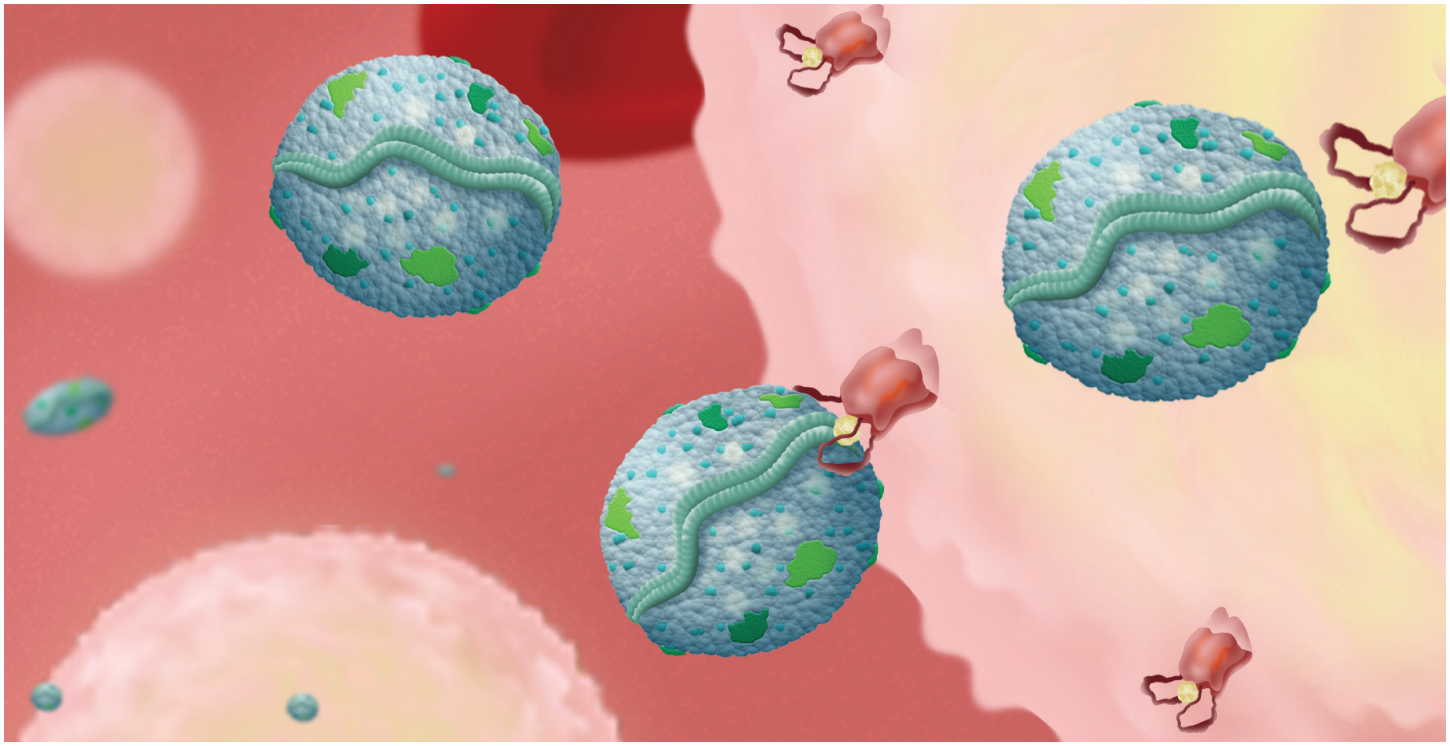
High-density Lipoprotein (HDL) Dysfuntion

Educational materials



Metagenics Institute

Introduction

High-density lipoprotein (HDL) is a particle most famous for its role in collecting and transporting excess cholesterol to the liver, where it can be excreted or recycled. By removing excess cholesterol, HDL is thus thought as protective against cardiovascular disease. However, we now know that under certain circumstances, HDL particles become dysfunctional and lose their protective properties.

In the traditional lipid panel, HDL-cholesterol (HDL-C) refers to the amount of cholesterol carried inside the HDL particles. It has long been believed that a higher HDL-C level translates to lower cardiovascular disease risks. As it turns out, the story is not that simple. Furthermore, research supports that measuring HDL-C provides no information on whether the HDL particles are functioning properly, or silently causing harm.

In this downloadable eBook, we review scientific information to debunk the myth about HDL-C and provide clinical tools to help you identify patients at risk of HDL dysfunction, including a step-by-step procedure—designed by Mark Houston, MD—for improving the function of HDL particles. We also include in-depth information on lab tests relevant to HDL and compile clinical evidence demonstrating improvements in HDL function and cardiovascular health via lifestyle and nutritional approaches.

For additional information related to HDL, please visit
www.MetagenicsInstitute.com/HDL-Dysfunction

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HDL-cholesterol myth:

Higher HDL-cholesterol level means lower cardiovascular disease risk.

HDL-cholesterol truth:

HDL-cholesterol level is only a part of the HDL story; it doesn't give an indication if the HDL particle is functioning properly.

How did this myth start?

The concept that high-density lipoprotein cholesterol (HDL-C) is beneficial dates back to research from nearly 70 years ago.[1]

And in 1977, the prominent Framingham cohort study demonstrated that low HDL-cholesterol concentrations were associated with increased risks of coronary heart disease.[2]

In 1986, the second HDL measurement from the Framingham cohort became available for long-term analysis:[3] Individuals in the lowest 20% of HDL-cholesterol levels had twice the risk of coronary heart disease compared to those in the highest 20% of HDL-cholesterol levels.[3]

This inverse association between HDL-cholesterol level and disease risk has been replicated in multiple, large-scale observational studies such as the Münster Heart Study, the Atherosclerosis Risk in Communities (ARIC), and the Emerging Risk Factors Collaboration (ERFC) combined analysis.[4-6]

Low HDL-cholesterol level was thus believed to be a risk factor for heart health.

1951
Study found HDL-cholesterol level was reduced in patients with coronary heart disease

1977
The Framingham Study 4-year data

1986
The Framingham Study 12-year data

1997
The Münster Heart Study

2001
The ARIC study

2009
ERFC combined analysis

HDL assumption called into question by multiple studies

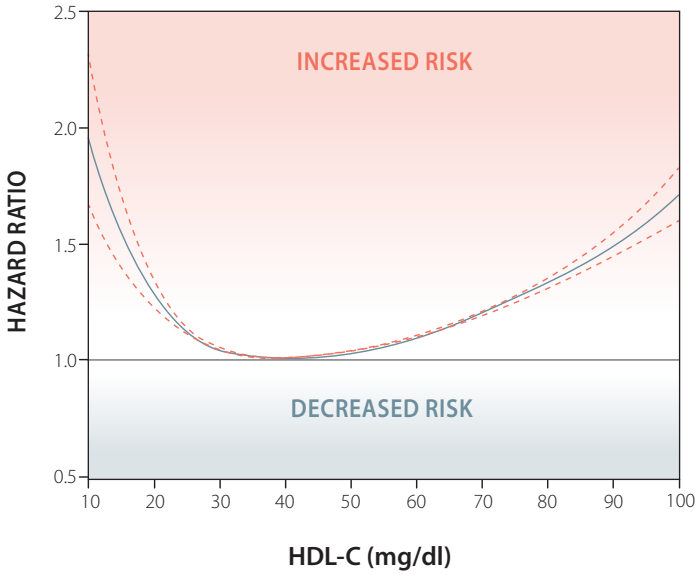
However, recent large-scale genetic studies have raised doubt about the accuracy of that assumption.

