atherosclerosis

Atherosclerosis newsletter

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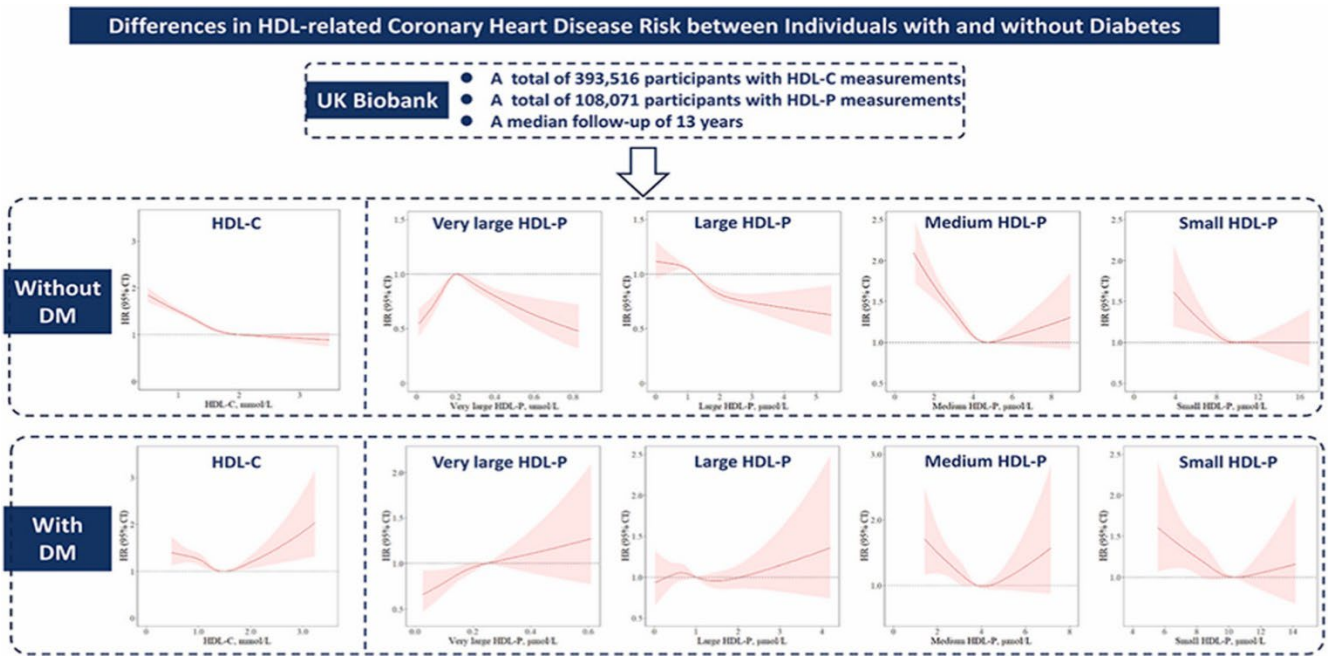
Because of the inverse relationship of HDL-cholesterol with risk of atherosclerotic cardiovascular diseases and many vasoprotective activities *in vitro* and *in vivo* HDL was considered for a long time as an anti-atherogenic lipoprotein. However, futile randomized controlled trials on the efficacy of HDL-cholesterol raising drugs or infused HDL-like particles , as well as findings of Mendelian Randomization studies, questioned causal relationships of HDL in the pathogenesis of atherosclerosis and hence its suitability as a therapeutic target. Thus, HDL-cholesterol is now widely considered as an indirect marker of cardiovascular risk, which reflects other pathogens, notably triglyceride-rich lipoproteins. Nevertheless, several researchers continued to search for HDL-related biomarkers beyond HDL-cholesterol with direct / causal disease-relationships, for example HDL subclasses or distinct proteins or lipids or functions of HDL.

The September and October issues of *Atherosclerosis* contain several articles on structure-function-disease relationships of HDL, classical functions of HDL, namely cholesterol efflux and reverse cholesterol transport, as well as less explored functions such as inhibition of proteolysis.

Differences in HDL-related coronary heart disease risk between individuals with and without diabetes



Inverse association of high-density lipoprotein cholesterol (HDL-C) with coronary heart diseases (CHD) risk has been established by epidemiological studies for decades. However, large-scale randomized clinical trials or Mendelian randomization studies failed to find a protective causal effect of HDL-C on CHD risk. This arose more detailed investigations into the diversity of HDL structure and function, with a novel focus on HDL particle (HDL-P), which emerged as a stronger predictor of cardiovascular risk than conventional HDL-C. Some recent studies have found that biological functions of HDL-P differed in sizes, with smaller HDL-P displaying more potent atheroprotective properties. Epidemiologic studies also supported heterogeneity across HDL subclasses by sizes. In the present study, using data from the UK Biobank, Chen et al. aimed to investigate the potential modifying effect of diabetes on the dose-response associations of conventional chemometric measurement of HDL-C and novel HDL-P subclasses measured by nuclear magnetic resonance (NMR) with the risk of CHD.

