Preβ1-High-Density Lipoprotein in Cardiovascular Diseases

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Abstract

Serum preβ1-high-density lipoprotein (preβ1-HDL) was defined by two-dimensional non-denaturing linear gel electrophoresis and apolipoprotein A-I immunoblotting. However, there are still debatable questions for its quantification and coronary artery disease (CAD) relevance. We have established a novel and simple method for human serum preβ1-HDL quantification. We found that human lower preβ1-HDL is an independent predictor for severer coronary artery stenosis. In this chapter, we will discuss all these.

Keywords

Preβ1-HDL · Preβ1-HDL measurement · HDL · LDL · Cardiovascular diseases

12.1 Regulation of Blood Lipids and Residual Cardiovascular Risks

Atherosclerotic cardiovascular disease is a serious threat to human health [1], but coronary heart disease can be prevented and controlled by regulating blood lipids with basic strategies of reducing the pathogenic effect of risk factors and enhancing the anti-atherosclerotic effect of protective factors. For the primary risk factor of low-density lipoprotein cholesterol (LDL-C), statins and PCSK9 inhibitors can significantly reduce LDL-C levels and stabilize plaque and the incidence of cardiovascular events [2]. However, even if LDL-C is reduced, it is difficult to completely inhibit the progress of atherosclerotic plaque lesions, and the residual risk of cardiovascular disease remains a challenging problem worldwide currently [3].

As we know, reverse cholesterol transport (RCT) is the key mechanism for plaque regression. As a protective factor against atherosclerosis, high-density lipoprotein (HDL) transports excess cholesterol from peripheral cells to the liver for metabolism [4]. Unfortunately, all previous clinical trials of drugs that attempted to increase HDL levels, including cholesteryl ester transfer protein (CETP) inhibitors, did not achieve the expected beneficial results [5]. CETP inhibitors, such as evacetrapib, can significantly increase large HDL particles but fail to effectively reduce cardiovascular endpoint events [6]. Therefore, it is necessary to reconsider the composition and function of HDL [7, 8]. As an extracellular receptor, small particles of HDL, especially nascent HDL (nHDL), promote cellular cholesterol efflux [9], which is an important focus of research in this field.

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12.2 Particle Characteristics of nHDL

Owing to differences in the physical and chemical properties of their constituents, HDL particles possess a complex heterogeneity [10]. Free apolipoprotein A-I (apo A-I) receives cholesterol from cells and initially produces nHDL [11]. This type of particle has the smallest diameter of 5 nm. Because the particle shows preβ1 mobility during gel electrophoresis, it is also known as preβ1-HDL. The preβ1-HDL mainly comprises apo A-I and trace lipids (phospholipids and free cholesterol) at an extremely high density, reaching 1.210 g/mL; in fact this preβ1-HDL particle may be defined as a kind of very high-density lipoprotein (VHDL) [12]. Notably, the lipoprotein particles comprise apolipoprotein and lipid, with a hydrophilic phospholipid-coated surface and a hydrophobic core of lipids such as cholesterol esters and triglycerides. apo A-I exists mainly in the form of lipoprotein particles with lipids, as well as in lipid-free forms, including precursors [13], monomers, and polymers [14]. Although lipid-free apo A-I and preβ1-HDL both exhibit electrophoretic characteristics of preβ1 migration, there is a fundamental conceptual difference between them.

12.3 Detection Methods for nHDL

Based on the physical and chemical characteristics of the particles, the main separation techniques for preβ1-HDL include gel electrophoresis, ultrafiltration, and chromatography. The quantitative methods mainly rely on protein immunity, but quantitative methods of lipid staining have also been reported recently.

The first detection method is protein immunoassay. In 1985, Kunitake [15] obtained lipoproteins containing apo A-I through immunoadsorption. After separation using agarose gel electrophoresis, he first reported that the lipoproteins containing apo A-I exhibited preβ electrophoretic migration characteristics. In 1988, Castro [16] established a method to separation and quantitative detection of apo A-I-containing particles using two-dimensional gel electrophoresis and Western blotting and reported that this two-dimensional polyacrylamide gel electrophoresis could distinguish lipid-free apo A-I (possibly a precursor or monomer) from preβ1-HDL. In 1993, Asztalos [17] improved the two-dimensional electrophoretic separation and used a radioactive 125I-antibody to quantify preβ1-HDL, which has since been widely used for a long time (Boston Heart HDL Map®). In addition, other methods that take advantage of the difference in the particle size of the lipoproteins have been reported in the literature, including ultrafiltration (membrane pore size 100 kDa) [18] and high-performance size-exclusion chromatography [19] for separation and purification of small particles of lipoproteins containing apo A-I (preβ1-HDL or Sm Lp-AI) and quantification of lipoprotein composition by apo A-I antibody immunoassay. In 2000, Miyazaki [20] reported the use of a specific apo A-I monoclonal antibody (MAb55201) to detect plasma preβ1-HDL via ELISA. But in another study in 2014 [21], he found no difference between the electrophoretic migration of preβ1-HDL purified from plasma with MAb55201 and lipid-free apo A-I. Based on the observation that no phospholipid or cholesterol was detected by chemical analysis, this study confirmed that the preβ1-HDL detected in the plasma by immunoblotting was a lipid-free apo A-I monomer. Therefore, to avoid interference from lipid-free apo A-I, the prerequisite for quantitative assessment of preβ1-HDL by protein immunoassay is to completely distinguish the lipid-free apo A-I (including monomers and polymers) or selective recognition through specific antibodies.