Multiple studies involving tens of thousands of individuals have found that genetically higher or lower HDL-cholesterol concentrations do not change risks of cardiovascular disease or type 2 diabetes.[7-10]

These data indicate HDL-cholesterol level itself isn't likely to be protective against these diseases.

In fact, higher HDL-cholesterol level itself is not always better. Data from the more recent cohort studies found a U-shaped association.[11-12]

Figure 1: U-Shaped Curve with HDL-C and All-Cause Mortality
(figure adapted from Bowe B et al. *Clin J Am Soc Nephrol.* 2016;11(10):1784-1793.[21]



In a cohort of 1,764,986 men in the US followed for ~10 years, very-low and high HDL-C concentrations were associated with increased risk of all-cause mortality.

Having very low or very high HDL-cholesterol levels is linked to increased mortality.

2012

Genetically low HDL-cholesterol levels were not associated with increased risk of myocardial infarction.

Genetically high HDL-cholesterol levels did not lower risk of myocardial infarction.

2015

Genetically low HDL-cholesterol levels were not associated with increased risk of type 2 diabetes.

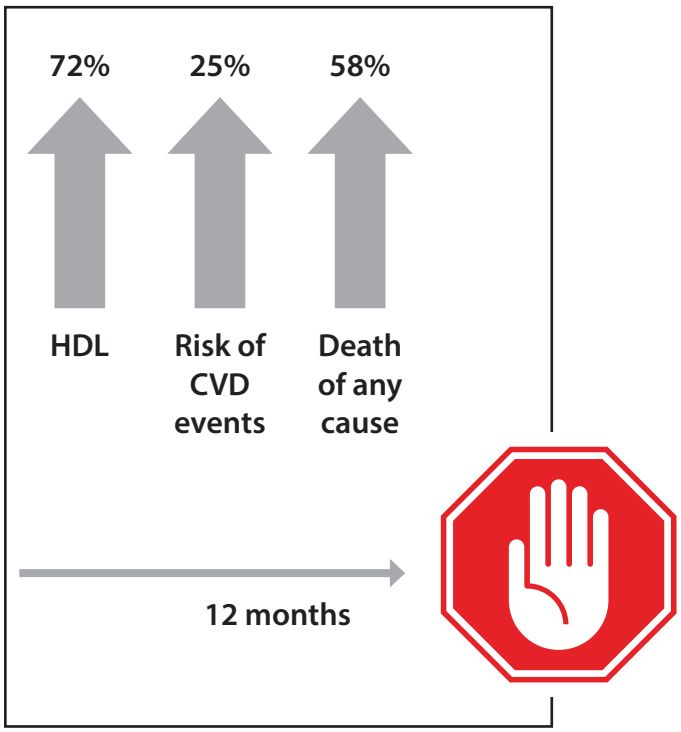
2017

Review of multiple studies: HDL-cholesterol level itself is unlikely to be causally protective against coronary heart disease.

Trials raising HDL-cholesterol did not reduce risks

When drug trials tested the assumption that increasing HDL-cholesterol concentration would translate into clinical benefits, the results were disappointing.

For example, in the ILLUMINATE Study that involved patients at high cardiovascular risk, the HDL-raising drug increased HDL cholesterol levels by 72% at 12 months, but the trial was terminated early because the risk of death and cardiac events increased.[13]



Raising HDL-cholesterol levels via different drugs failed to reduce the risk of cardiovascular events, including when combined with statins.[13-18]

2005
The FIELD Study
Fenofibrate

2007
The ILLUMINATE Study
CETP inhibitor torcetrapib + statin

2011
The AIM-HIGH Study
Pharmaceutical niacin + statin

2012
The dal-OUTCOMES Study
CETP inhibitor dalcetrapib

2014
The HPS2-THRIVE Study
Pharmaceutical niacin + laropiprant

2017
The ACCELERATE Study
CETP inhibitor evacetrapib

The FIELD Study: The Fenofibrate Intervention and Event Lowering in Diabetes Study; **The ILLUMINATE Study:** The Ibrutinib plus Obinutuzumab versus Chlorambucil plus Obinutuzumab in First-line Treatment of Chronic Lymphocytic Leukaemia Study; **The AIM-HIGH Study:** The Atherothrombosis Intervention in Metabolic Syndrome with low HDL/HIGH Triglycerides Study; **The dal-OUTCOMES Study:** The Dalcetrapib in Patients Hospitalized for an Acute Coronary Syndrome Study; **The ACCELERATE Study:** Assessment of Clinical Effects of Cholesteryl Ester Transfer Protein Inhibition with Evacetrapib in Patients at a High Risk for Vascular Outcomes Study

HDL-cholesterol truth:

HDL-cholesterol level is only a part of the HDL story; it doesn't give an indication if the HDL particle is functioning properly.

Quantity does not reflect quality

HDL-cholesterol levels measure the amount of cholesterol carried in the HDL particles but don't give information about HDL particle function. Only functioning particles effectively reduce cardiovascular risk.[19]

HDL function can be assessed through:[20]

- The number of HDL particle (HDL-P)
- The various sizes of HDL particles
- The integrity of HDL particle components
- Myeloperoxidase (MPO) and oxidized low-density lipoprotein (oxLDL)

Summary

Focusing only on HDL-cholesterol level (i.e., the amount of cholesterol transported by HDL particles) is no longer sufficient for determining risk. HDL cholesterol is a static measurement that poorly reflects the dynamic HDL function.[20] Assessing HDL particle function is vital to fully appreciate cardiovascular risk!

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Who develops HDL dysfunction?

Learn the specific conditions that exhibit HDL dysfunction

In certain patient populations, inflammation, oxidative stress, and high blood glucose damage the HDL particles, impairing their cardioprotective function. Patients present with normal or high HDL-cholesterol levels, and further evaluation reveals the threatening nature of dysfunctional HDL. Due to the ubiquity and versatility of HDL particles, multiple medical conditions influence their activity. Below are examples of conditions affecting HDL function and the specific HDL mechanisms harmed.

Cardiovascular disease

- Coronary heart disease
- Coronary artery disease
- Acute coronary syndrome
- Hypertension

Metabolic disease

- Obesity
- Metabolic syndrome
- Type 2 diabetes
- Nonalcoholic fatty liver disease
- Polycystic ovary syndrome

Chronic inflammatory and autoimmune disease

- Type 1 diabetes
- Rheumatoid arthritis
- Systemic lupus erythematosus
- Psoriasis
- Periodontitis

Kidney disease

- Chronic kidney disease
- End-stage renal disease

Other conditions

- Cardiac surgery
- Obstructive sleep apnea
- Hyper-homocysteinemia
- Hyper-alphalipoproteinemia
- HIV infection

Cardiovascular disease

Disease/condition	Evidence of HDL dysfunction
Coronary heart disease[1,2]	• Reduced PON1 activity in HDL • Reduced inhibition of monocyte binding to endothelial cells • Reduced inhibition of oxidation of LDL
Coronary artery disease[3,4,5]	• Reduced PON1 activity in HDL • Increased apoC-III level in HDL • Reduced cholesterol efflux capacity from macrophages • Reduced stimulation of NO production in endothelia cells • Reduced antioxidative capacity in endothelial cells • Reduced VCAM-1 expression in endothelial cells • Reduced inhibition of endothelial-monocyte adhesion • Reduced endothelial repair following carotid artery injury
Acute coronary syndrome[6,7,8,9]	• Reduced PON1 activity in HDL • Increased apoC-III level in HDL • Reduced inhibition of LDL oxidation • Impaired HDL-apoA-I exchange
Hypertension[10]	• Reduced serum PON1 activity • Reduced inhibition of LDL oxidation

apoA-I= apolipoprotein A-I; **apoC-III**= apolipoprotein C-III; **HDL**= high-density lipoprotein; **LDL**= low-density lipoprotein; **NO**= nitric oxide; **PON1**= paraoxonase 1; **VCAM-1**= soluble vascular cell adhesion molecule-1

