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BRIEF REVIEW

Proceedings of the Ninth HDL (High-Density Lipoprotein) Workshop

Focus on Cardiovascular Disease

Annabelle Rodriguez, Bernardo L. Trigatti, Chieko Mineo, Darcy Knaack, John T. Wilkins, Daisy Sahoo, Bela F. Asztalos, Samia Mora, Marina Cuchel, Henry J. Pownall, Corina Rosales, Pascal Bernatchez, Amanda Ribeiro Martins da Silva, Godfrey S. Getz, Jacob L. Barber, Gregory C. Shearer, Angela M. Zivkovic, Uwe J.F. Tietge, Frank M. Sacks, Margery A. Connelly, Michael N. Oda, W. Sean Davidson, Mary G. Sorci-Thomas, Tomas Vaisar, Giacomo Ruotolo, Kasey C. Vickers, Catherine Martel

ABSTRACT: The HDL (high-density lipoprotein) Workshop was established in 2009 as a forum for candid discussions among academic basic scientists, clinical investigators, and industry researchers about the role of HDL in cardiovascular disease. This ninth HDL Workshop was held on May 16 to 17, 2019 in Boston, MA, and included outstanding oral presentations from established and emerging investigators. The Workshop featured 5 sessions with topics that tackled the role of HDL in the vasculature, its structural complexity, its role in health and disease states, and its interaction with the intestinal microbiome. The highlight of the program was awarding the Jack Oram Award to the distinguished professor emeritus G.S. Getz from the University of Chicago. The tenth HDL Workshop will be held on May 2020 in Chicago and will continue the focus on intellectually stimulating presentations by established and emerging investigators on novel roles of HDL in cardiovascular and noncardiovascular health and disease states.

Key Words: American Heart Association ■ cardiovascular disease ■ cholesterol ■ health ■ lipoproteins

The HDL (high-density lipoprotein) Workshop was established in 2009 to advance our understanding of HDL biology.[1] In clinical medicine, the measurement of the cholesterol (C) component of HDL, HDL-C, remains a standard-of-care requirement for cardiovascular disease (CVD) risk prediction. In 2019, the American Heart Association and other professional societies issued an updated guideline on cholesterol management for primary and secondary CVD prevention.[2] This document targeted clinical management of LDL (low-density lipoprotein)-C and non–HDL-C, while providing no updates on HDL, HDL-C, or HDL functional metrics in CVD risk assessment. The American Heart Association and the American College of Cardiology did provide an online risk calculator to help clinicians assess CVD risk in their patients.[3] The risk factors included in the calculator are sex, age, race, total cholesterol, LDL-C, HDL-C, statin treatment, systolic blood pressure, hypertension treatment, history of diabetes mellitus, current smoker, and aspirin therapy. Another well-known CVD risk calculator, the Framingham Risk Score, also incorporates HDL-C as a component in the 10-year risk assessment and assigns a score of −1 if patients have HDL-C≥60 mg/dL.[4]

The latter would suggest that higher HDL-C levels are cardioprotective, but this paradigm is now being challenged, and many argue for a reassessment of HDL-C in these risk calculators. Many clinical investigators agree that the results of recent genetic association studies, as well as large clinical trials targeting increases in HDL-C to reduce CVD risk as a primary end point, have been disappointing.[5–8] Such observations leave the field wide open in better understanding the complexities of HDL biology and where this research might be of help to

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Nonstandard Abbreviations and Acronyms

ApoA-I	apolipoprotein A-I
CEC	cholesterol efflux capacity
CHD	coronary heart disease
CVD	cardiovascular disease
EPA	eicosapentaenoic acid
EVs	extracellular vesicles
FC	free cholesterol
HDL	high-density lipoprotein
HDL-p	HDL particle
JUPITER	Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin
LDL	low-density lipoprotein
LVs	lymphatic vessels
PUFA	polyunsaturated fatty acid
oxLDL	oxidized LDL
RCT	reverse cholesterol transport
SOF	serum opacity factor
SR-B1	scavenger receptor class B type I
WT	wild-type

Highlights

- HDL (high-density lipoprotein) Workshop celebrates its ninth year as a forum for academic basic scientists, clinical investigators, and industry researchers to have candid discussions about HDL biology.
- Presenters discussed novel aspects of scavenger receptors on cardiomyocyte function, LDL (low-density lipoprotein) transendothelial cytosis, and lipid raft distribution.
- Presenters discussed many methodologies to evaluate the HDL proteome and association with cardiovascular disease.
- Presenters discussed influences like serum opacity factor, omega-3 fatty acids, egg consumption, exercise, and the intestinal microbiome on different aspects of HDL biology.
- G.S. Getz provided a comprehensive discussion about apoA-I and HDL proteome in atherosclerosis as well as new in vivo approaches to assess HDL functionality.

clinicians. A major objective of the HDL Workshop has been, and will continue to be, a forum for academic basic scientists, clinicians, and industry researchers to share their research findings and perspectives. Over the past 10 years attendance has steadily grown demonstrating that interest in HDL biology is alive and well. Therefore, we now present the first of many future HDL Workshop Proceedings with the hopes of engaging scientists and clinicians to attend and present their research on this fascinating topic.

In this year's program, the HDL Workshop took place in Boston, MA, over a 2-day period. It included oral presentations from invited established investigators as well as short talks from trainees. A new feature was a poster presentation session. The highlight of the event was the annual Jack Oram Research Award, honoring the late Dr Jack Oram and generously funded by the Dyslipidemia Foundation of Boston. This year's award was presented to the distinguished professor emeritus Dr G.S. Getz from the University of Chicago.

HDL WORKSHOP PROGRAM

The program was chaired by Dr Catherine Martel and co-chaired by Dr Rodriguez. Over the course of the 2-day program, there were 5 sessions of oral presentations. The topics of each session were the following: HDL and the Vasculature, HDL Structural Complexity, Clinical Perspectives on HDL Measurement, HDL in Health and Disease, and Microbiota and HDL Metabolism. Please note that each of the following abstracts were written by the presenter with references included for the reader's benefit.

