Review

Assessing High-Density Lipoprotein: Shifting Focus from Quantity to Quality in Cardiovascular Disease Risk Assessment

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Abstract: High-density lipoprotein cholesterol (HDL) has long been regarded as a protective factor against cardiovascular disease (CVD). However, recent research challenges this notion, suggesting that HDL functionality rather than its quantity may be a more accurate predictor of CVD risk. While epidemiological studies have traditionally found that higher HDL levels are associated with reduced CVD risk, intervention trials aiming to elevate HDL levels have yielded inconsistent results. Moreover, observational studies have reported that unusually high HDL levels are associated with increased mortality rates. These discrepancies underscore the complexity of the role of HDL in CVD. Reverse cholesterol transport, facilitated by HDL, plays a crucial role in preventing atherosclerosis by removing cholesterol from peripheral tissues. Additionally, HDL exhibits anti-inflammatory properties by inhibiting endothelial adhesion molecules and suppressing pro-inflammatory cytokines. Recent studies have highlighted the importance of HDL particle number, size, and functionality in assessing CVD risk. For instance, increased HDL particle number and larger particle size have been associated with reduced CVD risk, independent of HDL cholesterol levels. Furthermore, HDL’s cholesterol efflux capacity has emerged as a promising biomarker for predicting CVD risk, with higher efflux capacity correlating with lower CVD incidence and mortality. This article reviews the latest findings regarding the role of HDL in CVD risk assessment, emphasizing the need to focus on HDL quantity and HDL quality.

Keywords: HDL; cholesterol; cardiovascular disease; HDL dysfunction; HDL quality

1. Introduction

It is widely recognized by both the general population and the scientific community that increased high-density lipoprotein cholesterol (HDL) is associated with risk for cardiovascular disease (CVD) [1]. The negative correlation between HDL and CVD has been established through epidemiological research with researchers reporting that increasing plasma levels of HDL are associated with a decline in cardiovascular events [2]. Two large longitudinal studies, the Framingham Heart Study and the Prospective Cardiovascular Münster (PROCAM) study, have reported a negative association between HDL and CVD [3]. However, it should be noted that multiple intervention trials have yielded evidence to support reduction in CVD events, with others suggesting that HDL may not always provide a protective effect [4]. In addition, authors of observational studies have reported that unusually high levels of HDL are associated with a higher death rate from CVD events [5]. The researchers have also observed that there was no protective effect of genetically enhanced HDL in lowering CVD events [5]. Study authors have reported a number of associations between HDL functions and the risk of CVD [1], with the most recent discoveries indicating both the protective and harmful impacts of HDL on CVD underscoring the growing necessity for a comprehensive and current understanding of HDL. Scientists are exploring the diverse roles of HDL, focusing on its protective effects as well as potential adverse impacts on CVD and related disease mechanisms [2]. All these findings raise the question of evaluating the quality of HDL rather than the quantity.
This review aims to present a concise overview of the most recent research advances addressing HDL. It will address an understanding of HDL, its impact on disease and a discussion regarding measuring HDL quantity and quality with a view to enabling researchers and clinicians to identify areas of opportunity for further investigation and treatment.

2. Origins of HDL

The genesis of HDL begins with the formation of Apolipoprotein A1 (APOA1), which is formed in the liver and gut [6], exhibiting a low lipid content [7]. Hepatocytes possess ATP-binding cassette transporter A1 (ABCA1) on their surface [8]. APOA1 interacts with ABCA1, followed by APOA1 acquiring cholesterol and phospholipids from cell sources, and this process creates a new HDL particle with a discoid shape, which is usually called pre-beta HDL [9]. The circulating HDL collects free cholesterol and phospholipids from chylomicrons, very low-density lipoproteins (VLDL), apolipoproteins, and peripheral tissues [10]. Lecithin cholesterol acyltransferase (LCAT) interacts with the surface cholesterol of HDL to generate cholesteryl ester [9]. This cholesteryl ester then accumulates in the core of the HDL molecule, leading to the transformation of the discoid-shaped HDL into a spherical form [11].