Metabolic disease	
Disease/procedure	**Evidence of HDL dysfunction**
Obesity[11,12,13,14]	• Reduced PON1 activity in HDL • Increased levels of lipid hydroperoxides in HDL • Increased SAA levels in serum
Metabolic syndrome[15,16]	• Increased triglycerides and decreased cholesteryl esters in HDL • Reduced apoA-I in HDL • Reduced inhibition of LDL oxidation
Type 2 diabetes[17,18,19,20,21]	• Increased MPO activity in HDL • Reduced PON1 activity in HDL • Increased triglycerides in HDL • Reduced stimulation of NO production in endothelial cells • Reduced antioxidative capacity in endothelial cells • Reduced endothelial repair following carotid artery injury • Reduced inhibition of LDL oxidation • Reduced cholesterol efflux capacity • Reduced inhibition of LDL-induced monocyte chemotactic activity in endothelial cells
Nonalcoholic fatty liver disease[22,23]	• Reduced cholesterol efflux capacity • Reduced circulating apoA-I • Reduced circulating preβ1-HDL
Polycystic ovary syndrome[24,25]	• Reduced cholesterol efflux capacity • Reduced circulating apoA-I levels • Increased intrinsic HDL oxidation levels

apoA-I= apolipoprotein A-I; **HDL**= high-density lipoprotein; **LDL**= low-density lipoprotein; **MPO**= myeloperoxidase; **NO**= nitric oxide; **PON1**= paraoxonase 1; **SAA**= serum amyloid A

Chronic inflammatory or autoimmune disease

Disease/condition	Evidence of HDL dysfunction
Type 1 diabetes[26,27,28]	• Reduced PON1 activity in HDL • Glycoxidation in HDL • Reduced ability to counteract oxLDL-mediated actions
Rheumatoid arthritis[29,30,31,32]	• Increased MPO in HDL and plasma • Reduced PON1 activity in HDL • Increased SAA in HDL • Reduced plasma LCAT activity • Reduced cholesterol efflux capacity from macrophages • Reduced inhibition of LDL oxidation
Systemic lupus erythematosus[33,34]	• Reduced inhibition of LDL oxidation • Reduced cholesterol efflux capacity
Psoriasis[35]	• Reduced apoA-I in HDL • Reduced cholesterol efflux capacity
Periodontitis[36,37]	• Reduced production of NO in endothelial cells • Increased production of superoxide in endothelial cells • Reduced serum PON activity • Reduced apoA-I in plasma

apoA-I= apolipoprotein A-I; **HDL**= high-density lipoprotein; **LCAT**= lecithin cholesterol acyltransferase; **LDL**= low-density lipoprotein; **MPO**= myeloperoxidase; **NO**= nitric oxide; **oxLDL**= oxidized LDL; **PON**= paraoxonase; **SAA**= serum amyloid A

Kidney disease	
Disease/condition	**Evidence of HDL dysfunction**
Chronic kidney disease[38]	• Reduced stimulation of NO production in endothelial cells • Reduced endothelial repair following carotid artery injury • Reduced inhibition of endothelial monocyte adhesion • Reduced inhibition of endothelial VCAM-1 expression • Increased superoxide production in endothelial cells
End-stage renal disease[39,40,41,42,43]	• Reduced apoA-I in HDL • Increased triglycerides in HDL • Reduced apoA-II in HDL • Increased apoC-III in HDL • Increased SAA in HDL • Reduced inhibition of LDL oxidation • Reduced cholesterol efflux capacity • Reduced inhibition of oxLDL-stimulated VCAM-1 expression in endothelial cells • Reduced inhibition of oxLDL uptake in monocytes • Reduced inhibition of MCP-1 production in endothelial cells

apoA-I= apolipoprotein A-I; **apoA-II**= apolipoprotein A-II; **apoC-III**= apolipoprotein C-III; **HDL**= high-density lipoprotein; **LDL**= low-density lipoprotein; **MCP-1**= monocyte chemoattractant protein-1; **NO**= nitric oxide; **oxLDL**= oxidized LDL; **SAA**= serum amyloid A; **VCAM-1**= soluble vascular cell adhesion molecule-1

Other conditions

Disease/procedure	Evidence of HDL dysfunction
Cardiac surgery[44]	• Reduced PON1 activity in HDL • Reduced inhibition of LDL-induced monocyte chemotactic activity • Reduced inhibition of MCP-1 expression
Obstructive sleep apnea[45,46]	• Reduced inhibition of LDL oxidation • Reduced cholesterol efflux capacity
Hyper-homocysteinemia[47]	• Reduced cholesterol efflux capacity • Reduced inhibition of IL-6 release from endothelial cells
Hyper-alphalipoproteinemia[48]	• Reduced apoA-I in HDL • Reduced cholesterol efflux capacity
HIV infection[49,50,51,52]	• Reduced PON1 activity in HDL • Reduced LCAT activity in HDL • Reduced inhibition of LDL oxidation

apoA-I= apolipoprotein A-I; **HDL**= high-density lipoprotein; **IL-6**= interleukin-6; **LCAT**= lecithin cholesterol acyltransferase; **LDL**= low-density lipoprotein; **MCP-1**= monocyte chemoattractant protein-1; **PON1**= paraoxonase 1

