

High-density lipoprotein: Quality is more important than quantity!

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ABSTRACT

High density lipoproteins (HDLs) are complex lipid carriers which replace their own structures and functions according to different conditions including healthy or pathological status. HDL has substantial roles in reverse cholesterol transport system for regulation of lipid metabolism. Also HDL has many functions such as antiinflammation, antioxidation, antithrombotic and vasodilator actions. However, these functions may be compromised under pathological conditions. Nowadays, it's known that the function of HDL is more important than it's dose for preventing against cardiovascular diseases. This review mostly summarizes available information of HDL structure, metabolism and the real information related to the physicochemical changes of HDLs causing their different functions. Also HDLs, that lost their protective functions have been associated with oxidative stress and systemic inflammation diseases.

Keywords: Antiinflammatory, antioxidant, dysfunctional HDL, high-density lipoproteins

1. INTRODUCTION

Lipoproteins carry apolar lipids in the center of their structures including cholesterol esters and triglycerides. Also, they have hydrophilic membrane carrying phospholipids, cholesterol, and apolipoproteins. These lipid carriers are grouped according to their size, apolipoproteins and lipid composition. Lipoproteins are grouped as chylomicrons, chylomicron remnants, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), high density lipoproteins (HDL) and Lp(a). Table 1 shows the classification of lipoproteins and the major lipids and major apoproteins that these lipoproteins contain (Table 1) [1,2].

HDLs, which will be discussed, have different roles such as antiinflammatory, antioxidant and

reverse cholesterol transport. These roles of HDLs can be observed under both physiological and pathological situations. Such as, HDLs undertake antiinflammatory roles in the immune system when inflammatory conditions occurs and change their structures for carrying proinflammatory molecules [3]. This review reports the novel knowledges linking up the relationships between physicochemical characteristics of HDLs and those changes found in the pathological circumstances such as oxidative stress and systemic inflammation.

HDL Structure:

HDLs have complex and heterogeneous structures among other class of lipoproteins by carrying important differences in composition, shape, size and function [4]. HDLs have small (5-17 nm diameter) and most dense (1,063-1,25 g/mL) structures in the

Table 1. Lipoprotein classes

Lipoprotein	Density (g/ml)	Major Lipids	Major Apoproteins
Chylomicrons	<0.930	Triglycerides	Apo B-48, Apo C, Apo E, Apo A-I, A-II, A-IV
Chylomicron Remnants	0.930- 1.006	Triglycerides Cholesterol	Apo B-48, Apo E
VLDL	0.930- 1.006	Triglycerides	Apo B-100, Apo E, Apo C
IDL	1.006- 1.019	Triglycerides Cholesterol	Apo B-100, Apo E, Apo C
LDL	1.019- 1.063	Cholesterol	Apo B-100
HDL	1.063- 1.210	Cholesterol Phospholipids	Apo A-I, Apo A-II, Apo C, Apo E
Lp (a)	1.055- 1.085	Cholesterol	Apo B-100, Apo A

group of lipoproteins. HDLs have different shaped forms such as discoidal (nascent) and spherical (mature).

Nascent HDL consists of 100 to 150 phospholipid molecules in phospholipid bilayer as found in plasma membrane and 2 to 5 Apo A-I molecules creating a band around the lipid structure. Nascent HDL contains approximately 10% of overall circulating HDL. Apo A-I and Apo A-II are the most existing protein contents of the nascent HDL. Numerous phospholipids and few triglycerides, free cholesterol and cholesteryl esters are also found in this unique structure. Nascent HDL is converted into mature HDL, that is the main type of HDL in the blood stream [5]. The structure of these mature HDL particle contains of 3–5% cholesterol, approximately 5% triglycerides, 15–20% esterified cholesterol, 26–32% phospholipids and 45–55% apolipoprotein molecules. The lipid core of HDL is consist of cholesterol esters (CE). Apo A-I and Apo A-II are main apoproteins of this mature HDL and few Apo E and Apo C are also found around of lipid structure. HDL is divided into three classes considering their densities: HDL1, HDL2 and HDL3 which have densities of 1,050-1,063 g/ml, 1,063-1,12 g/ml and 1,12-1,21 g/ml respectively.