3. Composition of HDL

HDL is a diverse and complex spherical lipoprotein that is composed of a combination of proteins, lipids, microRNAs (miRNA), and metabolites [12]. This lipoprotein size varies from 6.5 to 15 nm and has the highest density (1.063–1.21 g/mL) in comparison to other cholesterol molecules [12], with half of the HDL mass comprised of protein [13]. Apolipoprotein A II (ApoA-II) constitutes 15–20% of total HDL protein and is the second most prevalent HDL apolipoprotein, followed by apoA-IV, the C apolipoproteins, apoE, and apoM [2]. Approximately 110 proteins have been identified in HDL and are categorized into various groups according to their function. These include enzymes, lipid transfer proteins, proteinase inhibitors, acute phase response proteins, and complement components [12]. The enzymes lecithin–cholesterol acyltransferase (LCAT), cholesterol ester transfer protein (CETP), and phospholipid transfer protein (PLTP) play a pivotal role in the maturation of HDL [13]. Furthermore, two enzymes, paraoxonase-1 (PON) and platelet-activating factor acetyl hydrolase (PAF-AH), are associated with HDL and play a significant role in defining the biological functions of HDL [14]. PON and PAF–AH degrade platelet-activating factor (PAF) and exhibit antioxidant and anti-inflammatory properties [13].

Lipids, comprising approximately 50% of HDL’s overall mass, also regulate the function of other lipoproteins, including HDL [13]. These lipids include phospholipids, free cholesterol, cholesterol esters, and triglycerides [9]. Specifically, phospholipids constitute 36% to 40% of the total lipid content, while glycerophospholipids contribute 35% to 50% of the overall HDL lipid composition [15]. Additionally, sphingolipids constitute 5% to 10% of HDL lipids. Notably, phosphatidylcholine (PC) and sphingomyelin (SM) emerge as the principal glycerophospholipids in HDL [15]. Additionally, HDL extracted from healthy normolipidemic adults contains over 200 known lipid compounds [16] with cholesterol being the most measured lipid due to its potential role as an independent negative risk factor for CVD [13]. Major components of the HDL molecule are summarized in Table 1.

Table 1. Major Components of an HDL molecule.

<table>
<thead>
<tr>
<th>Major Components of HDL</th>
<th>Protein</th>
<th>Enzymes</th>
</tr>
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<tbody>
<tr>
<td>Apolipoprotein</td>
<td>ApoA-I, ApoA-II, ApoA-IV, C apolipoproteins, ApoE, ApoM etc</td>
<td></td>
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<tr>
<td>Enzymes</td>
<td>lecithin–cholesterol acyltransferase (LCAT), cholesterol ester transfer protein (CETP), and phospholipid transfer protein (PLTP), paraoxonase-1 (PON), platelet-activating factor acetyl hydrolase (PAF-AH) etc.</td>
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Table 1. Cont.

<table>
<thead>
<tr>
<th>Major Components of HDL</th>
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<tbody>
<tr>
<td>Free Cholesterol</td>
</tr>
<tr>
<td>cholesterol esters</td>
</tr>
<tr>
<td>Lipids</td>
</tr>
<tr>
<td>Glycerophospholipids: Phosphatidylcholine (PC) and sphingomyelin (SM)</td>
</tr>
<tr>
<td>Triglycerides</td>
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<tr>
<td>Phospholipid</td>
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<tr>
<td>MicroRNAs (miRNA) and metabolites</td>
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</table>

4. HDL Sub-Classes

HDL is heterogenous in its density and composition [17]. Scientists have used different techniques, including ultracentrifugation, gradient gel electrophoresis, denaturing polyacrylamide gel electrophoresis, and nuclear magnetic resonance (NMR), for separating distinct HDL subgroups [18]. The categorization and nomenclature of HDL subclasses demonstrate heterogeneity based on the particular analytical methodologies utilized, whereas each subclass includes distinct subpopulations [19]. Ultracentrifugation categorizes HDL into two subgroups, HDL2 (1.063–1.125 g/mL) and HDL3 (1.125–1.21 g/mL), based on density [19]. The gradient gel electrophoresis technique classifies the HDL subclasses into five groups according to their particle size. These include HDL3c with a diameter of 7.2–7.8 nm, HDL3b with 7.8–8.2 nm, HDL3a with 8.2–8.8 nm, HDL2a with 8.8–9.7 nm, and HDL2b with 9.7–12.0 nm [20]. Agarose gel electrophoresis can divide the HDL-C into α-migrating particles and preβ-migrating particles based on their surface charge [20]. Nuclear magnetic resonance (NMR) can categorize HDL subclasses into three groups: large HDL (8.8–13.0 nm diameter), medium HDL (8.2–8.8 nm), and small HDL (7.3–8.2 nm) [21]. High-performance liquid chromatography (HPLC) using gel permeation columns can also classify HDL into five sub-groups, which correspond to the categorization obtained through NMR [22]. 2D gel electrophoresis has revealed five prominent HDL particles, which are as follows: (a) pre-β-1 HDL, a very small discoidal precursor with a diameter of about 5.6 nm; (b) α-4 HDL, a very small discoidal particle with a diameter of about 7.4 nm; (c) α-3 HDL, a small spherical particle with a diameter of about 8.0 nm; (d) α-2 HDL, a medium-sized spherical particle with a diameter of about 9.2 nm; and (e) α-1 HDL, a large spherical particle with α mobility [23]. Subclasses of HDL identified by major analytical techniques are summarized in Table 2.