Session 1: HDL and the Vasculature

1. Bernardo L. Trigatti, PhD, Professor, Department of Biochemistry and Biomedical Sciences, McMaster University and Thrombosis and Atherosclerosis Research Institute, McMaster University and Hamilton Health Sciences, Hamilton, Ontario, Canada. *HDL and SR-B1 in Cardiovascular Disease: Atherosclerosis and Beyond.*

Global knockout of the HDL receptor, SR-B1 protein (scavenger receptor class B type I (*Scarb1* gene), in atherogenic mice increases their susceptibility to high-fat diet induced aortic sinus atherosclerosis.[9] In addition, when fed highly atherogenic, high fat and high cholesterol diets, *Scarb1/Ldlr* double knockout mice develop occlusive coronary artery atherosclerosis, extensive myocardial fibrosis, and early death.[10] The extensive myocardial fibrosis is undoubtedly triggered by the occlusive coronary atherosclerosis in these mice, but we wondered if it might also reflect a role for SR-B1 in cardiomyocytes themselves. SR-B1 is expressed in cardiomyocytes, and SR-B1's ligand, HDL, is able to protect cultured mouse and human cardiomyocytes from apoptosis induced by the anti-cancer drug doxorubicin.[11] This protection is lost when SR-B1 is inactivated. HDL induces phosphorylation of Akt in mouse and human cardiomyocytes in an SR-B1 dependent manner and Akt1 or Akt2 knockdown impair HDL-mediated protection against doxorubicin mediated apoptosis.[12] In vivo, treatment of mice with doxorubicin over 5 weeks triggers greater cardiomyocyte apoptosis in *Scarb1*$^{-/-}$ than

wild-type (WT) mice. Transplantation studies implicate SR-B1 in the heart itself. Finally, human apoA-I (apolipoprotein A-I) transgenic overexpression or injection protect WT but not *Scarb1*$^{-/-}$ mice against doxorubicin induced cardiomyocyte apoptosis and cardiac dysfunction.[11,12] In conclusion, while global knockout studies clearly demonstrate a protective role for SR-B1 against atherosclerosis (likely by virtue of its function in liver mediated reverse cholesterol transport [RCT]), SR-B1 and HDL appear to have cardioprotective roles beyond atherosclerosis. Notably, this includes protection of cardiomyocytes against cytotoxicity by the chemotherapeutic agent doxorubicin.

2. Chieko Mineo, PhD, Associate Professor, Center for Pulmonary and Vascular Biology, Department of Pediatrics and Cell Biology, University of Texas Southwestern Medical Center, Dallas, Texas. *SR-B1 and Atherosclerosis: A New Paradigm for an Old Story.*

The biology of the high-affinity HDL receptor SR-B1 is best understood in the liver, where the receptor plays a key role in RCT, facilitating cholesterol transfer from HDL to hepatocytes for disposal in bile.[13] The global SR-B1 knockout mice show exaggerated atherosclerosis in the setting of hypercholesterolemia. In addition to the liver, SR-B1 is expressed in multiple cell types that are relevant to atherogenesis, including macrophages, platelets, and endothelium. In endothelial cells, SR-B1 has been shown to promote production of the antiatherogenic molecule nitric oxide.[14] To study how endothelial SR-B1 impacts atherosclerosis, we generated mice lacking SR-B1 selectively in the endothelium by crossing floxed *Scarb1* mice with VE-Cadherin Cre mice (*Scarb1*$^{\Delta EC}$).[15] Contrary to our prediction, *Scarb1*$^{\Delta EC}$ mice were protected from hyperlipidemia-induced atherosclerosis without affecting the plasma lipid profiles. Recognizing that SR-B1 binds both HDL and LDL and that LDL uptake into the vascular wall is the initial step of atherogenesis, we hypothesized that SR-B1 facilitates LDL transport through the endothelial layer. Using multiple loss-of-function approaches in vivo, we demonstrated that SR-B1 promotes LDL uptake into the aorta. Studies in cultured endothelial cells further revealed that transendothelial transport of LDL requires SR-B1 C-terminal cytoplasmic domain that recruits DOCK4. DOCK4 initiates LDL–SR-B1 internalization via activation of the small GTPase Rac1. Our study revealed that expression of SR-B1 and DOCK4 is elevated in human atherosclerotic arteries compared with normal arteries. These findings suggest that the mechanisms that influence LDL transport across endothelium might be targeted to provide novel interventions to prevent atherosclerosis.

3. Catherine Martel, PhD, Associate Professor, Canada Research Chair in Lymphatics and Cardiovascular Medicine, Department of Medicine, Université de Montréal, Montreal Heart Institute, Montreal, Quebec, Canada. *Extracellular Vesicles at the Heart of Lymphatic Function.*

Lymphatic vessels (LVs) form a whole-body network that maintains fluid balance, dietary lipid absorption, and pathogen clearance from peripheral tissues. In the past years, the direct role of LVs in atherosclerosis has been more fully assessed. We reported that functional adventitial LV are essential to first mobilize cholesterol out of the vessel wall before reaching the circulation.[16] A defect in the propelling capacity of the collecting LV, rather than a defect in the absorptive capacity of the lymphatic capillaries would be the instigating element of this atherosclerosis-related lymphatic dysfunction.[17] We are now pursuing the mechanisms that contribute to the loss of collecting LV function during atherosclerosis, and how can they be modulated. Cell fragments called extracellular vesicles (EVs) are important cell-cell messengers released on cell activation or death and are found in atherosclerotic lesions. We reported for the first time that EVs of heterogeneous origins are also present in lymph and that these small vesicles are even more abundant in atherosclerotic mice.[18] With preliminary data suggesting that specific subsets of EVs could be harmful to the lymphatic endothelium,[19] we hypothesize that EVs accumulation could be, at least in part, responsible for the atherosclerosis-related lymphatic dysfunction. This massive accumulation of EVs in the artery wall could, in turn, be due to the underlying reduced LV function. Altogether, these data suggest that EVs might be the connection between abnormal lymphatic function and atherosclerosis progression.

4. Darcy Knaack, PhD, graduate student (Dr Daisy Sahoo, mentor), Department of Biochemistry, Division of Endocrinology, Medical College of Wisconsin, Milwaukee, WI. *The roles of SR-B1 and CD36 in Maintenance of Macrophage Cholesterol Homeostasis.*

Atherosclerosis is a chronic inflammatory disease characterized by buildup of cholesterol-rich macrophages within the endothelium. Two scavenger receptors that regulate macrophage cholesterol homeostasis and cholesterol transport are SR-B1 and CD36, which bind HDL and oxLDL (oxidized LDL), respectively. Their individual roles in modulating atherosclerosis have been widely studied, but how they influence each other's functions has yet to be investigated.[20,21] To test our hypothesis that SR-B1 and CD36 are functional partners that mediate macrophage cholesterol homeostasis, we performed 4 different assays in peritoneal macrophages from WT, *Scarb1*$^{-/-}$, and *Cd36*$^{-/-}$ mice. First, coimmunoprecipitation assays demonstrated a potential interaction between SR-B1 and CD36 that was enhanced with oxLDL treatment in WT macrophages. Next, we examined how cholesterol transport functions of these receptors were affected when one receptor was absent. We provide evidence that when CD36 is absent, the ability of HDL to bind surface receptors was impaired, as was the ability of the cell ability to internalize HDL-cholesterol as compared to WT or *Scarb1*$^{-/-}$ mice. We further show

that membrane distribution of cholesterol is altered when either receptor is absent. Last, we used sucrose-gradient fractionation to examine whether absence of SR-B1 or CD36 impacts membrane localization of the other receptor. Our data suggest that, in WT cells, SR-B1 and CD36 reside within lipid raft microdomains, but when one receptor is absent, the other migrates to nonraft domains. Based on these observations, there seems to be a cooperative partnership between SR-B1 and CD36, suggesting they may work together to promote macrophage cholesterol homeostasis.