Moreover, these classes of HDLs are also consists of five different subclasses such as HDL2 and 2b, HDL3a, 3b, and 3c, according to their size. HDL3 which is the first small and mature form of HDL is the best acceptor of free cholesterol. When the amount of esterified free cholesterol increases, the particle size increases and HDL2 occurs. HDL2 is further enhanced by cholesterol esters and also obtain apoE. This apo E-containing particle (HDL1)

actually constitute a small fraction of HDL but is known as metabolically active subclass [6,7].

Besides, HDL particles comprise some other components with different biological activity such as vitamins, hormones and microRNAs. HDL successfully takes these functional miRs to their target cells. Hence, microRNAs consolidate structure of HDL particles [8].

Apo A-I is an effective molecule that contributes in nearly all known functions of HDL particle. Apo A-I provides the coaction of HDL particle with ATP-binding cassette transporters A1 (ABCA1), G1, scavenger receptor B1 and lecithin cholesterol acyltransferase (LCAT) [9]. These cholesterol-phospholipid transporters expressed in hepatocytes, enterocytes, macrophages and other tissues [10]. The main structure of high-density lipoprotein is shown in figure 1.

HDL Metabolism and Function:

Firstly, Apo A-I is synthesized in the metabolism of HDL particles. Nearly 80% of these Apo A-I molecules are obtained by de novo synthesis in hepatocytes and others secreted by intestinal mucosa (20%). The name of this firstly synthesized nascent protein and phospholipid structure is the form of pre- β 1 HDL. In blood stream, coaction of Apo A-I or pre- β 1 HDL with ABCA1 transporters is an important process for beginning of HDL functions. The Apo A-I also starts activation of LCAT enzyme. These mentioned receptors cause transportation of phospholipids and free cholesterol from peripheral tissues to HDL particle and transformation of initial particles into nascent HDL (α 4 HDL). Cholesteryl esters obtained by LCAT enzyme located with