Table 2. HDL subclasses based on different methods of separation.

<table>
<thead>
<tr>
<th>Ultracentrifugation</th>
<th>Gradient Gel Electrophoresis</th>
<th>Nuclear Magnetic Resonance</th>
<th>2-D Gel Electrophoresis</th>
<th>Agarose Gel Electrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL2</td>
<td>HDL2b 9.7–12 nm</td>
<td>Large 8.8–13 nm</td>
<td>pre-β-1 HDL 5.6 nm</td>
<td>α-migrating particles</td>
</tr>
<tr>
<td>HDL3</td>
<td>HDL2a 8.8–9.7 nm</td>
<td>Medium 8.2–8.8 nm</td>
<td>α-4 HDL 7.4 nm</td>
<td>preβ-migrating particles</td>
</tr>
<tr>
<td></td>
<td>HDL3a 8.2–8.8 nm</td>
<td></td>
<td>α-3 HDL 8.0 nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HDL3b 7.8–8.2 nm</td>
<td></td>
<td>α-2 HDL 9.2 nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HDL3c 7.2–7.8 nm</td>
<td></td>
<td>α-1 HDL large spherical</td>
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</tbody>
</table>

HDL is heterogenous in its density and composition [17]. Scientists have used different techniques, including ultracentrifugation, gradient gel electrophoresis, denaturing polyacrylamide gel electrophoresis, and nuclear magnetic resonance (NMR), for separating distinct HDL subgroups [18]. The categorization and nomenclature of HDL subclasses demonstrate heterogeneity based on the particular analytical methodologies utilized, whereas each subclass includes distinct subpopulations [19]. Ultracentrifugation categorizes HDL into two subgroups, HDL2 (1.063–1.125 g/mL) and HDL3 (1.125–1.21 g/mL), based on density [19]. The gradient gel electrophoresis technique classifies the HDL subclasses into five groups according to their particle size. These include HDL3c with a diameter of 7.2–7.8 nm, HDL3b with 7.8–8.2 nm, HDL3a with 8.2–8.8 nm, HDL2a with 8.8–9.7 nm, and HDL2b with 9.7–12.0 nm [20]. Agarose gel electrophoresis can divide the HDL-C into α-migrating particles and preβ-migrating particles based on their surface charge [20]. Nuclear magnetic resonance (NMR) can categorize HDL subclasses into three groups: large HDL (8.8–13.0 nm diameter), medium HDL (8.2–8.8 nm), and small HDL (7.3–8.2 nm) [21]. High-performance liquid chromatography (HPLC) using gel permeation columns can also classify HDL into five sub-groups, which correspond to the categorization obtained through NMR [22]. 2D gel electrophoresis has revealed five prominent HDL particles, which are as follows: (a) pre-β-1 HDL, a very small discoidal precursor with a diameter of about 5.6 nm; (b) α-4 HDL, a very small discoidal particle with a diameter of about 7.4 nm; (c) α-3 HDL, a small spherical particle with a diameter of about 8.0 nm; (d) α-2 HDL, a medium-sized spherical particle with a diameter of about 9.2 nm; and (e) α-1 HDL, a large spherical particle with α mobility [23]. Subclasses of HDL identified by major analytical techniques are summarized in Table 2.
It should be noted that all methods of HDL classification have strengths and weaknesses, therefore subclasses are not uniform among the different techniques. As such, the relationship between HDL subgroup data and CVD generated by various analytical techniques is minimal [19]. This creates a significant challenge in establishing an association between disease and HDL subgroups [18].