Session 2: HDL Structural and the Vasculature

1. John T. Wilkins, MD, Associate Professor, Department of Medicine, Division of Cardiology, Northwestern University, Chicago, IL. Henrique Seckler, MS, Departments of Chemistry and Molecular Biosciences, the Chemistry of Life Processes Institute, and the Proteomics Center of Excellence, Northwestern University, Evanston, IL. *Differential ApoA-I Proteoform Quantification across HDL Particle Size Subtypes.*

HDL particle (HDL-p) size subtypes differ in proteome, lipidome, and functional characteristics.[22] We recently reported that apoA-I has specific post-translational modifications that occur at precise amino acid residues, yielding 14 distinct specific molecular forms (proteoforms) of apoA-I.[23] Fatty acid-modified proteoforms of ApoA-I (Acyl-K88 apoA-I) are associated with HDL efflux, but it is unknown if apoA-I proteoforms vary with HDL-p size. We hypothesized that HDL subtypes have distinct apoA-I proteoform profiles. Using pooled samples from 30 healthy Coronary Artery Risk Development in Young Adults participants we adapted CN-GELFrEE, a native acrylamide-gel electrophoretic technique, to separate HDL-p by size. We used Western blot to quantify apoA-I content and average particle size of the electrophoretic fractions and then submitted apoA-I-containing fractions to liquid-chromatography mass spectrometry for proteoform quantification. We observed 36 distinct HDL size fractions covering the size range of HDL (5–11 nm). Proteoform quantification revealed a significantly higher proportion of Acyl-K88 apoA-I in the pre-β-1 (5–7.5 nm) and α-1 (9–11 nm) size ranges in comparison to medium size subtypes (7.5–9 nm; mean fold difference: 1.4× and 1.2×, respectively). Glycated and oxidized proteoforms were significantly more abundant (mean fold difference: 1.7× and 1.8×, respectively) in the medium size ranges. We concluded that CN-GELFrEE is a novel, high-resolution HDL size separation technique and is compatible with downstream proteomics. These data suggest the profile of apoA-I proteoforms in HDL-p is size-related and that apoA-I proteoforms may be important markers or mediators of different functional characteristics across HDL size subspecies.

2. Daisy Sahoo, PhD, Professor of Medicine, Division of Endocrinology, Medical College of Wisconsin, Milwaukee, WI. *The importance of HDL clearance: lessons from Dr Jekyll and Mr Hyde.*

HDL protects against CVD, primarily due to its role in delivering peripheral cholesterol to the liver for excretion via RCT. The last step in RCT requires the binding of HDL to SR-B1. Paradoxically, despite high circulating levels of HDL-C, humans harboring common and rare SR-B1 (*SCARB1* gene) mutations have higher CVD risk, paralleling the failure of HDL-raising therapeutics to protect against CVD.[5–8,24,25] Thus, the field is appreciating that efficient cholesterol flux via RCT is critical to maintaining the protective functions of HDL. We tested the hypothesis that impaired HDL clearance promotes HDL dysfunction through increased oxidative modification. We observed that HDL modified by reactive aldehydes such as acrolein and malondialdehyde impaired the atheroprotective functions of HDL in macrophages. Compared to native HDL, acrolein- and malondialdehyde-modified HDL have impaired abilities to promote migration of primary murine peritoneal macrophages. Furthermore, malondialdehyde-HDL increased the ability of macrophages to generate reactive oxygen species. Given these findings, our laboratory took a structure-function approach to understand how the SR-B1-HDL interaction can enhance cholesterol clearance via RCT. In our recently solved nuclear magnetic resonance spectroscopy structure of an SR-B1 peptide, we have identified a short amphipathic α-helix that, when mutated along its hydrophobic face in full-length SR-B1, exhibits impaired HDL binding and delivery of HDL-C into cells. Using electron paramagnetic resonance spectroscopy, we are testing whether this region is a juxtamembrane helix that mediates membrane/receptor interactions to facilitate cholesterol transport. Together, an improved understanding of SR-B1 structure will help us develop strategies to enhance RCT and reduce CVD risk.

3. Bela Asztalos, PhD, Scientist I, Human Nutrition Research Center,Tufts University, Boston, MA. *Analyses on HDL composition and function.*

The composition and functional relationship of HDL-p is poorly understood. We tested the hypothesis that the functionality of HDL-p is significantly influenced by their lipid composition. We determined apoA-I and 8 lipid classes in 5 different-size HDL subpopulations isolated from coronary heart disease (CHD) patients having low pre-β-1 functionality (as defined by pre-β-1-concentration-normalized cholesterol efflux capacity) and control subjects having either low or high pre-β-1 functionality. The average number of apoA-I and lipid molecules were 4 and 426 in α-1, 3 and 227 in α-2, 2 and 112 in α-3, 2 and 96 in α-4, and 2 and 57 in pre-β-1, respectively. Interestingly, one-third of the lipid molecules in the discoid-shaped pre-β-1 particles were core lipids (CE and TG). The phospholipid/CE and free cholesterol/

CE ratios were significantly higher in pre-β-1 compared with α-mobility particles. The pre-β-1 phospholipid/CE ratio was significantly higher in low pre-β-1 functionality subjects independent of the presence or absence of CHD. There were strong positive correlations between pre-β-1 particle functionality and the abundance of major lipids in subjects with high pre-β-1-functionality but not in subjects with low-pre-β-1-functionality and CHD. The lipid composition of the large-HDL-p was significantly different between the CHD and the control groups. In CHD patients, the high TG/CE and phospholipid/CE ratios were associated with increased functionality of large-HDL-p. These data do not support clear causative relationships between the lipid composition and the functionality of HDL-p. Considering these results, we hypothesize that other factors, for example, the lipid-binding capacity of apoA-I, regulate HDL-p functionality.