The second detection method is lipid staining. Recognition of lipoproteins by lipophilic dyes such as Sudan Black B can completely avoid the interference of lipid-free apolipoprotein. In a previous study, we built the MEDLiPO system and set three layers of polyacrylamide gels at concentrations of 3.0%, 3.6%, and 7.0% as the electrophoretic medium. The serum lipoproteins were prestained by Sudan Black B for electrophoretic separation, and the HDL with the fastest electrophoretic migration showed relatively an

isolated staining band [12]. After analyzing the particle size, density, charge, and chemical composition, it was proved that this isolated fast-moving lipoprotein was the so-called preβ1-HDL. In the study, Sudan Black B was dissolved in a mixed solvent of isopropyl alcohol and ethylene glycol in a volume ratio of 4:1, which improved the stability of lipoprotein staining. Using BeneScan-1000 scanner customized by BENEFI and MICROTEK Technology Co., Ltd (Shanghai, China), the separated gel images and gray scale of the lipid staining were acquired and quantitatively analyzed by measuring the optical density. Then, the absolute content and percentage of total lipid staining of preβ1-HDL were quantified. After repeated experiments, the results showed that the intra- and inter-assay coefficients of variation of serum preβ1-HDL were <5%. The MEDLiPO system was easy to operate and could meet the actual requirements of clinical testing with a unique performance.

12.4 nHDL and Coronary Heart Disease

Most clinical studies have reported an increase in plasma preβ1-HDL levels in patients with coronary heart disease, with a significantly positive correlation [22]. Guey et al. [23] reported that preβ1-HDL was an independent predictor of myocardial infarction. Sethi et al. [24] showed that preβ1-HDL levels in patients with ischemic heart disease (IHD) were twice as high as those in the control group, and the high preβ1- HDL and low activity of lecithin cholesterol acyltransferase (LCAT) were considered risk factors for IHD, independent of HDL-C. Because preβ1-HDL exerts a protective effect by promoting cholesterol efflux from peripheral cells, the main reason for the increase in preβ1-HDL content in patients with coronary heart disease is that the accumulated preβ1-HDL cannot be converted into large particles of mature HDL, leading to reverse cholesterol transport disorders. Under this circumstance, preβ1-HDL accumulates and increases in patients with Tangier disease [25]. The pathogenesis of Tangier disease is owing to a defect in the ATP-binding cassette (ABC) transporter gene *ABCA1* and impaired efflux of cellular cholesterol. Further, ABCA1 deficiency results in the inability of lipid-free apo A-I to receive cholesterol from cells, and theoretically, no preβ1-HDL is formed in this situation. However, immunoquantitative testing showed a controversial result. The MEDLiPO system demonstrated that preβ1-HDL and HDL were missing in the samples obtained from patients with Tangier disease [12]. Very few studies have reported a decrease in plasma preβ-HDL [26] or preβ1-HDL levels in patients with coronary heart disease [12, 27]. The reasons are more related to the detection methods and the differences observed in the included cases.

Using the MEDLiPO system can effectively avoid the interference of lipid-free apo A-I and accurately detect preβ1-HDL by quantitative determination of lipid staining. In 2016, we reported that the MEDLiPO system detected a decrease in serum preβ1- HDL levels in patients with coronary heart disease and the decrease was independently negatively correlated to the degree of coronary stenosis [12]. At the beginning of 2018, we fortunately obtained some blood samples from the ACCENTUATE clinical trial [28]. And using the MEDLiPO system, we found that plasma preβ1-HDL was significantly reduced after treatment with the CETP inhibitor evacetrapib [29]. These results were completely contrary to those of previous reports [30]. The preβ1-HDL reduction could give a clue to understand the failure of CETP inhibitors on cardiovascular outcomes.