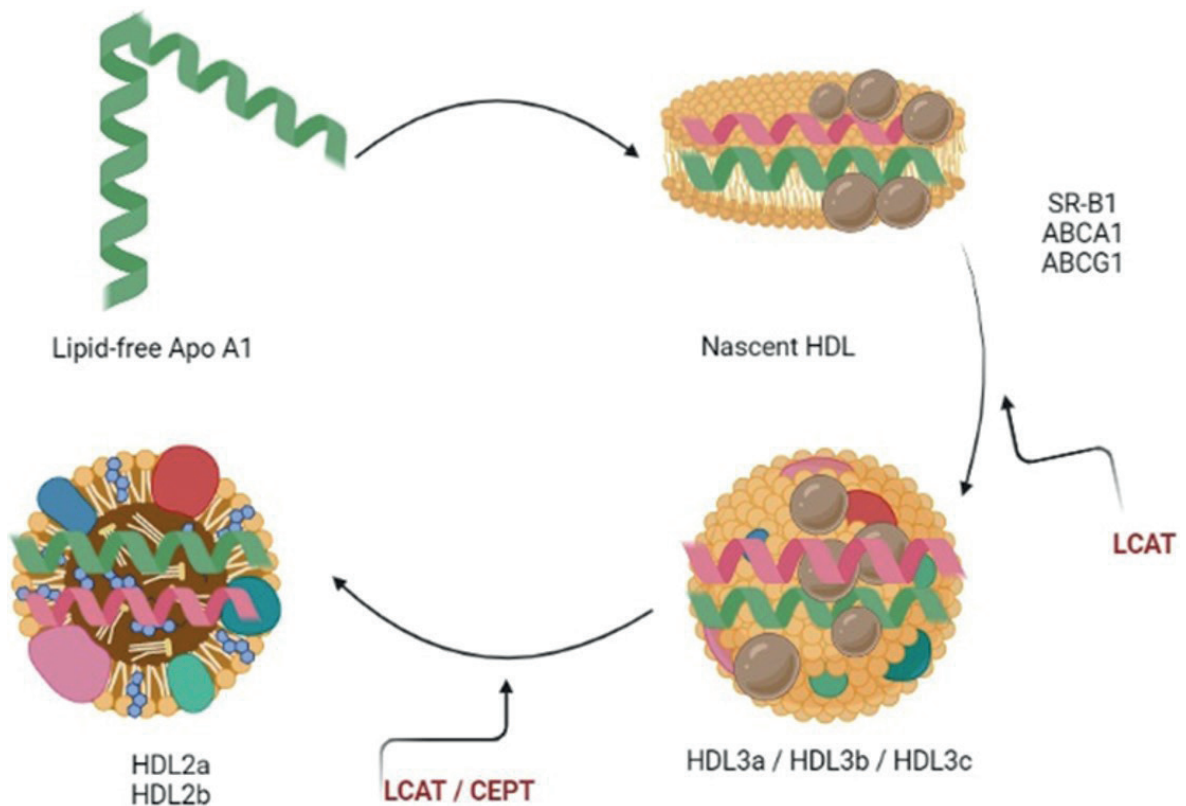


Figure 1. The main structure of high-density lipoprotein

triglycerides in the hydrophobic core of discoidal HDL. Formation of mature HDL structure begins with accumulation of triglycerides and cholesteryl ester. This spherical HDL form is the main particles in circulation. Conformational changes in this Apo A-I are critical for formation of spherical HDL and functions for transport of cholesterol from peripheral tissues to HDL. The spherical HDL is developed by continuous of the LCAT enzyme effect and the activities of lipid transfer proteins. Mature HDL formation is further continued by different proteins such as cholesteryl ester transfer protein (CETP) and the phospholipid transfer protein (PLTP). HDL concentrations and structure are controlled by these proteins in circulation [5]. Another important feature of HDL is that it contains paraoxonase 1 (PON-1) enzyme in its structure [10].

Apo A-I has the HDL associated enzymes PON-1 and platelet activating factor acetylhydrolase (PAF-AH) which suppress LDL oxidation by hydrolyzing oxidized lipids [11]. HDL performs multiple physiological functions. Especially HDL

is a substantial molecule for continuity of whole lipid metabolism [12]. The most important role of HDL is the continuous activity of RCT pathway which removes cholesterol from peripheral tissues to the liver exerting antiatherosclerotic character of this lipoprotein. It is therefore considered an antiatherosclerotic lipoprotein. This process is called the HDL cycle and protects endothelial cells. Actually, HDLs prevent toxic accumulation of cholesterol and cause transformation of cholesterol into bile acids [13]. HDL has roles to interfere with nitric oxide (NO) metabolism because of its preventive effects on endothelial cells and vascular function. Except that Apo A-I, HDL related enzymes and apolipoproteins such as PON-1 and Apo E stimulate eNOS and increase NO production [14]. Antiinflammatory action of HDL is associated with Apo A-I, PON-1 and sphingosine-1-phosphate contents. HDL prevents migration and infiltration of monocytes and macrophages to the arterial wall by inhibiting monocyte chemoattractant protein 1 (MCP 1) which induced by LDL [15].

The other indispensable role of HDL is its antioxidant action. HDL success this action by preventing of lipid oxidation and removing oxidized products. Because of interaction of inflammation and oxidative stress, HDL's antioxidant characteristic contributes to it's antiinflammatory behaviors [6]. Furthermore, HDL has antithrombotic effects because of it's roles on the prostacyclin signaling pathway. It's known that prostacyclins interact with NO to protect against platelet activation and aggregation. HDL also induce the expression of cyclooxygenase 2, that induce prostacyclin synthesis in endothelial cells. Moreover, HDLs protect against apoptosis of endothelial cells by reduction of CPP32-like protease activity, resulting in a decrease in the effect of tumor necrosis factor (TNF- α) [16]. HDL-C physiological functions are shown in figure 2.

To summarize the functions of HDL; HDL transfers apoproteins to other lipoproteins. It takes lipids from other lipoproteins and esterifies cholesterol by the LCAT reaction. By transferring appropriate esters to other lipoproteins, it ensures the transport of cholesterol to the liver. Also, antithrombotic

property of HDL is it's ability to induce the generation of prostacyclin. HDL particles are highly antiatherogenic and inhibit endothelial cell apoptosis and prevents against LDL oxidation.

What conditions make HDL go bad?

HDLs obtained from healthy people have frequently antiinflammatory characteristics. On the other hand, when systemic inflammation occurs in organism, HDL can turn into proinflammatory form as part of an acute phase response. Coronary atherosclerosis, surgery, diabetes mellitus, influenza, sepsis, chronic systemic inflammation are a good examples of this effect [17].