Session 3: Clinical Perspectives on HDL Measurement

1. Annabelle Rodriguez, MD, Professor, Cell Biology, Linda and David Roth Chair of Cardiovascular Research, Center for Vascular Biology, University of Connecticut Health, Farmington, Connecticut. *High HDL-C Paradox: A Clinical Vignette.*

A clinical vignette was presented to highlight the case of a 52-year-old woman with elevated HDL-C (110 mg/dL) and elevated LDL-C (170 mg/dL) levels with a positive family history of premature CVD. The patient had no known personal history of CVD, smoking, or history of diabetes mellitus, but on physical examination was found to have bilateral carotid bruits. The patient was reluctant to start statin therapy as she and her primary care physician believed that the HDL-C levels would provide cardioprotection. This case demonstrated the high HDL-C paradox and the need for more research in this group of patients to uncover the underlying genetic and nongenetic etiologies of increased CVD risk.[24,25]

2. Samia Mora, MD, Director, Center for Lipid Metabolomics, Associate Professor of Medicine, Department of Medicine, Divisions of Preventive and Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA. *Clinical perspectives on HDL measurement.*

While HDL-C is a strong inverse indicator of CVD risk, this has not yet translated into clinical benefit. Given the extreme heterogeneity of HDL structure and function, measuring only the cholesterol content of HDL will, at best, only partially reflect the potential role of HDL in CVD risk assessment and therapeutic drug development. This has led to interest in developing HDL metrics that might better indicate the atheroprotective functions of HDL. Proposed measurements include HDL-p, average size, subclasses, and functional assays. Of the alternate HDL metrics, the number of HDL-p has potential utility in CVD prevention. At present, it can be measured clinically by nuclear magnetic resonance spectroscopy (LabCorp) and ion mobility analysis (Quest Diagnostics). Most studies have been based on the nuclear magnetic resonance spectroscopy method. Data from the Multi-Ethnic Study of Atherosclerosis and the placebo arm of the JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) showed that HDL-C is no longer predictive of CVD after adjusting for HDL-p, while HDL-p remained inversely associated with CVD after adjusting for HDL-C.[26,27] HDL-p might also be of potential value in targeting residual CVD particularly for primary prevention as indicated by data from the rosuvastatin arm of JUPITER, where HDL-p, but not HDL-C, was inversely predictive of CVD.[28]

2. Marina Cuchel, MD, PhD, Research Associate Professor, Department of Medicine, Division of Translational Medicine and Human Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA. *Clinical perspectives on HDL measurement.*

Low levels of HDL-C are associated with increased CVD risk. However, contrary to LDL-C, the HDL-C association with CVD risk is not linear and, in recent years, several data have emerged highlighting the absence of protection against CVD, or even an increase in CVD risk, at high HDL-C concentrations, suggesting the presence of a U-shaped curve between HDL-C and all-cause mortality.[29,30] Furthermore, HDL-C is not an adequate biomarker of HDL function, and several additional biomarkers are currently being investigated. RCT is one of the most important atheroprotective functions of HDL. Cholesterol efflux capacity (CEC), an in vitro assay assessing the ability of HDL to efflux cholesterol from macrophages (the first step of RCT), has been inversely associated with both prevalence and incidence of CVD in several studies.[31,32] Unfortunately, it is not available in routine clinical care, and its results may be affected by underlying conditions, such as diabetes mellitus and an inflammatory status. Recently, a method to assess macrophage-specific RCT in vivo has been developed that could be of use during drug development.[33] Excitingly, thanks to the greater awareness of its remarkable heterogenicity in size and composition, our understanding of HDL's role in the physiopathology of many diseases and conditions is expanding beyond its role in the progression of atherosclerosis and RCT.

Session 4: HDL in Health and Disease

1. Henry J. Pownall, PhD, Professor of Bioenergetics, and Corina Rosales PhD, Assistant Professor of Bioenergetics, Institute for Academic Medicine, Houston Methodist, Weill Cornell Medical College, Houston, TX. *HDL and serum opacity factor.*

The optimal range for a low risk of atherosclerotic CVD among humans is HDL-C between 60 and 80

mg/dL with higher and lower concentrations associated with higher risk. Whereas low plasma HDL-C levels are associated with metabolic syndrome, the cause of atherosclerotic CVD among patients with high plasma HDL-C levels is unknown and interventions that rescue HDL function have not been identified. *Scarb1*$^{-/-}$ mice, which have high plasma HDL levels present with multiple metabolic abnormalities; they are atherosusceptible, and the females are infertile. Notably, HDL from *Scarb1*$^{-/-}$ mice is free cholesterol (FC)-rich. These observations support our hypotheses that the metabolic abnormalities associated with high plasma HDL levels are due to high HDL-FC bioavailability and that reduction of HDL-FC bioavailability prevents/reverses these abnormalities. Our hypothesis is supported by other observations. FC transfer from HDL is fast, t=<5 minutes and reversible; in mice the majority of HDL- and nascent (n)HDL-FC transfers to the liver with $t_{1/}2$<5 min without esterification; over longer times, nHDL-derived FC appears in all tissues.[34] Whereas FC-free recombinant HDL supports cellular FC efflux, recombinant HDL containing ≥15 mol% FC drives FC influx; the magnitude of recombinant HDL-FC influx is proportional to HDL-FC content.[35] Bacterial serum opacity factor disrupts HDL structure and lowers plasma total HDL levels in mice.[36–38] Adeno-associated virus delivery of serum opacity factor to *Scarb1*$^{-/-}$ mice reduces HDL-FC to nil and constitutively rescues fertility among the female *Scarb1*$^{-/-}$ mice within ≈24 hours. In conclusion, serum opacity factor-mediated reduction of HDL-p number reduces HDL-FC bioavailability and could reduce metabolic problems due to high plasma HDL levels.