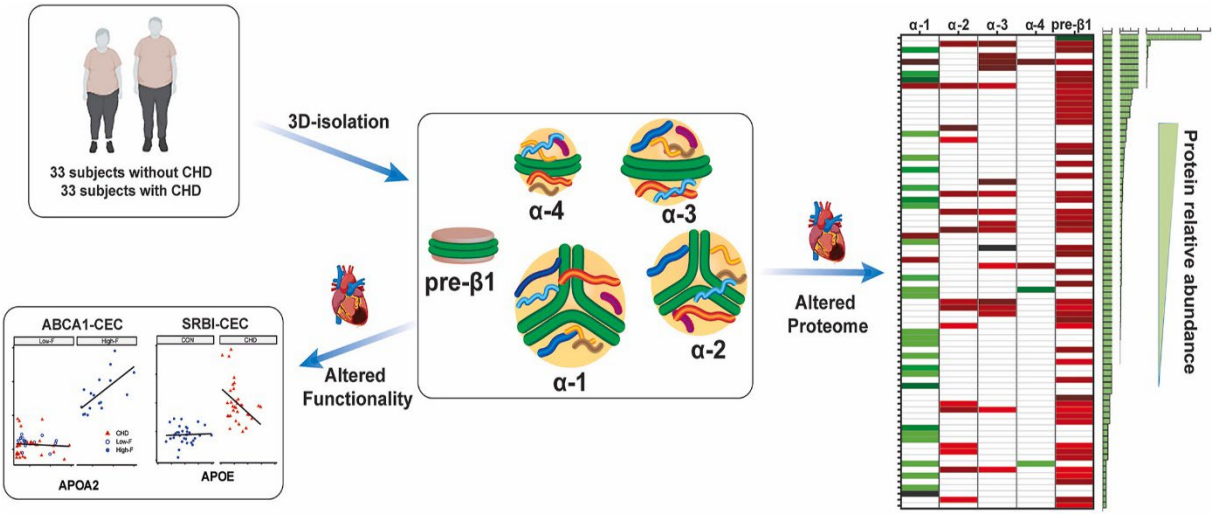
They included 393,516 participants (20,691 diabetics and 372,825 nondiabetics) from the UK Biobank. Restricted cubic splines cooperated with Cox model were used to estimate associations of HDL with CHD.

During a median follow-up of 13.0 years, 3398 (16.4 %) and 24,772 (6.6 %) incident CHD events occurred among diabetics and nondiabetics, respectively. HDL-C showed inverse associations with CHD among nondiabetics, whereas U-shaped associations among diabetics. Compared to individuals with normal HDL-C, those in the top percentile had lower CHD risks among nondiabetics, but higher risks among diabetics. As for HDL-P, there were inverted U-shaped associations of very large HDL-P and

linearly negative associations of large HDL-P with CHD among nondiabetics; however, linearly positive associations of very large HDL-P and null associations of large HDL were observed among diabetics. L-shaped associations of medium and small HDL-P were found both in diabetics and nondiabetics.

Very high HDL-C levels were associated with lower CHD risks in nondiabetics, but higher risks in diabetics. Smaller HDL-P was negatively, whereas very large HDL-P was positively associated with CHD risk in diabetics.

Relationships between HDL subpopulation proteome and HDL function in overweight/obese people with and without coronary heart disease



High-density lipoprotein (HDL) is the most heterogeneous lipoprotein class with particles varying in size, composition, and functions. Depending on the isolation and detection methods, omics studies have identified several hundred protein and lipid species in HDL and in its subfractions. The structure-function relationships of high-density lipoprotein (HDL) subpopulations are not well understood. Vaisar et al. aimed to examine the interrelationships between HDL particle proteome and HDL functionality in subjects with and without coronary heart disease (CHD).