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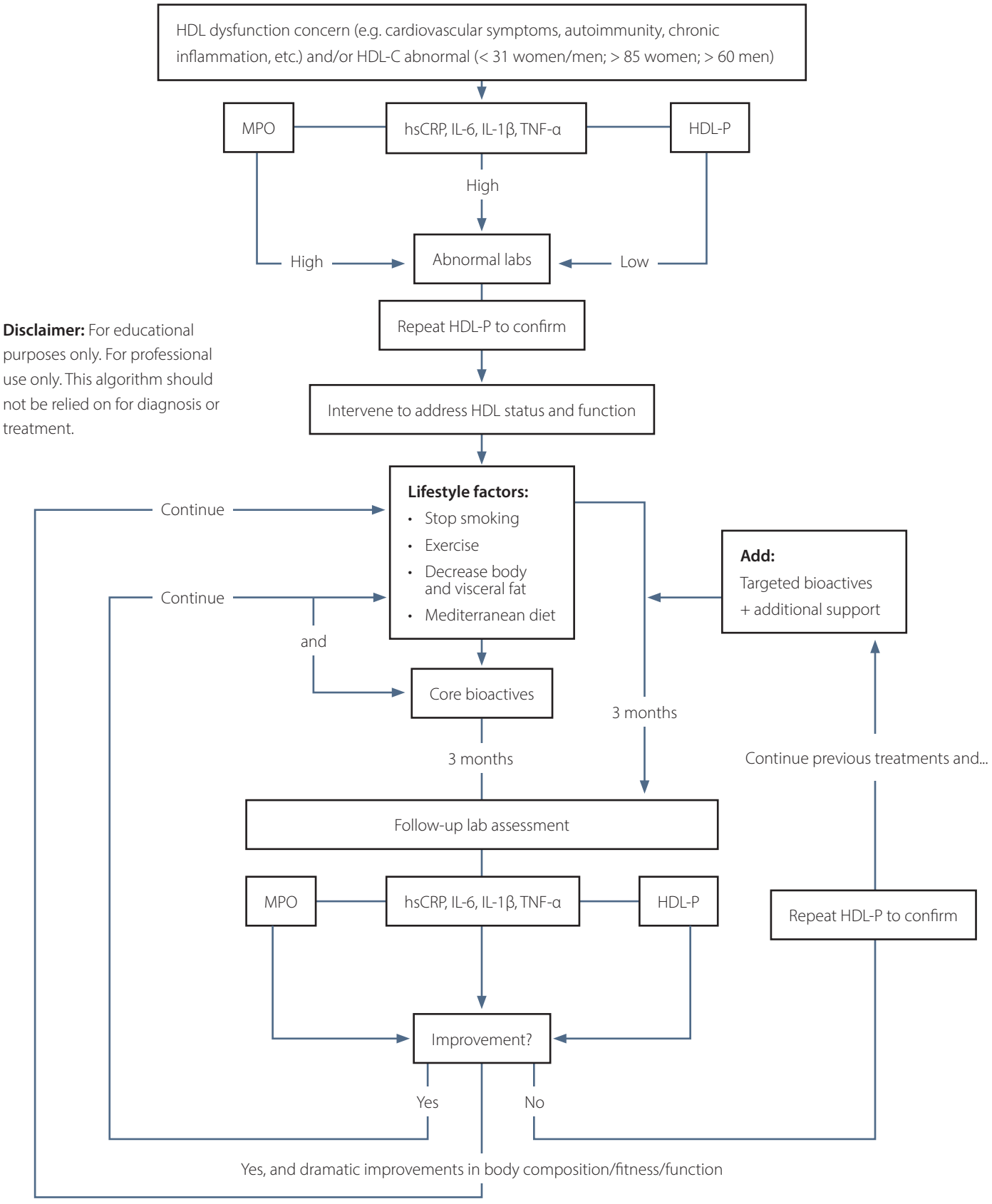
HDL Assessment by Mark Houston, MD

a companion to: HDL dysfunction protocol



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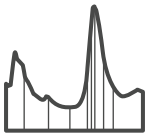
Labs to Identify High-density Lipoprotein (HDL) Risk

Learn which lab tests can help you better understand HDL and cardiovascular (CV) risk





HDL-C testing | HDL-P testing | HDL mapping | HDL-size and density | Surrogate markers

HDL-Cholesterol (HDL-C)

What does this test measure?	• This blood test measures the amount of cholesterol present within the HDL particles (HDL-P) in circulation.
Why would I test?	• In large cohort studies, very low levels of HDL-C were associated with increased risk of developing cardiovascular disease (CVD).[1-6] • HDL-C is closely linked with insulin resistance and is often part of an atherogenic dyslipidemia phenotype.[7] Finding low HDL-C can add to a picture of general metabolic risk.
What should I know?	• The relationship between HDL-C and CV risk is not linear (i.e., higher HDL-C is not more protective). • In a large cohort of 24,510 men and women, HDL-C levels above 75 mg/dL for men and 90 mg/dL for women did not provide any additional cardioprotective effects.[8] In a Japanese cohort, HDL-C greater than or equal to 90 mg/mL in both men and women was associated with increased CV mortality risk,[9] a finding echoed in a Northern European cohort, where it was found that HDL-C greater than or equal to 116 mg /dL for men and 135 mg /dL in women was linked with greater risk of CV mortality.[10] • In a cohort of 1,764,986 men who were United States veterans followed for ~10 years, a U-shaped curve was identified with HDL-C and all-cause mortality. Individuals at both ends of the curve (low and very high) were seen to be at greater risk of all-cause mortality.[11] • Clinical studies with cholesterol ester transfer protein (CETP) inhibitors failed to reduce CV risk despite raising HDL by 40-133%.[12-14] • Large cohort studies have shown that individuals with high HDL-C due to genetic variation are not protected against CVD.[15] • These newer data suggest that HDL-C measurement alone is not sufficient to understand CV risk. Additionally, HDL-C does not provide any information on HDL function. Studies show HDL can be dysfunctional when HDL-C are below or within normal values.[16]
Normal reference ranges?*	• Women: 50-90mg /dL • Men: 40-90mg /dL • Values > 90-135mg /dL for women and > 90-116mg /dL for men have been associated with increased CV risk[9,10]

*Reference ranges may differ between clinical laboratories

HDL-Particle Number	
What does this test measure?	• This blood test measures the number of HDL particles in circulation.
Why would I test?	• Data from multiple cohort studies has demonstrated that HDL-P is superior to HDL-C in assessing CVD.[17,18]
What should I know?	• The JUPITER trials showed HDL-P is a greater predictor of residual risk than HDL-C, cholesterol efflux capacity, or apoAI, when using potent statins as a therapy.[17,19] • Clinical cohort studies measuring HDL-P by both nuclear magnetic resonance (NMR) or Ion Mobility Spectrometry, have shown that HDL-P is inversely related to risk of developing heart disease.[20] • HDL-P was shown to correlate with cholesterol efflux capacity (spearman correlation coefficient of 0.39; $p < 0.0001$).[17]
Normal reference ranges?*	• Women and men: > 7,000 nmol/L[13,14]

*Reference ranges may differ between clinical laboratories

HDL Mapping

What does this test measure?

- HDL Mapping provides information on the 5 different subspecies of HDL molecules from pre-β-1 (small discoid particles with low levels of cholesterol and high capacity to accept cholesterol) through α-4, α-3, α-2, and α-1 particles, which get progressively larger and more spherical as they accumulate more cholesterol.

Why would I test?

- Lower levels of the larger α-1 particles are associated with the development of cardiovascular disease. Functional smaller pre-β-1 particles have a greater ability to accept cholesterol from peripheral cells through ABCA-1.[20] Larger particles (α-1) are responsible for efflux of cholesterol through SR-BI (from peripheral cells or to the liver).[21] Both components provide insights into the reverse cholesterol transport process and, HDL particle size may not be as important as initially thought.[21,22]

What should I know?

- In male participants of the Framingham offspring study, higher levels of the lipid-poor pre-β-1 HDL particles and lower levels of cholesterol-rich larger α-1 HDL particles were correlated with coronary heart disease risk.[22] These results suggest that maturation of pre-β-1 HDL particles into larger cholesterol-rich particles was impaired, which resulted in inadequate reverse cholesterol transport.[23]
- In the HDL-Atherosclerosis Treatment Study using a niacin-simvastatin combination, participants with the greatest increase in α-1 HDL particles halted progression of arterial stenosis.[24]
- In a group of subjects with raised triglycerides, pre-β-1 concentration was reduced compared to healthy controls.[21] However, the function and capacity for cholesterol efflux was significantly increased in the group with high triglycerides.[22]
- In subjects with type 2 diabetes, pre-β-1 concentrations were increased compared with controls; however, their HDL function was impaired.[25]
- These results in total indicate that the size and concentration of different HDL subspecies is not as clear cut as initially thought. All subspecies play an important role in reverse cholesterol transport, and subspecies function is important to understand.