2. Pascal Bernatchez, PhD, Associate Professor, Department of Anesthesiology, Pharmacology & Therapeutics, University of British Columbia, Heart & Lung Innovation Centre, St. Paul's Hospital, Vancouver, British Columbia, Canada. *HDL, LDL, and Muscular Dystrophy.*

Lipid management pharmacotherapy has highlighted new links between LDL cholesterol-lowering approaches and skeletal muscle homeostasis.[39] For instance, statins can in rare cases cause rhabdomyolysis, but how this occurs is poorly understood. Using genetic models of muscular dystrophy (MD) that exhibit weak but chronic muscle degeneration (Duchenne and Limb-Girdle MD type 2b mice), we have observed drastically worsened skeletal muscle wasting and even loss of ambulation when non–HDL-C levels are increased via *APOE* gene inactivation and high-fat diets.[40] Double Duchenne/apoE or Limb-Girdle 2b/apoE mice can show severe muscle wasting compared to WT, apoE or single MD mice, with muscle lesion area jumping from 1% to 5% in skeletal muscles of Duchenne or Limb-Girdle 2b mice to up to 64% to 94% in double-mutant mice.[41] More interesting is the fact that muscle damage can reach levels that cause complete loss of ambulation, a phenotype never reported in these mice. Together, these data clearly showed an unexpected linked between plasma lipoprotein levels and muscle homeostasis in already genetically weakened tissues and even suggested that non–HDL-C may possess intrinsic toxic properties in muscles. In various settings of Duchenne MD, plasma lipoprotein metabolism was analyzed, and HDL-related abnormalities were also noted even in settings of low muscle damage as assessed by normal creatine kinase levels. Together, our data suggest a profound defect in LDL- and HDL-associated cholesterol metabolism in MD, which warrants further investigation.

3. Amanda Ribeiro Martins da Silva, PhD, Graduate student (Dr Graziella E. Ronsein, mentor), Institute of Chemistry, University of Sao Paulo, Sao Paulo, Brazil. *High-density lipoproteins subclasses mapping by targeted quantitative proteomics.*

HDL is a heterogeneous particle and linked to a variety of cardioprotective functions.[42] Close to 100 proteins have been identified as associated with HDL. However, APOA1 and APOA2, the 2 most abundant proteins in lipoprotein, make up to 90% of the protein content.[43] Thus, the proteins may be differentially localized to distinct HDL-p, and most of them are present in low abundance, which makes accurate quantification a challenge.[44] Mass spectrometry-based targeted quantitative proteomics is a powerful approach to accurately differentiate HDL subclasses due to its high sensitivity, specificity, and wide dynamic range.[45] We, therefore, quantified the proteome of HDL subclasses of 19 apparently healthy volunteers. HDL-p were separated into HDL_2 and HDL_3 subfractions by discontinuous density ultracentrifugation, and their proteome was quantified using 2 targeted methodologies, Data Independent Acquisition and Parallel Reaction Monitoring. Both methods worked equally well with high precision to differentiate HDL subclasses. The majority of proteins were shared by both subclasses, but their abundance varied considerably. Dense HDL_3 was significantly enriched with proteins related to antioxidant activity, such paraoxonase and apolipoprotein L1, apolipoprotein D, apolipoprotein A-IV, apolipoprotein H, apolipoprotein J, and phosphatidylcholine-sterol acyltransferase. While HDL_2 was enriched with APOA2, apolipoprotein Cs (apoC-I, apoC-II, and apoC-III), apolipoprotein E, and serum amyloid A-4. Thus, Data Independent Acquisition and Parallel Reaction Monitoring are attractive strategies to differentiate HDL subclasses proteome and may contribute to understand how HDL proteome affects its functionality.

Session 5: Microbiota and HDL Metabolism

1. Jacob L. Barber, PhD, Graduate student (Dr Mark A. Sarzynski, mentor). Department of Exercise Science, University of South Carolina, Columbia, SC. *Association of exercise-induced changes in cholesterol efflux capacity with changes in the HDL proteome.*

HDL function has recently been identified as a strong predictor of cardiovascular risk.[32] Exercise is known to effect HDL metabolism and CVD risk, however, the effects of exercise on measures of HDL function and composition are less well known.[46–48] We examined changes in CEC and concomitant changes in the HDL proteome in 19 white, nonrelated individuals from the HERITAGE Family Study. Measurement of the efflux of radiolabeled and BODIPY-labeled cholesterol from J774 macrophages to apolipoprotein B depleted plasma, along with untargeted HDL proteome profiling, was performed at baseline and following 20 weeks of endurance exercise. There was no effect of regular exercise on CEC; however, exercise training resulted in improvements in CEC in 9 subjects (responders) and either no changes or decreased CEC in 10 subjects (nonresponders). A total of 33 known HDL proteins were identified in all 19 subjects. There were no overall changes in abundance of any HDL proteins following training. Changes in abundance of 6 HDL proteins (Albumin, IGHA1, IGHG2, C6, SAA4, and APOE) were nominally negatively correlated with changes in CEC. Additionally, among responders, HDL APOE abundance was significantly decreased (P=0.03), while there was no change in HDL APOE abundance (P=0.78) among nonresponders. This pilot study provided limited evidence for a potential role of select HDL proteins as mediators of changes in CEC. Future studies with larger sample sizes are needed to further examine how alterations in the HDL proteome are related to HDL function.

2. Gregory C. Shearer, PhD, Associate Professor, Nutritional Sciences, The Pennsylvania State University, University Park, PA. *Eicosapentaenoate and other polyunsaturated fatty acids in HDL function.*

Recent clinical trials demonstrate 4 g/d of eicosapentaenoic acid (EPA) is associated with a 25% risk reduction for myocardial infarction.[49] Further, subjects with low HDL had a greater benefit from EPA than those with high HDL. EPA is an ω3-polyunsaturated fatty acid (PUFA). There are strong reasons to associate its effects with HDL: HDL transport PUFAs; HDL responds most to ω3-PUFA treatment; and HDL host more oxylipins than any other plasma pool (oxylipins are signaling metabolites derived from PUFAs, including EPA).[50,51] Increased ω3-PUFA intake is associated with large increases in HDL-EPA-oxylipins. We seek to explain PUFA action in general, and EPA action specifically, by testing whether HDL mediates the cellular efflux of oxylipins. Oxylipins are intracellular oxylipins and by acting as an oxylipin acceptor, HDL could regulate intracellular signaling. For detection purposes, we used 12-HETE as an oxylipin tracer. It is produced by 12-lipoxygenase from arachidonate. We hypothesized efflux of 12-HETE from activated macrophages would be apoA-I and ABCA1 dependent. RAW264.7 macrophages were provided d8-arachidonate and stimulated with 100 ng/mL LPS under 4 conditions: ±apoA-I, and ±ABCA1 expression by siRNA silencing. Tracer efflux was analyzed using compartmental modeling. The efflux of label into media phospholipids (as media esterified) was dependent on the presence of both apoA-I and ABCA1 and concurrently resulted in the lowest intracellular unesterified d8-12-HETE. Oppositely, the absence of apoA-I or ABCA1 resulted in high intracellular label appearance and little to no appearance of d8-12-HETE in the media phospholipid pool. Overall, our results suggest an important role of HDL in mediating oxylipin efflux.