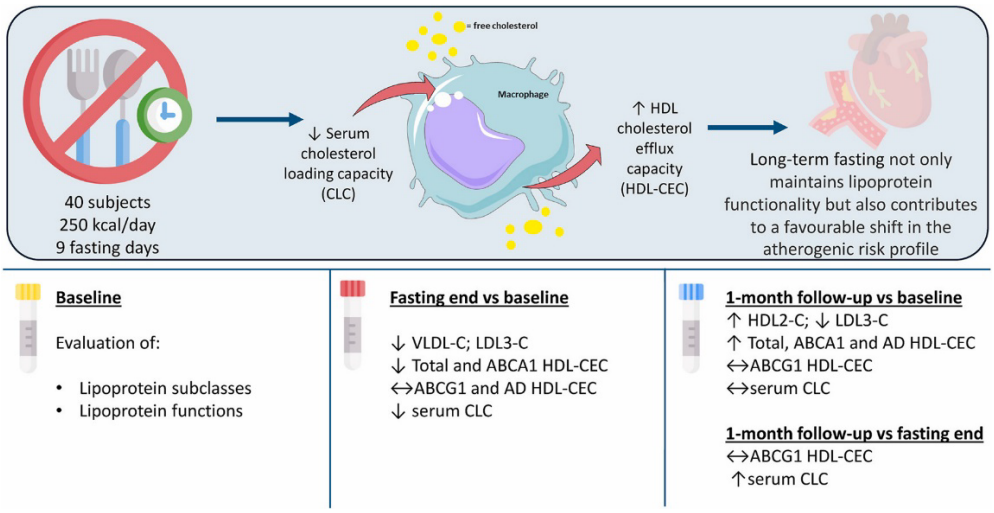
The authors isolated 5 different HDL subpopulations based on charge, size, and apolipoprotein A1 (APOA1) content from the plasma of 33 overweight/obese CHD patients and 33 age-and body mass index (BMI)-matched CHD-free subjects. The relative molar concentration of HDL-associated proteins was measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) and particle functionality was assessed.

110 proteins associated with the 5 APOA1-containing HDL subpopulations were quantified. The relative molar concentration of these proteins spanned five orders of magnitude. Only 10 proteins were present in >1% while 73 were present in <0.1% concentration. Only 6 of the 10 most abundant

proteins were apolipoproteins. Interestingly, the largest (α-1) and the smallest (preβ-1) HDL particles contained the most diverse proteomes. The protein composition of each HDL subpopulation was altered in coronary heart disease (CHD) cases as compared to controls, with the most prominent differences in preβ-1 and α-1 particles. APOA2 concentration was positively correlated with preβ-1 particle functionality (ABCA1-CEC/mg APOA1 in preβ-1), while APOE concentration was inversely correlated with large-HDL particle functionality (SRBI-CEC/mg APOA1 in α-1+α-2).

The protein composition of the different HDL subpopulations was altered differentially in CHD patients. The functionality of the small and large HDL particles correlated with the protein content of APOA2 and APOE, respectively.

HDL cholesterol efflux capacity and cholesterol loading capacity in long-term fasting: Evidence from a prospective, single-arm interventional study in healthy individuals



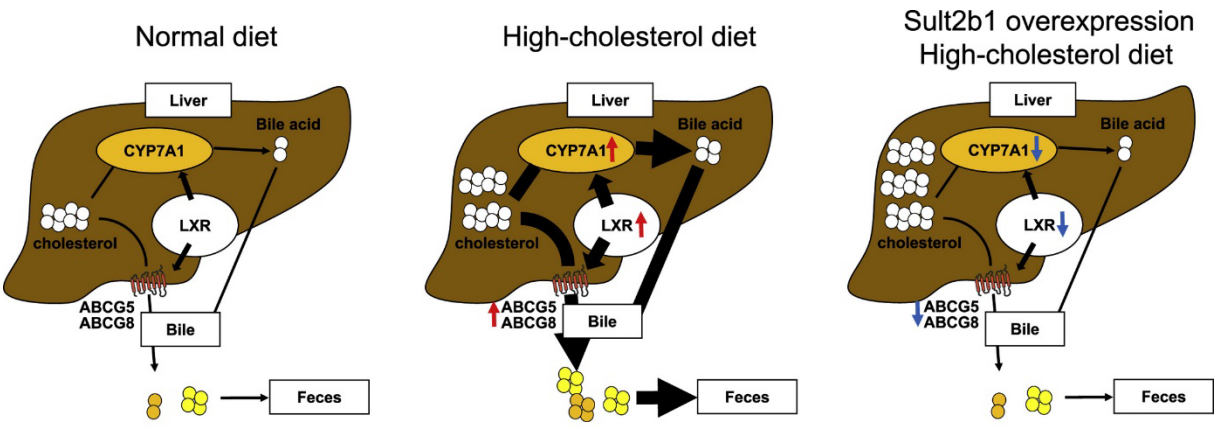
Long-term fasting (LF) can be defined as the abstinence from food for at least 4 days to several weeks and is increasingly emerging as a non-pharmacological approach to modulate risk factors associated with the development of atherosclerotic cardiovascular disease (ASCVD). The fasting metabolism is characterized by the switch from food glucose to adipose tissue-derived lipids and ketones as fuels. Blood glucose and insulin levels decrease as well as triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). However, protection from ASCVD is more tied to the functionality of high-density lipoprotein (HDL) than its plasma levels. In this prospective interventional study Grundler et al. focus on the functional properties of lipoproteins in modulating cholesterol homeostasis on peripheral cells and examines how LF may influence this and lipoprotein subclass composition. For that purpose, the authors investigated its impact on HDL-cholesterol efflux capacity (CEC), and on serum cholesterol loading capacity (CLC).