In response to this challenge, Rosenson et al. [23] meticulously examined various techniques and synthesized their findings. They introduced a systematic nomenclature that categorizes HDL into five distinct subgroups: very large, large, medium, small, and very small HDL [23]. Still, scientists are looking for the best acceptable method to identify HDL subclasses.

5. HDL Functions

HDL has demonstrated several potential protective effects, supported by several experimental studies [24–28]. The key functions of HDL include Cholesterol Efflux Capacity (CEC), antioxidative activity, antithrombotic activity, antiapoptotic activity, and anti-inflammatory activity [12].

6. Cholesterol Efflux and Reverse Cholesterol Transport

The key function of HDL is to facilitate reverse cholesterol transport, allowing cholesterol to move from peripheral tissues back to the liver [29]. Reverse cholesterol transport is of the utmost importance in the prevention of atherosclerosis since it enables the efficient removal of cholesterol from the walls of arteries, where it tends to accumulate [13], cause inflammation and is associated with CVD and myocardial infarction [30]. Apo A-I is produced by the intestine and the liver and collects cholesterol and phospholipids from peripheral cells by passive diffusion [31]. HDL capacity to promote the uptake and transfer of cholesterol from foam cells within atherosclerotic plaques back to the liver and bile underscores its role in combating atherogenesis and inflammation [32].

7. Anti-Inflammatory Functions

Inflammation accelerates the process of atherosclerosis, with findings from the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) [33] and the Colchicine Cardiovascular Outcomes Trial (COLCOT) [34] reporting that inflammation significantly enhances atherosclerosis outcomes [3]. Chronic inflammatory diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and inflammatory bowel disease (IBD), have been reported to be linked to a greater chance of developing CVD [35].

HDL stimulates the production of nitric oxide through the activation of nitric oxide synthase (eNOS), a protein that promotes the relaxation of blood arteries and inhibits the function of endothelial adhesion molecules [36]. This, in turn, prevents the movement of white blood cells across the vascular wall [37], with HDL inhibiting adhesion molecules (VCAM1) and induced cellular adhesion molecules (ICAM1) [38]. Apolipoprotein A-I inhibits the synthesis of many pro-inflammatory molecules, such as tumor necrosis factor-alpha (TNF-alpha) and interleukin 1, and also suppresses the activity of activated neutrophils [37]. HDL also has an anti-apoptotic function, shielding macrophages from apoptosis triggered by oxidized LDL [35].

8. Anti-Oxidative Functions

High levels of LDL cholesterol have been associated with a greater risk of CVD [3]. The process of atherosclerosis is bolstered by oxidized low density lipoprotein (OxLDL) [24]. In healthy middle-aged men, high OxLDL levels are associated with a 4× greater risk of developing coronary heart disease [39]. HDL can prevent the oxidation of LDL in various ways [37] through enzymes, including paraoxonase 1 (PON1) and Lecithin–cholesterol acyltransferase (LCAT), which can inhibit the oxidation of LDL [40]. HDL inhibits reactive oxygen species, which can cause oxidative stress and damage to the endothelium and prevents apoptosis in endothelial cells [37]. HDL also helps make new blood vessels and
repairs the endothelial barrier. Finally, HDL stops the loss of the glycocalyx layer that covers endothelial cells and makes the endothelial barrier stronger [37].

The fluidity and protein content of HDL can also affect the inhibition of oxidation of LDL by HDL [41]. Apolipoprotein A-I and Apolipoprotein M in HDL are essential for inhibiting the oxidation of LDL [42].

9. Antithrombotic Activity

HDL prevents blood clot formation in several ways [12]. Platelets loaded with cholesterol are more prone to clot formation, while HDL can help maintain the optimal cholesterol level in platelets [43]. HDL interacts with platelet HDL receptors, including apoER2 and the scavenger receptor class B type I (SR-BI) and this changes the signaling pathways in platelets and keeps them from becoming too active [43]. HDL increases activated protein C (APC) activity, where APC prevents coagulation [13]. Nitric oxide and prostacyclin are both produced by endothelial cells in response to HDL stimulation and these two substances are capable of inhibiting platelet activity [13].