3. Angela M. Zivkovic, PhD, Assistant Professor, Department of Nutrition, University California Davis, Davis, CA. *Diet-induced changes in HDL lipidome, glycoproteome, and functional capacity.*

There is growing evidence that the composition and functional capacity of HDL-p are important determinants of the protective effects of HDL in CVD. But we do not yet know how to improve the composition and function of HDL. In 2 controlled, randomized-order, cross-over feeding studies, we determined the effects of short-term diet and long-term diet on HDL composition and function. Four days of fast food versus Mediterranean diet widely remodeled the HDL lipidome to reflect dietary lipid composition, altered apoA-I content but not A1AT, A2HSG, apoC-III, apoE, SAA, and did not alter protein glycoprofiles, except apoC-III sialylation. Individual HDL lipid species and glycopeptides were highly correlated with CEC; however, the diet-induced compositional changes were not accompanied by changes in HDL CEC possibly due to differences in baseline total:HDL cholesterol ratios, study duration, or gut microbiome composition. Four weeks of 2 whole eggs versus egg whites per day, however, increased HDL CEC, which was unrelated to HDL ApoA-I content, particle size or distribution, or lipidomic compositional changes.[52] Our findings indicate that diet can alter HDL composition in as little as 4 days. High interindividual variability in response even in a tightly controlled study, may be partly explained by baseline lipid profiles and gut microbiome. Our results also indicate that diet can alter HDL CEC, and that HDL lipids and glycoproteins are highly correlated with HDL function, providing evidence that dietary strategies are a promising approach for improving the functional capacity of HDL.

4. Uwe J.F. Tietge, MD, PhD, Professor, Division of Clinical Chemistry, Karolinska Institutet, Stockholm, Sweden. *Impact of intestinal microbiota on reverse cholesterol transport.*

Mediating RCT is envisioned as a major antiatherosclerotic function of HDL-p. Available data lend strong support to a concept, in which, at least in rodents, the biliary secretion pathway accounts for ≈75% of RCT while the remaining 25% occurs from the nonbiliary pathway of transintestinal cholesterol excretion.[53,54] Within the biliary pathway, cholesterol can be secreted either directly or after metabolic conversion into bile acids. RCT studies

distinguishing between the respective excretion of the macrophage-derived cholesterol tracer within the fecal neutral sterol versus the fecal bile acid fraction demonstrated that a large proportion of RCT actually occurs via bile acids.[55] While primary bile acids are formed in the liver, secondary bile acids are metabolic products of the intestinal microbiota. Germ-free mice lack intestinal bacteria and thus the capacity for producing secondary bile acids. Interestingly, in the absence of a microbiota RCT is increased due to more secretion of RCT-relevant cholesterol within the fecal bile acid fraction.[56] Since mostly the rodent-specific muricholic acids were affected, more research in the human system is required to investigate, if such results can be translated. Nevertheless, bile acid sequestrants represent a classical successful antiatherosclerotic intervention strategy with relatively limited side effects. Conceivably, with the application of bile acid sequestrants RCT within the fecal bile acid fraction could be enhanced. As a secondary effect also RCT within the fecal neutral sterol fraction could increase, since the availability of bile acids is important also for cholesterol absorption.

JACK ORAM RESEARCH AWARD

Godfrey S. Getz, MD, PhD, Chairman Emeritus, Donald N. Pritzker Distinguished Professor, Departments Pathology, Biochemistry, Molecular Biology, University of Chicago, Chicago, IL. *Apolipoprotein A-I and HDL heterogeneity in humans and mice.*

Dr Getz focused on 3 topics. The first being differences between HDL and apoA-I in humans and mice, based on the role of apoA-I in distinguishing between HDL_2 (mouse only) and HDL_3 and HDL_2 in human plasma. Chimeric apoA-I containing mouse helices 7 and 8 in a human apoA-I background favored HDL_2 distribution. Second, it was noted that despite the strong evidence for atheroprotection by apoA-I derived from animal experiments, the *APOA1* gene does not appear as a causal gene from genome-wide association studies in humans and the Hybrid Diversity Mouse Panel. This may be reconciled by the possibility that apoA-I serves as a platform protein for HDL formation and the compositional heterogeneity of HDL. An example of the latter is the finding that plasminogen promotes ABCA1 dependent cholesterol efflux.[57] Plasminogen is found in a small cholesterol-free phospholipid containing particles. The fact that apoA-I does not appear as a major gene in Hybrid Diversity Mouse Panel is exemplified by the comparison of C57BL6 and FVB mice, atherosensitive and atheroresistant strains, respectively. The latter strain shows limited or no effect of apoA-I deficiency on atherosclerosis. The comparison of HDLs from the 2 strains shows a polymorphism of both apoA-I (2 amino acid differences) and apoA-II (3 amino acid differences). HDL of FVB mice is enriched in apoA-II. The latter protein of the 2 strains show differences in in vivo dynamics. HDL can be remodeled by exposure to tandem mimetic peptides; FVB HDL is much more stable to this remodeling. Advantage could be taken of this remodeling, combined with proteomics could be used to probe the structure of HDL by examining the release of other HDL proteins along with apoA-I. Last, a novel method of in vivo cholesterol efflux was described, involving the encapsulation of macrophages in an alginate capsule, allowing for the recovery of the macrophages for assessment of sterol homeostasis and gene expression in the macrophage subject to various global environments. The method could also be used to encapsulate other cells or 2 different cell types for their direct interaction at the level of gene expression.

PERSPECTIVES

HDL exists. A simple statement and a challenge for basic scientists and clinicians to now convincingly determine for what biological purpose(s).

As evidenced by the talented roster of established investigators and emerging ones from a variety of scientific backgrounds, there is a vibrant community of scientists exploring new paradigms of HDL biology. We are continuing to discover novel functions of HDL-p, including, for example, their role in protection from toxin-induced damage via SR-B1, their role in enhancing lymphatic transport, and their role as transporters/effluxers of lipid signaling molecules (ie, oxylipins). An area of continued focus was on the composition of HDL-p, from different methodologies to examine their number, size, proteome, and association with functionality that largely is still defined by cholesterol efflux assays. Environmental effects on the HDL proteome were examined by interventions with exercise and diet, with intriguing early results that need testing in larger populations. From a clinical perspective, results from the JUPITER trial suggest that measurement of HDL-p versus cholesterol might be more informative in CVD risk reduction. All of this speaks to an underlying friction in the need to distinguish the clinical utility of HDL-p versus the lipid content of heterogenous HDL molecules.