Forty healthy subjects (50 % females) underwent medically supervised 9-day fasting (250 kcal/day) in a specialised facility. Thirty-two subjects had a follow-up examination after one month of food reintroduction.

LF was well tolerated and increased self-reported energy levels. Fasting reduced triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and HDL cholesterol (HDL-C). Very-low-density lipoprotein cholesterol (VLDL-C) and LDL3-C showed sustained reductions at follow-up. Only HDL-C, specifically HDL2-C levels, increased at follow-up. Total HDL-CEC decreased during LF and increased above baseline at follow-up. Fasting decreased ATP binding cassette (ABC)A1-mediated HDL-CEC whereas ABCG1-mediated HDL-CEC remained unaffected. Aqueous diffusion increased at follow up. LF decreased serum CLC and then returned to baseline levels.

These results show that LF not only maintains lipoprotein functionality but also contributes to a favorable shift in the atherogenic risk profile, which persists even after food reintroduction. This further emphasizes the importance of considering HDL functionality alongside traditional lipid measurements to understand the potential for non-pharmacological interventions like LF to promote cardiovascular prevention and health.

Liver-specific Lxr inhibition represses reverse cholesterol transport in cholesterol-fed mice



Liver specific LXR inhibition represses RCT under High-cholesterol diet

Low plasma levels of HDL-cholesterol (HDL-C) have been consistently associated with increased risk of atherosclerotic cardiovascular diseases. It is therefore considered to be an anti-atherogenic lipoprotein. HDL exerts an anti-atherosclerotic effect via reverse cholesterol transport (RCT). Several phases of RCT are transcriptionally controlled by Liver X receptors (Lxrs). Although macrophage Lxrs reportedly promote RCT, it is still uncertain whether hepatic Lxrs affect RCT *in vivo*.

To gain further insights into the effects of hepatic Lxrs on RCT, Nishida et al. inhibited Lxr-dependent pathways in mouse livers, by performing hepatic overexpression of sulfotransferase family

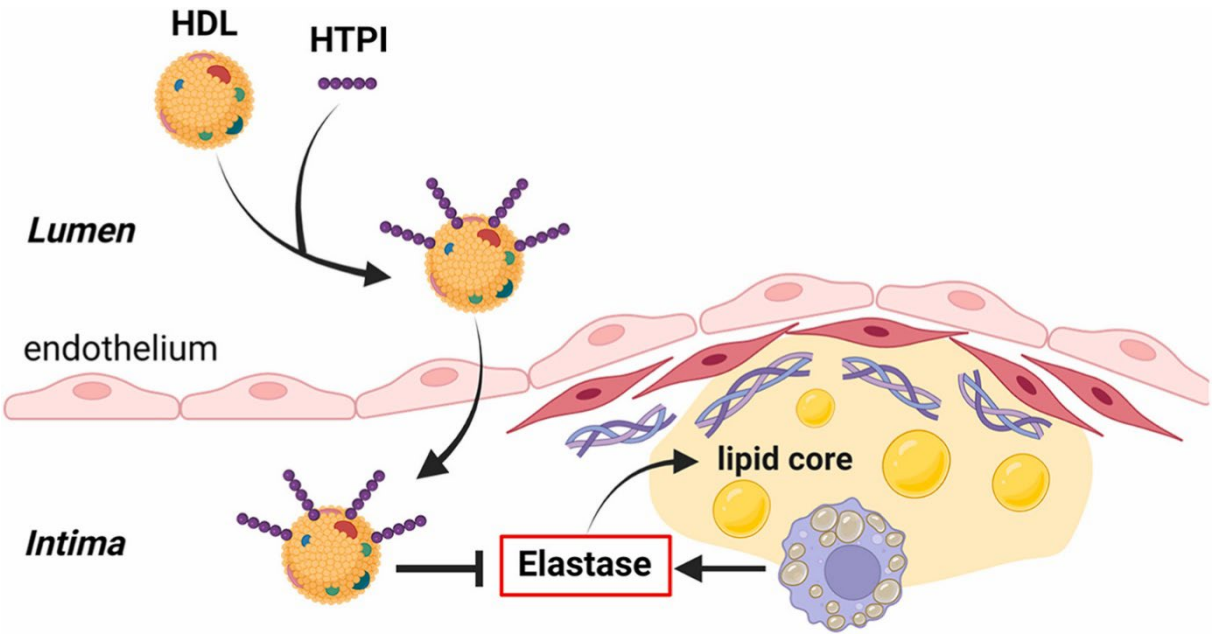
cytosolic 2B member 1 (Sult2b1) using adenoviral vector (Ad-Sult2b1). Ad-Sult2b1 or the control virus was intravenously injected into wild type mice and *Lxrα/β* double knockout mice, under a normal or high-cholesterol diet. A macrophage RCT assay and an HDL kinetic study were performed.