12.5 nHDL Particle Reconstruction and Hypothesis

The ABC transporter family mediates free cholesterol efflux from cells [31]. As an acceptor, activated lipid-free apo A-I accepts cell membrane phospholipids and free cholesterol by ABCA1 to form a nascent type of preβ1-HDL particle. This process is the initiation of the reverse cholesterol transport mechanism. ABCA1-dependent cellular cholesterol efflux is

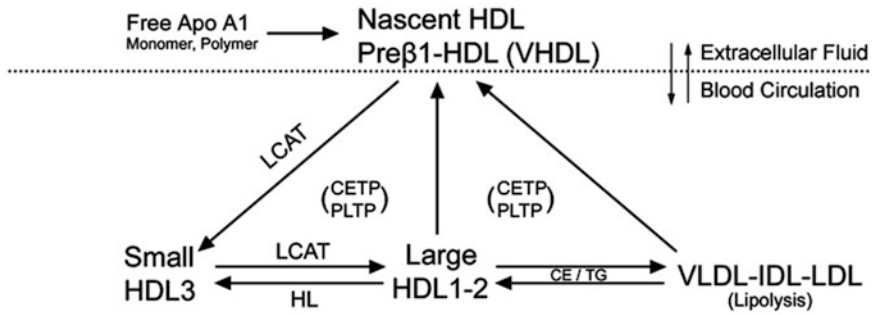

Fig. 12.1 *Generation and reconstruction of nHDL particles.* Extracellular activated apo A-I receives phospholipids and free cholesterol under ABCA1-mediated production of preβ1-HDL. The reconstruction of preβ1-HDL in blood circulation is in a state of dynamic equilibrium. The tissue barrier results in a difference in the composition and metabolism of extracellular fluid and plasma lipoproteins

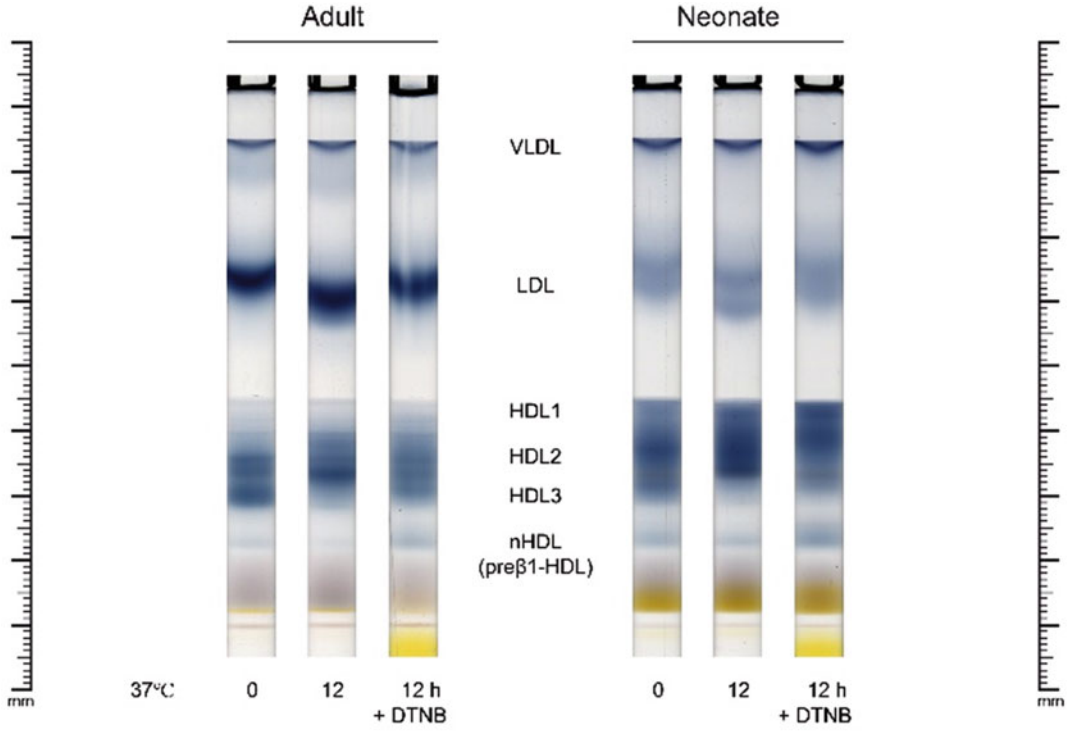

Fig. 12.2 *MEDLiPO system for lipoprotein detection*. Gel separation and quantification of blood lipoproteins through staining with Sudan Black B. 2-nitrobenzoic acid (DTNB), an LCAT inhibitor, inhibits the conversion of nHDL to mature HDL. The production amount of preβ1-HDL was calculated using preβ1-HDL content inhibited by a 37°C water bath for 12 h + DTNB minus the basic value (0 h). The amount of conversion is the value of the production amount plus the net content (12 h). The amount of serum preβ1-HDL produced in the water bath over the 12 h was less than the amount of transformation, and its net content decreased