Normal reference range*

Men	**Optimal**	**Borderline mg/dL**	**Increased Risk**
α-1	> 35.0	25.0-35.0	< 25.0
α-2	> 55.0	45.0-55.0	< 45.0
α-3	< 20.0	20.0-25.0	> 25.0
α-4	< 20.0	20.0-25.0	> 25.0
Preβ-1	< 20.0	20.0-25.0	> 25.0

Women	**Optimal**	**Borderline mg/dL**	**Increased Risk**
α-1	> 45.0	35.0-45.0	< 35.0
α-2	> 65.0	55.0-65.0	< 55.0
α-3	< 20.0	20.0-25.0	> 25.0
α-4	< 20.0	20.0-25.0	> 25.0
Preβ-1	< 20.0	20.0-25.0	> 25.0

*Reference ranges may differ between clinical laboratories

HDL Subclasses

What does this test measure?	• HDL subclasses measure the concentration of larger, more buoyant HDL2b and the smaller, less buoyant HDL3 in circulation.
Why would I test?	• Concentrations of HDL2 and HDL3 have been associated with reduced risk of cardiovascular disease.[26] • In more recent studies, higher levels of HDL3 were associated with reduced risk of developing arterial stiffness and reduced risk of developing coronary heart disease.[27]
What should I know?	• The literature supporting the link between HDL2, HDL3, and cardiovascular disease is mixed. A review of 80 studies highlights this variability, wherein several studies report a link between higher HDL2 and reduced cardiovascular risk; other studies report that higher HDL3 concentrations drive risk reduction.[26]
Normal reference range?*	• HDL-2 Cholesterol: 9-38 mg/dL • HDL-3 Cholesterol: 22-35 mg/dL

*Reference ranges may differ between clinical laboratories

Myeloperoxidase (MPO)

What does this test measure?	• This test measures levels of circulating a pro-oxidant protein expressed in and secreted from proinflammatory immune cells.
Why would I test?	• Circulating MPO is linked with increased risk of developing CVD and to greater risk of adverse events following a myocardial infarction (MI).[28,29] • HDL particles and the apoA-I proteins on HDL can be modified by MPO, causing them to become dysfunctional.[30]
What should I know?	• Several human studies implicate MPO in the development of atherosclerotic plaques.[31] • In a study of ~3,300 men and women, circulating MPO concentrations at baseline predicted the risk of development of coronary artery disease over an 8-year follow-up.[32] • Circulating MPO levels predict adverse outcomes following MI.[28,29] • MPO-modified apoA-I recovered from human atherosclerotic plaque showed impaired cholesterol efflux capacity and had potent proinflammatory activity on endothelial cells.[33] • Elevated circulating levels of MPO-modified apoA-1 was associated with increased CV risk in humans.[33] • In HDL isolated from healthy people, exposure to a pro-oxidant metabolite of MPO led to a reduction in HDL function as seen by a reduction in cholesterol efflux capacity and a failure to activate endothelial nitric oxide (eNOS).[34] Additionally, MPO-modified HDL increased the expression of vascular inflammation markers.[34]
Normal reference range?*	< 470pmol/L* cardiovascular risk; other studies report that higher HDL3 concentrations drive risk reduction.

*Reference ranges may differ between clinical laboratories

High-Sensitivity C-Reative Protein (hsCRP)

What does this test measure?	• This is a high-sensitivity assay to assess the concentration of C-reactive protein in circulation.
Why would I test?	• hsCRP is a biomarker of inflammation and, increased circulating concentrations are associated with an increased risk for developing cardiovascular disease.
What should I know?	• An inflammatory environment is associated with HDL dysfunction. In individuals participating in the the PREVEND (Prevention of Renal and Vascular End-stage Disease) study, higher hsCRP concentrations correlated significantly with lower cholesterol efflux capacity.[35]
Normal reference range?*	• < 1.0 mg /L is optimal

*Reference ranges may differ between clinical laboratories

Circulating ApolipoproteinA-I (apoA-I)

What does this test measure?	• This test measures the concentration of apoA-I in circulation.
Why would I test?	• apoA-I is the major protein on HDL and is involved in the transport of cholesterol from peripheral cells into the HDL particle.
What should I know?	• The JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial showed HDL-P is a greater predictor of residual risk than HDL-C, cholesterol efflux capacity, or apoA-I, when using potent statins as a therapy.[17,19] • MPO-modified apoA-I recovered from human atherosclerotic plaque showed impaired cholesterol efflux capacity and had potent proinflammatory activity on endothelial cells.[33] • Elevated circulating levels of MPO-modified apoA-I was associated with increased CV risk in humans.[33]
Normal reference range?*	• Men: > 160 mg/dL • Women: > 180 mg/dL

*Reference ranges may differ between clinical laboratories

Research tests	
Paraoxonase (PON)	
What does this test measure?	• This test measures PON protein concentration associated with HDL. • Tests can also measure the activity of PON.
Why would I test?	• PON is a protein with antioxidant capacity that protects low-density lipoprotein (LDL) from oxidation.[29,30,36]
What should I know?	• Antioxidant activity of PON decreases with age.[37,38] • Several preclinical studies suggest that PON1 may play a role in supporting cholesterol efflux capacity.[39] • In the PREVEND study (n=6,029 people followed over 9 years), increased PON activity was associated with increased risk of developing CVD.[42] However, PON activity did not provide additional improvement in risk assessment beyond what traditional biomarkers could predict.[42] • In subjects with high HDL-C and hsCRP, decreased PON activity is associated with incident CVD risk.[41]
Normal reference range?	• Research tool

Cholesterol Efflux Capacity	
What does this test measure?	• This test commonly measures the movement or efflux of cholesterol from macrophages or foam cells into HDL.
Why would I test?	• Cholesterol efflux capacity is a measure of HDL function and is considered a surrogate marker of reverse cholesterol transport.[42] Cholesterol efflux capacity is inversely associated with the development of CVD and survival following MI.[35,43,44]
What should I know?	• In the PREVEND study (n=8592 with a follow-up of 12 years), a comparison of cases with cardiovascular disease compared with healthy controls with identical HDL-C and apoA-I levels, cholesterol efflux capacity from foam cells was significantly lower in cases.[35] Cholesterol efflux capacity was inversely associated with incident cardiovascular events.[35] • In a generally healthy population of men participating in the Health Professionals Follow-up Study, baseline cholesterol efflux capacity predicted the risk of developing cardiovascular disease.[45] However, controlling for baseline HDL-C removed the predictive power of cholesterol efflux capacity, indicating this test might not improve clinical decision making.[37] • In patients hospitalized with acute MI (n=1609), higher cholesterol efflux capacity was a strong predictor of survival.[46] Patients with higher cholesterol efflux capacity experienced a markedly lower rate of mortality after 6 years.[36] • The JUPITER trials showed HDL-P is a greater predictor of residual risk than HDL-C, cholesterol efflux capacity, or apoAI, when using potent statins as a therapy.[10,12]
Normal reference range?*	• Varies depending on method and cell type used • Research tool

HDL-Apolipoprotein E (apoE) content

What does this test measure?	• This measures the concentration of the apoE on HDL particles.
Why would I test?	• In a study of 3696 men and women, higher apoE reduced the risk of developing coronary heart disease.[38] Those in the highest quintile of HDL-apoE had a 35% reduction in risk.[38]
What should I know?	• Only ~4% of HDL contains apoE.[38] • Tracer studies in humans show that presence of apoE on HDL leads to faster clearance through the liver, with this more rapid metabolism thought to reflect enhanced reverse cholesterol transport.[38] • apoE protective properties are blocked by the presence of apoCIII on the HDL particle.[47] This is thought to delay HDL metabolism and clearance, reflecting slowed reverse cholesterol transport.[38]
Normal reference range?*	• Research tool