10. Shifting the Focus from HDL Quantity to HDL Quality

Numerous epidemiological studies have documented a negative association between low HDL cholesterol levels and adverse cardiovascular outcomes [4,33,44–47], with selected studies listed in Table 3. When LDL is adequately maintained with a statin or through diet and exercise, HDL remains a reliable indicator for the future incidence of CVD events in populations [29]. However, attempts to mitigate the risk by elevating HDL cholesterol levels using drugs and infusion have been ineffective in reducing CVD events [2]. Several study authors have demonstrated that having a very high level of HDL has been associated with an elevated risk of mortality from all causes including CVD [26,28,32,48,49]. Observational and genetic research have established a correlation between individuals with significantly low levels of HDL and the occurrence of several non-cardiovascular diseases, such as infections, autoimmune disorders, cancer, type 2 diabetes, kidney diseases, and pulmonary diseases [50]. Studies in human genetics have reported that extremely low or high levels of HDL do not act either as a risk factor nor a protective factor for CVD. This could indicate that the quantity of HDL alone is not the determining factor for the development of CVD [4].

Different clinical trials [25,27,51], genetic studies, and epidemiological studies [7,26,27,52–54] have reported conflicting and paradoxical results that suggest an explanation of these differences is warranted. A plausible explanation is that HDL quality and functionality, rather than the level of HDL cholesterol, may be more predictive of CVD outcomes [54]. Previous and more recent studies are emerging that suggest HDL particle number and size rather than HDL quantity only is warranted [13]. HDL particles have varying quantities of apolipoprotein A-I (apoA-I) molecules per particle, ranging from 2–5, which can be quantified using nuclear magnetic resonance (NMR) spectroscopy [29]. Studies have demonstrated that the number of HDL particles, and the density, rather than HDL quantity only, has an inverse association with coronary artery disease [29].

The findings of a recent meta-analysis indicate that a high particle number for HDL is associated with a reduction in the risk of cardiovascular disease incidence [55], with a larger HDL particle providing more protection [55]. It is important to keep in mind that the typical clinical measurement of HDL predominantly reflects the levels of large HDL, rich in cholesterol [13].
<table>
<thead>
<tr>
<th>Author, Journal, Year, Country</th>
<th>Type of Study</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liu et al. [56], Jama Cardiology, 2022.</td>
<td>Multicenter Cohort study. The study included patients with CAD, 14,478 patients (median follow-up of 8.9 years), and 5467 patients (median follow-up of 6.7 years).</td>
<td>A U-shaped association of HDL with all-cause mortality was found. Both low and very high HDL levels (&gt;80 mg/dL) were associated with higher risk than normal (40–60 mg/dL).</td>
</tr>
<tr>
<td>Hamer et al. [57], Arteriosclerosis, Thrombosis, and Vascular Biology, 2018, United Kingdom.</td>
<td>A population-based observational study. Adult participants (N = 37,059) were recruited from the Health Survey for England and Scottish Health Survey.</td>
<td>A U-shaped association was found with all causes of mortality, and risk was not attenuated with the highest and lowest category of HDL.</td>
</tr>
<tr>
<td>Oh et al. [58], Atherosclerosis, 2019, South Korea.</td>
<td>Observational Study. The research incorporated 365,457 participants who were part of the Korean National Health Insurance Service–National Sample Cohort.</td>
<td>An extremely high HDL level was associated with an increased risk of all-cause mortality.</td>
</tr>
<tr>
<td>Hirata et al. [59], Journal of Clinical Lipidology, 2018, Japan.</td>
<td>A pooled analysis of observational cohort studies. It included 43,407 participants from 9 Japanese Cohort Studies.</td>
<td>Significant associations have been established between elevated levels of HDL and an increased susceptibility to mortality resulting from cardiovascular disease, coronary heart disease, and ischemic stroke.</td>
</tr>
<tr>
<td>Le et al. [60], The Journal of Clinical Endocrinology and Metabolism, 2019, United States.</td>
<td>Prospective cohort study. A total of 7756 elderly individuals were enrolled who had a median follow up of 5.9 years.</td>
<td>HDL concentrations &lt;61 mg/dL and &gt;87 mg/dL. These two-groups showed significantly higher risk for all-cause mortality than those who had 61 to 87 mg/dL HDL levels.</td>
</tr>
<tr>
<td>Zhong et al. [61], European Journal of Preventive Cardiology, Country (US, UK, Japan, Canada, Denmark, Iran, Peru, Israel, South Korea, Turkey).</td>
<td>Thirty-seven prospective cohort studies were analyzed. The study sample size was 3,524,505 participants.</td>
<td>The j-shaped association was found between HDL level and mortality from all causes, including CVD and cancer. The risk was higher in both low- and high-HDL groups.</td>
</tr>
<tr>
<td>Liu et al. [62], The American Journal of Cardiology, 2022, UK.</td>
<td>Prospective cohort study, Number of participants are 415,416 without CAD, median follow up was 9 years.</td>
<td>No significant difference was found in the risk of cardiovascular mortality between women with high (&gt;60 and &lt;80 mg/100 mL) or very high levels of HDL (&gt;80 mg/100 mL) and those with normal HDL levels. Men showed two-fold higher risk of CVD death after adjusting for confounding factors.</td>
</tr>
<tr>
<td>Yi et al. [63], European Journal of Preventive Cardiology, 2022, Republic of Korea.</td>
<td>Prospective cohort study, mean follow up was 8.8 years. Sample was 15,859,501 Korean Adults with no CVD or cancer.</td>
<td>Both high and low HDL were significantly associated with increased CVD mortality.</td>
</tr>
</tbody>
</table>