Looking forward, the tenth HDL Workshop will take place on May 2020 in Chicago. The chair will be Dr Rodriguez, and the co-chair will be Dr Kasey C. Vickers. The roster of speakers will continue to be innovative established investigators and emerging ones, with topics that will push the boundaries of what is known about HDL biology. The tentative program will include topics on HDL and reproduction, HDL as a drug platform, clinical perspectives of high HDL-C paradox, nontraditional HDL cargo, and HDL immunology.

We think that health care organizations such as the American Heart Association and others should issue treatment guidelines that specifically address the role of

HDL-C in the clinical management of patients at risk and those with CVD. The overwhelming evidence shows that low HDL-C adds predictive value for increased CVD risk while high HDL-C does not show evidence for cardioprotection. What about the question of HDL-p and functionality? Results from large clinical trials do point to the possibility of HDL-p and cholesterol efflux assays as providing utility in CVD risk reduction. However, much more work needs to be done to determine if these assays can be performed in a high throughput manner relevant to clinical medicine and insurance reimbursement.

We think it is important to expand into other fundamental aspects of HDL biology, as shown by our program development for the tenth HDL Workshop. These explorations will likely guide us into causal pathways influenced directly or secondarily by HDL that will be targets for future clinical diagnostic and therapeutic applications.

The clinical need is urgent and this collaborative effort of academic basic scientists, clinicians, and industry researches will certainly find the answers to why HDL exists.

ARTICLE INFORMATION

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Disclosures

Dr Rodriguez is the founder of Lipid Genomics and declares inventorship rights to issued patents related to HDL biology. B.L. Trigatti declares inventorship rights to issued patents related to *Scarb1*$^{-/-}$ mice. J.T. Wilkins declares having served as a consultant for NGM Bio. S. Mora declares having received research support from Atherotech Diagnostics and serving as a consultant to Quest Diagnostics and Pfizer. M. Cuchel declares receiving support for clinical trials unrelated to HDL from Akcea Therapeutics, Regeneron Pharmaceuticals, and Regenxbio. C.Rosales was supported by NIH HL129767. G.C. Shearer declares receiving speakership and advisory panel honoraria from Amarin Pharmaceuticals. F. Sacks declares being a consultant for Pfizer and AstraZeneca. M.A. Connelly is an employee of LabCorp, which offers lipoprotein profiling services via nuclear magnetic resonance spectroscopy (NMR). M.N. Oda is the founder of DRx BioLogics, Inc. T. Vaisar is a consultant for MedImmune/AstraZeneca. Dr Giacomo Ruotolo is an employee of Eli Lilly and Company. The other authors report no conflicts.

REFERENCES

1. https://hdlworkshop.org/. Accessed August 8, 2019.
2. 2018 AHA/ACC/AACBPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the management of blood cholesterol. *Circulation.* 2019;139:e1082–e1143.
3. American Heart Association. 2018 Prevention Guidelines Tool CV Risk Calculator. http://static.heart.org/riskcalc/app/index.html#!/baseline-risk. Accessed July 8, 2019.
4. Framingham Heart Study. Hard Coronary Heart Disease (10-year risk). https://www.framinghamheartstudy.org/fhs-risk-functions/hard-coronary-heart-disease-10-year-risk/. Accessed July 8, 2019.
5. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, Hindy G, Hólm H, Ding EL, Johnson T, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet.* 2012;380:572–580. doi: 10.1016/S0140-6736(12)60312-2
6. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J, Chaitman BR, Holme IM, Kallend D, Leiter LA, et al; dal-OUTCOMES Investigators. Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N Engl J Med.* 2012;367:2089–2099. doi: 10.1056/NEJMoa1206797
7. Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W; AIM-HIGH Investigators. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *N Engl J Med.* 2011;365:2255–2267. doi: 10.1056/NEJMoa1107579
8. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, Lopez-Sendon J, Mosca L, Tardif JC, Waters DD, et al; ILLUMINATE