HDL-Apolipoprotein CIII (apoCIII) content

What does this test measure?	• This test measures the concentration of the apoCIII on HDL particles.
Why would I test?	• Increased apoCIII content of HDL is associated with an increased risk of developing coronary heart disease.[39]
What should I know?	• 6-8% of HDL particles have apoCIII present.[39] • The presence of apocIII on HDL appears to block protective properties of apoE. This is thought to delay HDL metabolism and clearance, reflecting slowed reverse cholesterol transport.[38] • The MESA (Multi-Ethnic Study of Atherosclerosis) study (n= 5675 men and women aged 52-72 years followed for up to 13 years) and DCH (Danish Diet, Cancer, and Health) study (n= 3642 men and women aged 51-64 years followed for up to 16 years) cohorts demonstrated higher apoCIII content of HDL increased the risk of developing cardiovascular disease.[39] • In nested case-control cohorts from the Nurses' Health Study and the Health Professionals Follow-Up Study higher levels of HDL without apoCIII lowered the risk of developing coronary heart disease.[40] • In a study of subjects with (n=140) and without (n=99) coronary artery disease, the apoCIII content of HDL was inversely correlated with cholesterol efflux capacity, indicating the presence of apoCIII impacts HDL-mediated cholesterol efflux capacity and HDL function.[41]
Normal reference range?*	• Research tool

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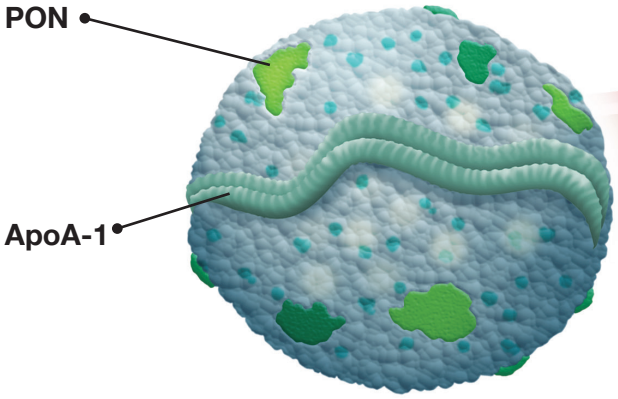
Treating HDL Dysfunction

Bioactives and lifestyle factors can improve HDL function.
Here you'll see the mechanisms supported.



Endothelial effects:
- Vasodilation
- Flow-mediated vasodilation
- Reduction of ischemia
- Endothelial nitric oxide production

HDL composition effects:
- HDL-P
- HDL size/maturation
- HDL type
- Activity/expression/modification of:
  - PON
  - apoA-I
  - LCAT
  - CETP
  - SAA

Cholesterol loaded cell effects:
↑Increase cholesterol efflux
↓Reduce macrophage cytokine secretion
↓Reduce LDL oxidation
↓Reduce intima-media thickness
↓Reduce reactive oxygen species

Liver effects:
Increased expression and activity of cholesterol transporters involved in accepting cholesterol from HDL particles

KEY:

P pomegranate polyphenols	N niacin	Q quercetin
L lycopene	α-T α-tocopherol	M Mediterranean diet
exercise	no smoking	

Other abbreviations:

- **ApoA-I**: apolipoprotein A-I; responsible for cholesterol transport into HDL particle.
- **CETP**: cholesteryl ester transfer protein; serum protein involved in lipid exchange between lipoprotein classes. Inhibiting this protein has been a drug target action for reducing atherosclerosis.
- **LCAT**: lecithin-cholesterol acyltransferase; packs cholesterol into the HDL core.
- **PON**: paraoxonase; protects HDL particle
- **SAA**: Serum amyloid A; an acute-phase inflammatory protein that can displace functional proteins on HDL particles.

Pomegranate Clinical Evidence Related to HDL Function and Cardiovascular Health

Ingredient	Study Design	Main Findings
Pomegranate juice (130 mg/day GAE polyphenols)[1]	Clinical study in men with T2D (n=6); 50 mL/day for 4 wk	• Increased PON1 binding to HDL via reduction in oxidative stress
Pomegranate juice (130 mg/day total polyphenols) or pomegranate polyphenol extract (650 mg/day total polyphenols)[2]	Clinical study in adults with T2D (n=30) ; 50 mL/day juice for 4 wk or 5 mL day extract for 6 wk	• Increased PON1 activity vs. baseline • Increased PON1 protein binding to HDL vs. baseline • Decreased serum oxidative stress vs. baseline
Pomegranate juice*[3]	Clinical study in adults with T2D (n=50); 200 mL/day for 6 wk	• Increased PON activity vs. baseline • Decreased serum MDA vs. baseline • Improved serum fasting glucose, total cholesterol, and LDL cholesterol vs. baseline
Pomegranate juice*[4]	Clinical study in patients with CAS; 10 were supplemented for 1 year, and 5 continued for 3 years	• Increased serum PON1 activity vs. baseline • Reduced intima-media thickness vs. baseline • Reduced LDL oxidation at vs. baseline • Increased serum total antioxidant status vs. baseline
Pomegranate juice (1.5 mM/day total polyphenols)[5]	Clinical study in healthy male volunteers (n=3 or 13); 50 mL/day for 2 wk	• Increased serum PON activity vs. baseline • Reduced HDL oxidation *ex vivo* vs. baseline • Increased plasma total antioxidant status vs. baseline
Pomegranate juice (1.5 mM/day total polyphenols)[6]	Clinical study in healthy men (n=10) and men with T2D (n=10); 50 mL/day for 3 mo	• Increased serum PON1 activity vs. baseline • Reduced serum lipid peroxides vs. baseline • Reduced oxidative stress in macrophages vs. baseline
Pomegranate juice*[7]	RCT in subjects at moderate risk for CHD (n=289); 240 mL/day or placebo for up to 18 mo	• Slowed carotid intima-media thickness progression in subjects with increased oxidative stress
Pomegranate extract (650 mg/day GAE polyphenols)[8]	RCT in hypercholesterolemic adults receiving statin (n=23); 650 mg/day or placebo for 2 mo	• Decreased oxidative stress in monocyte-derived macrophages vs. baseline
Pomegranate extract[9]	RCT in individuals with BMI ≥ 25 (n=48); 1,000 mg/day or placebo for 1 mo	• Decreased inflammatory biomarkers, serum hs-CRP and IL-6 • Decreased lipid peroxidation biomarker, serum MDA • Improved serum fasting glucose, insulin, total cholesterol, HDL cholesterol, and triglycerides
Pomegranate juice*[10]	RCT in patients who had CHD and MI (n=45); 240 mL/day or placebo for 3 mo	• Decreased stress-induced ischemia
Concentrated pomegranate juice (350 mg/day total polyphenols)[11]	Clinical study in patients with T2D and hyperlipidemia (n=22); 40 g/day for 8 wk	• Improved serum lipids (total cholesterol, LDL cholesterol, total cholesterol/HDL cholesterol, LDL cholesterol/HDL cholesterol) vs. baseline
Concentrated pomegranate juice (3.15 mg/day total polyphenols)[12]	Clinical study in adults with T2D (n=40); 50 g/day for 4 wk	• Reduced serum levels of IL-6 vs. baseline • Increased serum total antioxidant capacity vs. baseline

*Amount of total polyphenols was not specified for these studies. Analysis of commercially available pomegranate juice has indicated total polyphenol content ranging from 3.15–437.5 mg/50 mL (/50 g).