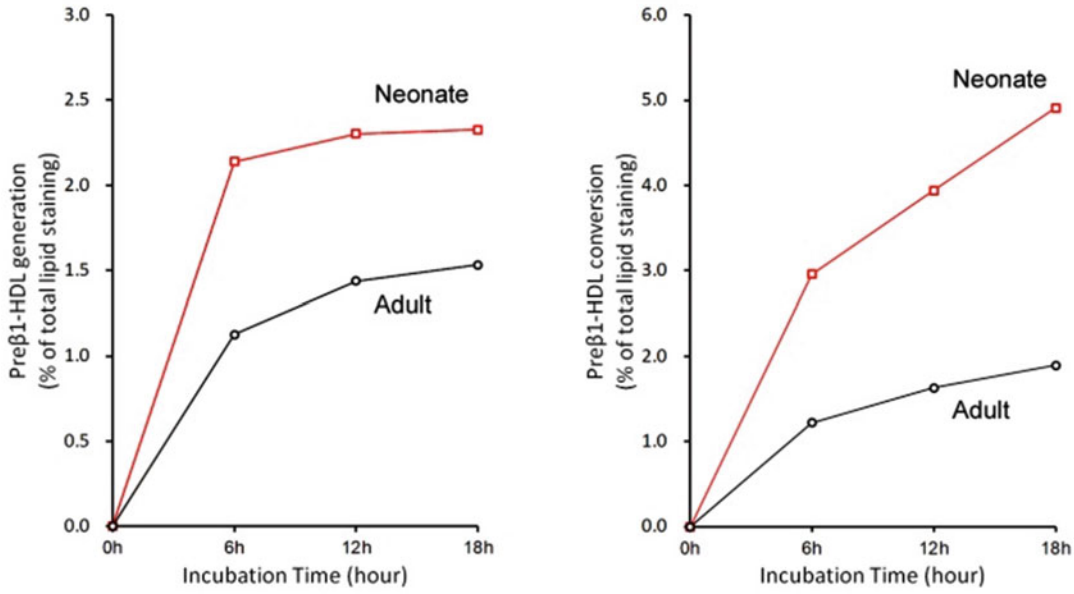

Fig. 12.3 *Reconstruction curve of nHDL particles.* The generation (left) and transformation (right) curves show that serum nHDL particle reconstruction is in a state of dynamic equilibrium. After the water bath experiments, the production and conversion rates of preβ1-HDL were calculated from the base values

a key mechanism by which HDL resists atherogenesis and reverses plaque. If the extracellular apo A-I or cell membrane ABCA1 is mutated or modified, it will cause dysfunction of the cholesterol efflux from the cell and accumulation of the lipid-free apo A-I, making it difficult to produce preβ1-HDL. There is a dynamic balance between production and transformation of preβ1-HDL in plasma or serum (Fig. 12.1). LCAT promotes the esterification of free cholesterol, and HDL is transformed from small particles to large particles. Hepatic lipase catalyzes the hydrolysis of lipids, and large particles of HDL are converted to small particles. Both CETP and phospholipid transporter are involved in lipid transfer between lipoprotein particles and in particle remodeling.

Neonatal umbilical cord blood is rich in preβ1-HDL. In vitro water bath experiments result in the inhibition of LCAT, and the metabolic activity of preβ1-HDL particles remodeling could be measured by detecting the rate of change in the production and conversion of preβ1-HDL (Fig. 12.2). The preβ1-HDL content and metabolic activity in neonatal cord blood are about twice of those in adults (Fig. 12.3). We speculate that preβ1-HDL plays a key role in cholesterol reverse transport and plaque reversal and this protective effect may diminish with age. It has been proposed [32] that blood lipid levels in newborns may be an ideal target for lipid-lowering therapy in patients with coronary heart disease. Neonatal blood lipids are characterized by extremely low LDL-C levels (<1.0 mmol/L) which is lower than HDL-C [33], whereas preβ1-HDL levels as newborns are significantly higher than those in adults. At present, the combined application of statins and PCSK9 inhibitors can achieve extremely low LDL-C levels in most patients with coronary heart disease. Regulating preβ1-HDL levels in newborns and its function of promoting cholesterol efflux from cells may be a promising way to prevent atherosclerotic cardiovascular disease and reduce residual cardiovascular risk in the future.

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