Hepatic Sult2b1 overexpression resulted in reduced expression of Lxr-target genes: ATP-binding cassette transporter G5/G8, cholesterol 7α hydroxylase and Lxrα itself, reducing or increasing cholesterol levels in HDL and apolipoprotein B–containing lipoproteins (apoB-L). A macrophage RCT assay revealed that Sult2b1 overexpression inhibited fecal excretion of macrophage-derived ^{3}H-cholesterol only under a high-cholesterol diet. In an HDL kinetic study, Ad-Sult2b1 promoted catabolism/hepatic uptake of HDL-derived cholesterol, thereby reducing fecal excretion. Finally, in *Lxrα/β* double knockout mice, hepatic Sult2b1 overexpression increased apoB-L levels, but there were no differences in HDL levels or RCT compared to the control, indicating that Sult2b1-mediated effects on HDL/RCT and apoB-L were distinct: the former was Lxr-dependent, but not the latter.

Hepatic Lxr inhibition negatively regulates circulating HDL levels and RCT by reducing Lxr-target gene expression.

These results may provide the basis for Lxr-targeted strategies against atherosclerosis and are further discussed in the editorial by Paolo Parini.

Enhancement of high-density lipoprotein-associated protease inhibitor activity prevents atherosclerosis progression



Elevated plasma concentrations of low-density lipoprotein (LDL) contribute to atherogenesis. LDL particles infiltrate the vascular wall where they become trapped and modified by oxidative processes. Uptake of modified LDL by resident macrophages results in foam cell formation and the recruitment of additional inflammatory cells to the lesion, including neutrophils and monocyte-derived macrophages. Inflammatory cells within atherosclerotic lesions secrete proteolytic enzymes that contribute to lesion progression and destabilization, increasing the risk for an acute cardiovascular event. Elastase is a serine protease, secreted by macrophages and neutrophils that may contribute to the development of unstable plaque. Mobilia et al. previously reported interaction of endogenous protease-inhibitor proteins with high-density lipoprotein (HDL), including alpha-1-antitrypsin, an inhibitor of elastase. These findings support a potential role for HDL as a modulator of protease activity. In this study, they describe a novel HDL-targeting protease inhibitor peptide, which binds predominantly to HDL in plasma and confers elastase-inhibitor activity. Using this peptide, the authors test the hypothesis that enrichment of HDL-associated elastase inhibitor activity is protective against lesion progression in a mouse model of atherosclerosis.

An HDL-targeting protease inhibitor (HTPI) that binds to HDL and confers elastase inhibitor activity was designed. Lipoprotein binding and the impact of HTPI on atherosclerosis were examined using mouse models. Histology and immunofluorescence staining of aortic root sections were used to assess the impact of HTPI on lesion morphology and inflammatory features.

Intravenous administration of HTPI to mice resulted in its binding to plasma HDL and increased elastase inhibitor activity on isolated HDL. Accumulation of HTPI within plaque was observed after

administration to *Apoe*$^{-/-}$ mice. To assess the effect of HTPI treatment on atherosclerosis, prevention and progression studies were performed using *Ldlr*$^{-/-}$ mice fed Western diet. In both study designs, HTPI-treated mice had reduced lipid deposition in plaque.

These data support the hypothesis that HDL-associated anti-elastase activity can improve the atheroprotective potential of HDL and highlight the potential utility of HDL enrichment with anti-protease activity as an approach for stabilization of atherosclerotic lesions.