins

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ABSTRACT

Lipoprotein-associated proteins form an intrinsic part of the major plasma lipoprotein classes. There is increasing evidence that the quantity of these proteins per lipoprotein particle determines lipoprotein function including redox, inflammatory and thrombotic properties and may impact on lipoprotein-related risks for developing heart disease. However, only limited information on the relative quantity of these proteins has been published and no comprehensive absolute quantitative data providing the stoichiometry of proteins associated with lipoproteins is available to date. To address this, we performed extensive absolute quantification by mass spectrometry of 17 lipoprotein-associated proteins on VLDL, LDL, Lp(a) and HDL from healthy subjects. For the first time we show the exact stoichiometry of apolipoproteins on different lipoprotein classes. The most distinct differences were seen in the abundance of all apoCs, apoE and apoF. We further revealed strong variations between individual samples, which indicates the complexity of the protein complement of lipoproteins and can provide additional insights into lipoprotein-related risk factors. This approach has the potential to determine alterations in the protein profiles of lipoproteins in disease states such as CVD or diabetes and, if performed on large cohorts, to translate into a tool for identifying new candidate biomarkers for risk of disease.

Biological significance
A more comprehensive picture about the protein complement on individual lipoprotein classes is the goal of lipoprotein proteomics analyses. Despite many such studies, there is a lack of absolute quantitative data on lipoproteins isolated from individual subjects. The stoichiometry of lipoprotein-associated proteins rather than their presence or absence could provide insights into an individual's predisposition for disease such as heart disease or diabetes. Our study provides a comprehensive overview of the absolute quantity of proteins on the major apolipoprotein classes VLDL, LDL, Lp(a) and HDL.

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1. Introduction

Lipoproteins are the main lipid transport particles in circulation. Very low density lipoprotein (VLDL) is synthesized and secreted by the liver and transformed into the smaller and more cholesterol-rich, low density lipoprotein (LDL) via lipolysis by lipoprotein and hepatic lipases [1–3]. VLDL and LDL deliver triglycerides and cholesterol to the peripheral tissues for lipid and energy metabolism. They both contain one molecule of the major structural protein apolipoproteinB-100 (apoB-100), a 550 kDa glycoprotein, and are therefore referred to as apoB-100-containing lipoproteins. Human lipoprotein(a) [Lp(a)] is similar to LDL but contains an additional large glycoprotein, apolipoprotein(a) [apo(a)], which is attached to apoB-100 by one disulfide bridge. High density lipoprotein (HDL) is the only non-apoB-containing lipoprotein in circulation and has apoA1 as its major structural protein. HDL is mainly involved in transporting lipids from the periphery back to the liver, in a process known as reverse cholesterol transport.

High levels of LDL are associated with an increased risk for CVD, whereas high HDL levels are favorable and implicate a reduced risk [4]. Increased levels of Lp(a) are an established independent and important risk factor for developing premature CVD [5]. Traditionally LDL-total and HDL-total cholesterol levels are used for CVD-risk predictions in patients. However, various studies have shown that particle number rather than cholesterol levels is a much better predictor for CVD [6] and that lipoprotein independent factors can improve the predictive power for CVD. For example, the addition of C-reactive protein measurements in conjunction with cholesterol levels is seen as a simple and inexpensive tool to improve CVD risk prediction [7]. The ratio between apoA1 and apoB-100 has been reported as a better predictor for CVD risk than LDL and HDL cholesterol levels alone [8]. It is likely that other apolipoproteins may serve as additional targets to refine the accuracy of predicting CVD risk in individual patients. The relative abundance of apoCs and apoE declines in the maturation of VLDL to LDL and other apolipoproteins may increase in abundance [9]. As well as structural components, apolipoproteins act as activators or inhibitors of lipases involved in lipolysis i.e. the apoCs [8] or as ligands for receptor-mediated triglyceride clearance in the liver and peripheral tissues i.e. apoE [10]. It is possible that the imbalance of apolipoproteins on specific lipoprotein particles is related to different disease states such as CVD and diabetes.

Proteomic studies of different lipoproteins show that their protein load is much more diverse than originally anticipated with many unexpected or novel proteins (summarized in [11,12]) such as protease inhibitors, acute phase response proteins and proteins involved in the complement pathway and immune functions. This diversity coincides with the multiple functions attributed to lipoproteins beyond lipid transport, e.g. redox regulation, hemostasis, inflammation and immunity in HDL (summarized in [13]). For example, response to wounding is the main biological process attributed to Lp(a)-associated proteins, a link that could explain Lp(a)'s atherogenic and prothrombotic properties within sites of vascular injury through interference with coagulation and certain immune responses [14]. The quantity of lipoprotein-associated proteins may have a strong impact on the function of the respective lipoprotein population.

Despite the great number of lipoprotein proteomic studies published to date, there is a lack of accurate quantitative information about the apolipoproteins and associated proteins on individual lipoprotein particles. The available information is very limited or spans only a small protein dataset [14]. For example, analyses of HDL-associated proteins are mainly of semi-quantitative nature [15,16] or have targeted reconstituted particles [17]. Larger scale quantitative analyses of apolipoproteins and associated proteins have only targeted whole plasma so far [18,19]. A global picture of their absolute amount on the most predominant classes of lipoproteins is still missing.

Here, for the first time we show the exact stoichiometry of apolipoproteins on individual lipoprotein classes. We targeted 17 common apolipoproteins and associated proteins that have been identified by most proteomic studies to date or been attributed with interesting properties in conjunction with lipoprotein function and metabolism (summarized in [11]). We determined their exact stoichiometry per particle for VLDL, LDL, and Lp(a) by high resolution/accurate mass (HR/AM) mass spectrometry using stable isotope labeled standard (SIS) peptides for absolute quantification (AQUA) of proteins.

The exact molar ratio of one apoB-100 per VLDL, LDL and Lp(a) particle allows for the absolute quantification of other lipoprotein-associated proteins against it and determination of their stoichiometry per particle. There is no protein with a constant number of molecules per HDL particle. Intensive work has established the number of apoA1 molecules per particle to range between 2 and 5 [20,21]. To avoid any bias we normalized the measured protein quantities to one apoA1 molecule which does not reflect the number of HDL particles. AQUA of proteins using SIS peptides has been successfully applied to many different types of biological samples such as yeast, mice and human tissues, in vitro systems, human cell lines [22] and Lp(a) particles [14]. Our approach allows for simultaneous quantification of many target proteins in individual samples with high precision [23]. This will be critical for determining alterations in lipoprotein proteomes in larger sample numbers and the comparison between healthy and disease state samples.

2. Material and methods

2.1. Blood sampling

Human plasma was isolated from 16 healthy participants after overnight fasting who had no self-reported personal or family history of cardiovascular disease. This study was approved by the local Ethics Committee and participants gave written informed consent. For the isolation of VLDL, LDL and HDL, we chose donors with low to non-detectable Lp(a) levels (0–3.8 mg/dl, samples 44, 57, 62, 64, 65, 75, 92 and 95) whereas for isolating Lp(a) we chose donors with elevated Lp(a) levels (>30 mg/dl, samples 53, 54, 56, 58, 112, 136, 139 and 141). Table 1 shows the lipid profile for each donor.

2.2. Isolation of lipoproteins

2.2.1. Isolation and purification of VLDL, LDL and HDL

VLDL, LDL and HDL were isolated from individual plasma samples by a combination of sequential density

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