A prospective cohort study including 402,783 participants followed for a median of 12.1 years reported a “U” shaped association between Apo-A1 levels and both cardiovascular and all-cause mortality [64]. Patients with the highest levels of ApoA1 were associated with higher cardiovascular and all-cause mortality [64]. Another study from China reported that Apo-A1 level is inversely associated with inflammatory biomarkers, including hs-crp, TNF-alpha, and Interleukin-1 [65].

Additionally, Li et al. [60] conducted a clinical trial of 3553 patients reporting that higher HDL levels were not necessarily associated with better clinical outcomes in patients with various diseases. Study authors reported that c-reactive protein and other inflammatory cytokines may play an increasingly important role in relation to diabetes and CKD, suggesting that HDL dysfunction may be associated with these disease states, and therefore higher levels may not offer a protective effect.
Another study \[52\] reported sex specific counterintuitive findings in HDL levels. In a study of 38,377 patients with non-dialysis CKD patients, those with HDL levels below 30 mg/dL and between 31–40 mg/dL were associated with increases in cardiovascular mortality. Levels above 60 mg/dL were associated with decreases in cardiovascular disease in women only, and men did not have a protective effect with higher levels of HDL. This suggests that the U-shaped mortality curve with HDL may not be extended to males.

During a median follow-up period of 9.4 years, 2924 participants were assessed for CEC along with HDL cholesterol and HDL particle number in another study \[64\]. Participants in the upper quartile of CEC showed a 67% decrease in the risk of CVD compared to those in the lower quartile, even after adjusting for traditional risk factors, including HDL cholesterol level and HDL particle concentration \[66\]. Another study reveals that increasing HDL cholesterol efflux capacity while conserving the same HDL level was associated with lower mortality in heart failure patients, even after adjustment for known risk factors \[67\].

Several intervention studies used various medications for therapeutic purposes to control cholesterol levels and increase HDL \[68\], \[69–71\]. It should be noted that studies that used medications to increase HDL levels failed to reduce cardiovascular disease risk even with increasing HDL levels. One study used HDL infusions (reconstituted HDL or rHDL), which did not reduce coronary artery atherosclerosis in statin-treated patients but showed some promising beneficial effects in those who were not on statins \[72\]. Borja \[69\] et al. followed prospectively 56 patients while supplementing niacin and omega-3 fatty acids for 16 weeks. Functional changes as measured by changes in HDL-apolipoprotein A-1 exchange occurred in HDL levels, with the authors reporting that measuring HDL function may play an important role in the discovery of novel therapies to decrease CVD.

According to recent studies \[73–75\], HDL should no longer be considered a protective factor or a risk factor for CVD based on quantity only. Consequently, attention is directed towards the quality of HDL as measured by size, number of particles, anti-inflammatory and anti-thrombotic activity, as well as enzymatic activity. The functional quality of HDL is an emerging valuable biomarker that goes beyond simply measuring HDL quantity.

As the discussion of HDL quality continues to be debated \[76\], most consider important factors that are beyond the simple approach of the amount of cholesterol in HDL, but should be inclusive of size, composition, oxidation and glycation of HDL. Normally, larger HDL particles have more cholesterol content, and a higher triglyceride content is associated with smaller HDL particles. The less protective CVD effect from HDL has been associated with HDL particles that contain more triglycerides, less apoA-1, are more glycated and are smaller in size \[77\]. Additionally, though age is associated with increases in CVD, there is evidence that age may play a role in HDL quality, with older individuals having HDL that is more oxidized and glycated \[78\].