BMI= body mass index; **CAS**= carotid artery stenosis; **CHD**= coronary heart disease; **GAE**= gallic acid equivalents; **HDL**= high-density lipoprotein; **hs-CRP**= high-sensitivity C-reactive protein; **IL**= interleukin; **LDL**= low-density lipoprotein; **MDA**= malondialdehyde; **MI**= myocardial ischemia; **mo**= month(s); **PON**= paraoxonase; **RCT**= randomized controlled trial; **T2D**= type 2 diabetes; **wk**= week(s)

Quercetin Clinical and Preclinical Evidence Related to HDL Function and Cardiovascular Health

Ingredient	Study Design	Main Findings
Onion extract[13]	Clinical study in healthy men (n=23); 4.3 g/day containing 51 mg/day quercetin for 1 mo	• Improved postprandial flow-mediated vasodilation vs. baseline
Quercetin[14]	Female C57BL/6 mice; 0.05-2 mg/g diet for 6 wk	• Increased hepatic mRNA and protein levels of PON1 vs. control
Quercetin[15]	LDLR$^{-/-}$ mice fed an atherogenic liquid diet; 0-25 mg/dL quercetin for 8 wk	• Decreased aortic plaques in the ≥ 12.5 mg/dL groups • Increased liver and serum PON1 mRNA expression in the ≥ 12.5 mg/dL groups
Quercetin[16]	*apoE1*$^{-/-}$ mice fed a high-fat diet; 12.5 mg/kg/day or placebo for 8 wk	• Increased cholesterol efflux from macrophages to HDL • Increased cholesterol transport to the liver and bile for excretion
Quercetin[17]	Rats; 10 mg/L or control for 4 wk	• Increased hepatic expression of PON1 • Increased serum and liver PON1 activities • Protected against LDL oxidation
Quercetin[18]	*apoE1*$^{-/-}$ mice fed a high-fat diet; 12.5 mg/kg/day or atorvastatin 2.06 mg/kg/day or control for 12 wk	• Reduced serum levels of oxLDL • Reduced TNF-α and IL-6 levels • Reduced areas of atherosclerotic plaque and increased plaque stability at the aortic root • Increased PPARγ, LXRα, and ABCA1 protein levels in aortas and livers
Quercetin[19]	Rabbits fed high-fat diets; 0.05 mg/kg/day or control for 12 wk (aortic atherosclerosis model) or 4 wk (injured carotid artery model)	• Reduced formation of atherosclerotic plaques in both models
Quercetin[20]	Male Wistar rats; receiving quercetin (dosage n/a) or control	• Increased expression and activity of hepatic cholesterol 7α-hydroxylase • Increased expression and activity of hepatic ABCG1
Quercetin[21,22]	*In vitro* model	• Enhanced cholesterol efflux from RAW264.7 macrophages • Increased ABCA1 mRNA and protein expression in macrophages
Quercetin[23]	*In vitro* model	• Increased cholesterol efflux from foam cells • Activated PPARγ-LXRα pathway to upregulate ABCA1 expression
Quercetin[24]	*In vitro* model	• Induced apoA-I protein and mRNA synthesis in HepG2 (hepatocytes) and Caco-2 (intestinal) cells

ABCA1= ATP-binding cassette transporter A1; **ABCG1**= ATP-binding cassette transporter G1; **apoA-I**= apolipoprotein A-I; **HDL**= high-density lipoprotein; **IL**= interleukin; **LDL**= low-density lipoprotein; **LXR**= liver X receptor; **mo**= month(s); **mRNA**= messenger RNA; **oxLDL**= oxidized LDL; **PON**= paraoxonase; **PPAR**= peroxisome proliferator-activated receptor; **TNF**= tumor necrosis factor; **wk**= week(s)

Lycopene Clinical and Preclinical Evidence Related to HDL Function and Cardiovascular Health

Ingredient	Study Design	Main Findings
Lycopene-rich diet or lycopene supplement[25]	RCT in overweight subjects (n=54); lycopene-rich diet (224-350 mg/wk) or lycopene supplement (70 mg/wk) or control diet for 12 wk	• Increased serum HDL2 and HDL3 • Decreased SAA levels in serum and HDL3 • Increased PON1 activity in serum, HDL2, and HDL3 • Increased LCAT activity in serum and HDL3 • Decreased CETP activity in serum
Tomato[26]	Clinical study in patients with T2D (n=32); 200 g/day tomato for 8 wk	• Increased apoA-I vs. baseline • Decreased systolic and diastolic blood pressure vs. baseline
Lycopene[27]	RCT in statin-treated patients with CVD (n=36) and healthy volunteers (n=36); 7 mg/day or placebo for 2 mo	• Improved endothelium-dependent vasodilatation in patients with CVD
Lycopene and lutein[28]	RCT in subjects with subclinical atherosclerosis (n=144); 20 mg/day lycopene + 20 mg/day lutein for 12 mo	• Decreased carotid artery intima-media thickness vs. baseline
Lycopene[29]	RCT in healthy men (n=126); 6 mg/day or 15 mg/day or placebo for 8 wk	• Increased plasma superoxide dismutase activity • Reduced DNA damage (oxidative stress) in lymphocytes • Increased endothelial function in 15 mg/day group vs. baseline • Decreased hs-CRP, sICAM-1, and sVCAM-1 in 15 mg/day group vs. baseline
Cooked tomato sauce[30]	Pigs fed a hypercholesterolemic diet; 100 g/day containing 21.5 mg/day lycopene or none for 10 days	• Improved HDL function associated with apoA-I and apoJ • Prevented diet-induced impairment of endothelial-dependent coronary vasodilation • Enhanced eNOS transcription and activation and diminished DNA damage in the coronary arteries • Reduced lipid peroxidation
Lycopene[31]	Diabetic rats and control rats; receiving 10 mg/kg/day or none for 1 mo	• Increased PON1 activity
Lycopene[32]	Rabbits fed a high-fat diet; 4-12 mg/kg lycopene or none for 4 and 8 wk	• Reduced serum MDA • Reduced oxLDL • Reduced IL-1 • Increased total antioxidant capacity • Increased nitric oxide • Reduced atherosclerotic plaques in the aorta
Lycopene[33,34]	*In vitro* model	• Increased expression of ABCA1
Lycopene[35]	*In vitro* model	• Reduced proinflammatory cytokine secretion and expression in THP-1 macrophages • Reduced reactive oxygen species production

ABCA1= ATP-binding cassette transporter A1; **apoA-I**= apolipoprotein A-I; **apoJ**= apolipoprotein J; **CETP**= cholesterol ester transfer protein; **CVD**= cardiovascular disease; **DNA**= deoxyribonucleic acid; **eNOS**= endothelial nitric oxide synthase; **HDL**= high-density lipoprotein; **hs-CRP**= high-sensitivity C-reactive protein; **IL**= interleukin; **LCAT**= lecithin cholesterol acyltransferase; **MDA**= malondialdehyde; **mo**= month(s); **oxLDL**= oxidized LDL; **PON**= paraoxonase; **RCT**= randomized controlled trial; **SAA**= serum amyloid A; **sICAM-1**= soluble intercellular adhesion molecule-1; **sVCAM-1**= soluble vascular cell adhesion molecule-1; **T2D**= type 2 diabetes; **wk**= week(s)

α-tocopherol Clinical and Preclinical Evidence Related to HDL Function and Cardiovascular Health