Proinflammatory properties of HDL

HDL has an antiinflammatory effect through two mechanisms. 1) Normal HDLs protect LDL-C against oxidation. Then it decrease attraction of monocytes into arterial tissue and occurrence of foam cells that caused by oxidized LDL. 2) Normal HDLs carry out reverse cholesterol transport by removing cholesterol from peripheral tissues.

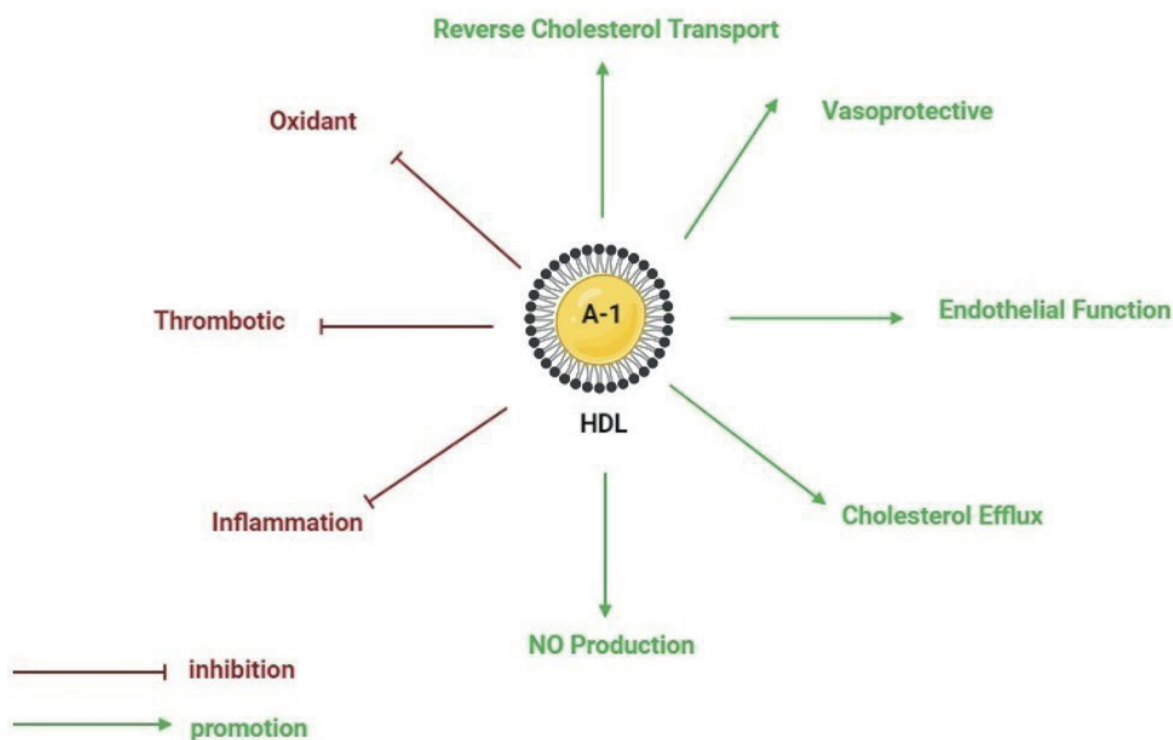


Figure 2. Physiological functions of HDL-C

HDLs have some antioxidant enzymes to work the continuity of an antiinflammatory statement. More than twenty years ago, it was shown that HDL can be in proinflammatory state during an acute phase response in humans and animal after a surgery [18].

During systemic inflammatory state antioxidants can decrease and HDL can increase oxidized lipids and proteins that enhance its proinflammatory state. Dysfunctional HDL causes releasing of proinflammatory cytokines that evoke transportation of monocytes into arterial walls, which eventually causes to occurrence of macrophage accumulation [19]. Atherosclerosis, in its simplest form, is accumulation of oxide LDL-C and triglycerides in peripheral vascular cells. ABCA1 and ABCG1 have roles in prevention and regression of atherosclerosis by removing of excess oxidized cholesterol from macrophages and forming of normal high density lipoprotein [20].

Dysfunctional HDL is also created by myeloperoxidase (MPO). MPO, released from macrophages in atherosclerotic lesions, causes oxidative damage to Apo A-I. It's known that HDLs isolated from the atherosclerotic lesions contain in large quantities of MPO modified proteins such as nitrated and chlorinated Apo A-I [21].