Terms that are used often in attempting to understand the quality of HDL dysfunctional HDL or HDL functionality. The functionality of HDL is normally associated with the ability to provide antioxidative and efflux activity. Moore and Fisher \[53\] in an earlier study suggested that HDL could be a risk marker rather than a risk factor for CVD, which is still being debated. The study authors suggested HDL can be transformed into an abnormal molecule that can cause endothelial dysfunction. A recent review \[51\] continues to support the notion that HDL function may be a more important role in the development of CVD. Additionally, the review authors summarized that dysfunction may be associated with disease states, such as obesity, diabetes and CVD. An additional study \[79\] reported that hypo-HDL was associated with increased levels of CVD, but that HDL trajectories were less predictive. Additionally, study authors reported that levels of dysfunctional HDL can be controlled through the reduction of many of the risk factors of CVD, such as aerobic exercise, a positive nutritional status, elimination of or never initiating smoking, reduction in diabetes and avoidance of developing chronic kidney disease \[79\].
11. Different Methods for HDL Quality Determination

A number of methods have been used by researchers to check the quality of HDL, such as analytical ultracentrifugation, nondenaturing gel electrophoresis, density gradient ultracentrifugation, vertical analytical profile, two-dimensional gel electrophoresis, nuclear magnetic resonance (NMR) spectroscopy, and more [22]. Density-gradient ultracentrifugation (UC) was the initial and commonly employed method for distinguishing the size of HDL particles, but this strategy requires a significant amount of time and financial resources in comparison to alternative approaches [23]. Additionally, it may encounter challenges in terms of precision, particularly when dealing with people who have underlying health conditions [23]. Nondenaturing gel electrophoresis is preferable for analyzing protein interactions and enzymatic activity, but it is not as precise as other techniques and is not appropriate for measuring molecular weight. Vertical analytical ultracentrifugation is a quick and less protein-destructive way to separate and measure cholesterol within HDL subfractions [23]. However, measurements may not be accurate all the time, especially for people whose HDL-C levels are low [80]. In addition, 2-Dimensional GEL Electrophoresis can separate HDL based on size and charge, allowing for the analysis of complex samples, but it is time-consuming and unable to detect all the proteins [80].

Nuclear magnetic resonance (NMR) spectroscopy is a highly advanced analytical technique that can offer a detailed and precise assessment of the quality of HDL and is capable of quantifying both the size and quantity of HDL particles [21]. This test is a recent and advantageous tool in a clinical environment as it offers the most precise data, which is highly valuable for the diagnosis, treatment, and prescription of medications for patients [81]. One disadvantage of NMR is its high cost [81].

12. Conclusions

Measuring HDL by quality rather than quantity may be essential in providing more protection against CVD events. HDL particles vary in size, density, and composition, and can vary in their functionality. Lower quality HDL particles that are dysfunctional are less likely to participate in reverse cholesterol transport, less likely to reduce LDL cholesterol, more glycosylated and oxidized, contribute to plaque formation and inflammation within the arteries, and increase the risk of CVD events despite the higher levels of HDL.

As more evidence emerges regarding the functionality and quality of HDL, providing a more comprehensive picture of cardiovascular risk as compared to a more traditional approach of HDL quantity, future research should measure the quality of HDL in additional to quantitative measurements. By focusing on HDL quality, healthcare providers can better identify individuals at risk for heart disease and implement targeted interventions to improve cardiovascular health.

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Conflicts of Interest: The authors declare no conflict of interest.

References


40. Hine, D.; Mackness, B.; Mackness, M. Coincubation of PON1, APO A1, and LCAT increases the time HDL is able to prevent LDL oxidation. IUBMB Life 2012, 64, 157–161. [CrossRef] [PubMed]

41. Kosmas, C.E.; Martinez, I.; Sourlas, A.; Bouza, K.V.; Campos, F.N.; Torres, V.; Montan, P.D.; Guzman, E. High-density lipoprotein (HDL) functionality and its relevance to atherosclerotic cardiovascular disease. Drugs Context 2018, 7, 212525. [CrossRef] [PubMed]

42. Brites, F.; Martin, M.; Guillas, I.; Kontush, A. Antioxidative activity of high-density lipoprotein (HDL): Mechanistic insights beyond potential clinical benefit. BBA Clin. 2017, 8, 66–77. [CrossRef]


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