Ingredient	Study Design	Main Findings
α-tocopherol[36]	RCT in individuals with hypercholesterolemia (n=69); 500 IU/day or placebo for 3 mo	• Increased apoA-I levels • Increased apoA-I/apoB ratio
α-tocopherol[37]	RCT in healthy subjects (n=32); 134 mg/day or 268 mg/day or placebo for up to 28 days	• Increased plasma apoA-I concentration in a time- and dose-dependent manner
α-tocopherol[38]	RCT in patients with T2D (n=83); 400 IU/day or placebo for 8 wk	• Increased PON1 activity and total antioxidant status
α-tocopherol[39]	Clinical study in athletes (n=10) in training; 200 mg/day for 1 mo	• Decreased PON1 postexercise; α-tocopherol prevented decreases in PON1 activity postexercise
α-tocopherol[40]	Clinical study in male patients with T2D (n=80); 300 mg/day for 4 wk	• Decreased SAA vs. baseline • Decreased TNF-α and hs-CRP vs. baseline
α-tocopherol[41]	Rabbits fed atherogenic diets; 125 IU/day or 1.25 IU/day (control) for 12 wk	• Reduced total cholesterol levels • Reduced esterified artery cholesterol levels

apoA-I= apolipoprotein A-I; **apoB**= apolipoprotein B; **hs-CRP**= high-sensitivity C-reactive protein; **mo**= month(s); **PON**= paraoxonase; **RCT**= randomized controlled trial; **SAA**= serum amyloid A; **T2D**= type 2 diabetes; **TNF**= tumor necrosis factor; **wk**= week(s)

Niacin Clinical Evidence Related to HDL Function and Cardiovascular Health		
Ingredient	**Study Design**	**Main Findings**
Niacin[42]	RCT in patients with a history of CVD (n=126) receiving atorvastatin 10-80 mg/day; 2 g/day or none for 1 yr	• Increased HDL-C by 39%, HDL-P by 14% and total cholesterol efflux capacity by 16% • Increased large HDL particles • Reduced small HDL particles
Extended-release niacin + laropiprant[43]	Crossover RCT in statin-treated patients (n=27) who had not achieved the LDL-C target; niacin/laropiprant 1 g/20 mg for 4 wk and then 2 g/40 mg for 8 wk or placebo for 8 wk (4 wk wash-out period)	• Significant reduction in total cholesterol, triglycerides, LDL-C, apoB, Lp(a), CETP activity, oxLDL, Lp-PLa2, lysoPC, MCP-1, SAA • No change in HDL antioxidant capacity or PON1 activity • 19.5% increase in cholesterol efflux capacity of HDL
Extended-release niacin[44]	Comparison crossover study in dyslipidemia patients (n=66) with low or normal HDL-C; fenofibrate 160 mg/day for 6 wk or niacin 0.5 g/day for 3 wk and then 1 g/day for 3 wk (4 wk wash-out period)	• Both treatments had a comparable increase in HDL-C and apoA-I, with minor changes in cholesterol efflux capacity
Extended-release niacin[45]	Crossover RCT in patients with metabolic syndrome (n=37); fenofibrate 160 mg/day for 6 wk or niacin 0.5 g/day for 3 wk and then 1 g/day for 3 wk (4 wk wash-out period)	• After either treatment patients had HDL with similar endothelial protective properties as from healthy control subjects • After treatment HDL particles improved endothelial nitric oxide production

apoA-I= apolipoprotein A-I; **apoB**= apolipoprotein B; **CETP**=cholesteryl ester transfer protein; **HDL-C**= HDL-cholesterol; **HDL-P**= HDL particle number; **LDL-C**= LDL-cholesterol; **Lp(a)**= lipoprotein(a); **Lp-PLa2**= lipoprotein phospholipase A2; **lysoPC**= lysophosphatidyl choline; **MCP-1**= macrophage chemoattractant protein; **oxLDL**= oxidized LDL; **PON**= paraoxonase; **RCT**= randomized controlled trial; **SAA**= serum amyloid A

Diet and Lifestyle Clinical Evidence Related to HDL Function and Cardiovascular Health

Diet or Lifestyle	Study Design	Main Findings
Mediterranean diet[46]	RCT of Mediterranean diet enriched with virgin olive oil (n=100) or nuts (n=100) compared to low-fat diet (n=96) in patients with high cardiovascular risk for 1 yr	• Improved HDL cardioprotective functions • Increased PON activity, cholesterol efflux capacity, vasodilation, and improved HDL composition • All diets increased percentage of large HDL particles
Mediterranean dietary components[47]	Secondary analysis of high cardiovascular risk patients (n=296) from PREDIMED clinical trial 1 yr data	• Increasing virgin olive oil (10 g/d) and whole grain (25 g/d) consumption increases cholesterol efflux capacity • Increasing nut (30 g/d), legume (25 g/d), and fish (25 g/d) intake improves PON activity
Fruits and vegetables[48]	RCT in subjects with obesity and T2D; 1 or ≥ 6 portions/day for 8 wk	• ≥ 6 portions/day increased PON1 and LCAT activities in HDL3
Improving dietary quality and increasing physical activity levels[49]	CT in men with obesity and dyslipidemia (n=113); 160 min/wk moderate intensity aerobic activity + moderate caloric restriction (-500 kcal/day) with macronutrient composition of 45-50% carbohydrate, 20-25% protein and 25-30% from fat for 1 yr; compared with control group (n=32)	• Increased cholesterol efflux capacity • Increased apoA-I levels
Smoking cessation[50]	CT in smokers (n=28) in a smoking cessation program using either varenicline or a transdermal nicotine patch for 12 wk	• Improved cholesterol efflux capacity and HDL inflammatory index compared to participants who were unable to quit smoking
Smoking cessation[51]	RCT in smokers (n=923); smoking cessation pharmacotherapies for 1 yr	• Participants who quit smoking (36.2%) had increases in HDL-C, HDL-P, and large HDL • Effects were more pronounced in women
Effect of smoking[52]	Clinical study in young smokers (n=21) and healthy controls (n=20) comparing lipoprotein parameters	• HDL from young smokers (< 10 cigarettes/d x 3 yrs) had lower antioxidant capacity, smaller particle size, and increased triglyceride content
Vigorous prolonged exercise[53]	RCT in 2 cohorts; first group with sedentary adults with prediabetes with BMI 25-35 (n=106) and the second group with similar but nondiabetic adults (n=90); varying levels of exercise for 6 mo	• Vigorous endurance exercise improved cholesterol efflux capacity • Non-ABCA1 cholesterol efflux capacity improved in highest intensity group • An exercise intensity or dosage threshold may need to be exceeded to see significant results • Exercise matures HDL particles (pre-β to α-HDL)
Exercise[54]	RCT of women with obesity (n=32); combined aerobic and resistance exercise (moderate-vigorous intensity, 40-60 min, 4x/wk) or control for 12 wk	• HDL subclasses were restored to "nonobese" state in this population • Decreased PON activity, antithrombotic actions, and distribution of small HDL particles—participants did not lose significant amount of weight • Cholesterol efflux capacity unchanged
Exercise-based cardiac rehabilitation[55]	Retrospective analysis of patients (n=57) with acute coronary syndrome who completed or dropped out (used as control) of an exercise-based program (gymnastics and aerobic exercise) 30 min, 3-5x/wk for 6 mo	• Increased HDL cholesterol efflux capacity in participants who successfully completed the program and stopped smoking • Program increased exercise capacity
Therapeutic lifestyle changes[56]	Prospective pilot study in patients with metabolic syndrome (n=25); 180 min/wk of exercise at 85% maximum heart rate for 12 wk	• Reduced oxidation molecules associated with HDL activity • Increased cholesterol efflux capacity

ABCA1= ATP-binding cassette transporter A1; **apoA-I**= apolipoprotein A-I; **BMI**= body mass index; **CT**= clinical trial; **HDL**= high-density lipoprotein; **LCAT**= lecithin cholesterol acyltransferase; **mo**= month(s); **PON**= paraoxonase; **RCT**= randomized controlled trial; **T2D**= type 2 diabetes; **wk**= week(s); **yr**= year(s)

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