Pro-oxidant properties of HDL

HDL may incorporate lipid peroxidation products and phospholipid containing hydroperoxides that given by oxidized LDL. Furthermore, HDL has roles in hydrolyzing oxidized phospholipids, such as F2-isoprostanes, formed during the oxidative modification of LDL [22]. On the other hand, oxidized HDL may induce the production of reactive oxygen species (ROS) and enhance the risk of cardiovascular diseases by upregulating expression of some proinflammatory genes such as tumor necrosis factor alfa, cyclooxygenase 2 and plasminogen activator inhibitor-1 (PAI-1) [23]. In addition to all this, the enrichment of HDL with triglycerides, MPO, phospholipase A2, ceruloplasmin and serum amyloid A decrease its antioxidant activity and cause the formation of prooxidant HDL [24]. It was shown that oxidized HDLs can not carry out cholesterol efflux from foam cells [25-28].

Methods of HDL Measurement in the Laboratory

Some *in vitro* and *ex vivo* reproducible methods were developed to determine HDL's heterogeneity and various functions. Therewithal detection of the presence of dysfunctional HDL in the patient will help to change the treatment process positively [24]. Standard method for measuring HDL-C; It is the precipitation of lipoproteins using a polyanion such as heparin $MnCl_2$, phosphotungstate $MnCl_2$, dextran sulfate $MnCl_2$ or polyethylene glycol, and then determining the cholesterol value by an enzymatic colorimetric test. These methods have limitations regarding insufficient precipitation of Apo B lipoproteins and assay environment that influence HDL-C measurement. These insufficiencies could produce inaccurate HDL-C measurement for the correct define of cardiovascular risk. On the other hand, electrophoresis, ultracentrifugation, chromatography and nuclear magnetic resonance can be seen more purified methods to separate HDL-C in plasma [29]. But this methods have also disadvantages because of requirement of specialized equipment or performing and learning of the methodology is difficult. Hence, the colorimetric methods are used to measure of HDL composition [30].

The increases or decreases in the change in fluorescence intensity (FI) caused by dichlorofluorescein diacetate (DCFHDA) oxidation can be measured to define the functional characteristics of HDL rather than quantity.

This method measure the capability of various doses of HDL to reduce the generation of intracellular H_2O_2 as reflected by the decrease in the generation of FI by DCF [31] (Figure 3).

2. CONCLUSION

The heterogeneity in the structure and content of HDL is the source of its functional diversity and importance. HDL components and their activity especially in the disease state undergoing significant modifications dynamically change. Such dysfunctional HDL particles appear as both a cause and a symptom of many diseases, especially atherosclerosis. Nowadays, routine lipid measurements in laboratories have

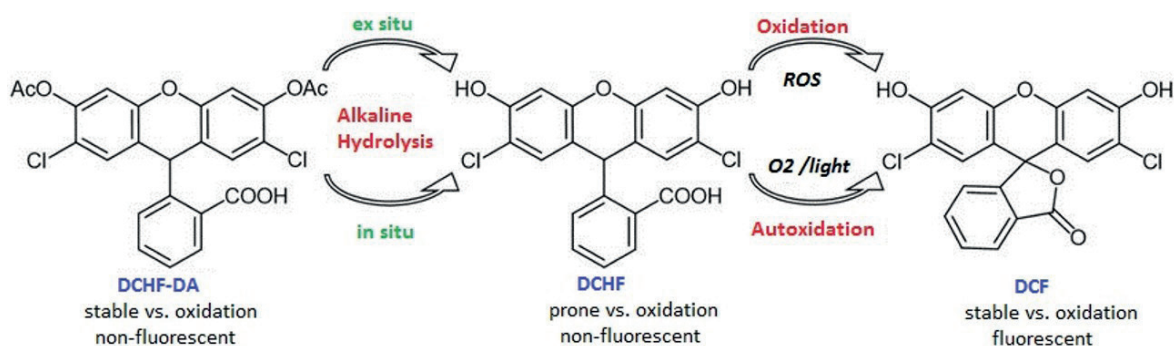


Figure 3. Oxidation of dichlorofluorescein diacetate

become a misleading indicator. In the light of current information, when assessing HDL in the body, it is clear that it is essential to make a qualitative analysis rather than a quantitative one.

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Author contribution

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Conflict of interest

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