Investigators. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med.* 2007;357:2109–2122. doi: 10.1056/NEJMoa0706628
9. PrabhuDas MR, Baldwin CL, Bollyky PL, Bowdish DME, Drickamer K, Febbraio M, Herz J, Kobzik L, Krieger M, Loike J, et al. A consensus definitive classification of scavenger receptors and their roles in health and disease. *J Immunol.* 2017;198:3775–3789. doi: 10.4049/jimmunol.1700373
10. Braun A, Trigatti BL, Post MJ, Sato K, Simons M, Edelberg JM, Rosenberg RD, Schrenzel M, Krieger M. Loss of SR-BI expression leads to the early onset of occlusive atherosclerotic coronary artery disease, spontaneous myocardial infarctions, severe cardiac dysfunction, and premature death in apolipoprotein E-deficient mice. *Circ Res.* 2002;90:270–276. doi: 10.1161/hh0302.104462
11. Durham KK, Kluck G, Mak KC, Deng YD, Trigatti BL. Treatment with apolipoprotein A1 protects mice against doxorubicin-induced cardiotoxicity in a scavenger receptor class B, type I-dependent manner. *Am J Physiol Heart Circ Physiol.* 2019;316:H1447–H1457. doi: 10.1152/ajpheart.00432.2018
12. Durham KK, Chathely KM, Trigatti BL. High-density lipoprotein protects cardiomyocytes against necrosis induced by oxygen and glucose deprivation through SR-B1, PI3K, and AKT1 and 2. *Biochem J.* 2018;475:1253–1265. doi: 10.1042/BCJ20170703
13. Linton MF, Tao H, Linton EF, Yancey PG. SR-BI: a multifunctional receptor in cholesterol homeostasis and atherosclerosis. *Trends Endocrinol Metab.* 2017;28:461–472. doi: 10.1016/j.tem.2017.02.001
14. Mineo C, Shaul PW. Regulation of signal transduction by HDL. *J Lipid Res.* 2013;54:2315–2324. doi: 10.1194/jlr.R039479
15. Huang L, Chambliss KL, Gao X, Yuhanna IS, Behling-Kelly E, Bergaya S, Ahmed M, Michaely P, Luby-Phelps K, Darehshouri A, et al. SR-B1 drives endothelial cell LDL transcytosis via DOCK4 to promote atherosclerosis. *Nature.* 2019;569:565–569. doi: 10.1038/s41586-019-1140-4
16. Martel C, Li W, Fulp B, Platt AM, Gautier EL, Westerterp M, Bittman R, Tall AR, Chen SH, Thomas MJ, et al. Lymphatic vasculature mediates macrophage reverse cholesterol transport in mice. *J Clin Invest.* 2013;123:1571–1579. doi: 10.1172/JCI63685
17. Milasan A, Dallaire F, Mayer G, Martel C. Effects of LDL receptor modulation on lymphatic function. *Sci Rep.* 2016;6:27862. doi: 10.1038/srep27862
18. Milasan A, Tessandier N, Tan S, Brisson A, Boilard E, Martel C. Extracellular vesicles are present in mouse lymph and their level differs in atherosclerosis. *J Extracell Vesicles.* 2016;5:31427. doi: 10.3402/jev.v5.31427
19. Jean G, Milasan A, Boilard E, Fortin C, Martel C. Extracellular vesicles can be friends or foes in atherosclerosis-related lymphatic dysfunction. *Atheroscler Suppl.* 2018;32:93.
20. Trigatti BL, Krieger M, Rigotti A. Influence of the HDL receptor SR-BI on lipoprotein metabolism and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2003;23:1732–1738. doi: 10.1161/01.ATV.0000091363.28501.84
21. Park YM. CD36, a scavenger receptor implicated in atherosclerosis. *Exp Mol Med.* 2014;46:e99. doi: 10.1038/emm.2014.38
22. Mutharasan RK, Thaxton CS, Berry J, Daviglus ML, Yuan C, Sun J, Ayers C, Lloyd-Jones DM, Wilkins JT. HDL efflux capacity, HDL particle size, and high-risk carotid atherosclerosis in a cohort of asymptomatic older adults: the Chicago Healthy Aging Study. *J Lipid Res.* 2017;58:600–606. doi: 10.1194/jlr.P069039
23. Seckler HDS, Fornelli L, Mutharasan RK, Thaxton CS, Fellers R, Daviglus M, Sniderman A, Rader D, Kelleher NL, Lloyd-Jones DM, et al. A targeted, differential top-down proteomic methodology for comparison of ApoA-I proteoforms in individuals with high and low HDL efflux capacity. *J Proteome Res.* 2018;17:2156–2164. doi: 10.1021/acs.jproteome.8b00100
24. Golden D, Kolmakova A, Sura S, Vella AT, Manichaikul A, Wang XQ, Bielinski SJ, Taylor KD, Chen YI, Rich SS, et al. Lymphocyte activation gene 3 and coronary artery disease. *JCI Insight.* 2016;1:e88628. doi: 10.1172/jci.insight.88628
25. Zanoni P, Khetarpal SA, Larach DB, Hancock-Cerutti WF, Millar JS, Cuchel M, DerOhannessian S, Kontush A, Surendran P, Saleheen D, et al; CHD Exome+ Consortium; CARDIoGRAM Exome Consortium; Global Lipids Genetics Consortium. Rare variant in scavenger receptor BI raises HDL cholesterol and increases risk of coronary heart disease. *Science.* 2016;351:1166–1171. doi: 10.1126/science.aad3517
26. Mackey RH, Greenland P, Goff DC Jr, Lloyd-Jones D, Sibley CT, Mora S. High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (multi-ethnic study of atherosclerosis). *J Am Coll Cardiol.* 2012;60:508–516. doi: 10.1016/j.jacc.2012.03.060
27. Mora S, Glynn RJ, Ridker PM. High-density lipoprotein cholesterol, size, particle number, and residual vascular risk after potent statin therapy. *Circulation.* 2013;128:1189–1197. doi: 10.1161/CIRCULATIONAHA.113.002671
28. Khera AV, Demler OV, Adelman SJ, Collins HL, Glynn RJ, Ridker PM, Rader DJ, Mora S. Cholesterol efflux capacity, high-density lipoprotein particle number, and incident cardiovascular events: an analysis from the JUPITER trial (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin). *Circulation.* 2017;135:2494–2504. doi: 10.1161/CIRCULATIONAHA.116.025678
29. Madsen CM, Varbo A, Nordestgaard BG. Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies. *Eur Heart J.* 2017;38:2478–2486. doi: 10.1093/eurheartj/ehx163
30. van der Steeg WA, Holme I, Boekholdt SM, Larsen ML, Lindahl C, Stroes ES, Tikkanen MJ, Wareham NJ, Faergeman O, Olsson AG, et al. High-density lipoprotein cholesterol, high-density lipoprotein particle size, and apolipoprotein A-I: significance for cardiovascular risk: the IDEAL and EPIC-Norfolk studies. *J Am Coll Cardiol.* 2008;51:634–642. doi: 10.1016/j.jacc.2007.09.060
31. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *N Engl J Med.* 2011;364:127–135. doi: 10.1056/NEJMoa1001689
32. Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, et al. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med.* 2014;371:2383–2393. doi: 10.1056/NEJMoa1409065
33. Cuchel M, Raper AC, Conlon DM, Pryma DA, Freifelder RH, Poria R, Cromley D, Li X, Dunbar RL, French B, et al. A novel approach to measuring macrophage-specific reverse cholesterol transport *in vivo* in humans. *J Lipid Res.* 2017;58:752–762. doi: 10.1194/jlr.M075226
34. Xu B, Gillard BK, Gotto AM Jr, Rosales C, Pownall HJ. ABCA1-derived nascent high-density lipoprotein-apolipoprotein AI and lipids metabolically segregate. *Arterioscler Thromb Vasc Biol.* 2017;37:2260–2270. doi: 10.1161/ATVBAHA.117.310290
35. Picardo M, Massey JB, Kuhn DE, Gotto AM Jr, Gianturco SH, Pownall HJ. Partially reassembled high density lipoproteins. Effects on cholesterol flux, synthesis, and esterification in normal human skin fibroblasts. *Arteriosclerosis.* 1986;6:434–441.
36. Gillard BK, Courtney HS, Massey JB, Pownall HJ. Serum opacity factor unmasks human plasma high-density lipoprotein instability via selective delipidation and apolipoprotein A-I desorption. *Biochemistry.* 2007;46:12968–12978. doi: 10.1021/bi701525w
37. Rosales C, Patel N, Gillard BK, Yelamanchili D, Yang Y, Courtney HS, Santos RD, Gotto AM Jr, Pownall HJ. Apolipoprotein AI deficiency inhibits serum opacity factor activity against plasma high density lipoprotein via a stabilization mechanism. *Biochemistry.* 2015;54:2295–2302. doi: 10.1021/bi501486z
38. Rosales C, Tang D, Gillard BK, Courtney HS, Pownall HJ. Apolipoprotein E mediates enhanced plasma high-density lipoprotein cholesterol clearance by low-dose streptococcal serum opacity factor via hepatic low-density lipoprotein receptors in vivo. *Arterioscler Thromb Vasc Biol.* 2011;31:1834–1841. doi: 10.1161/ATVBAHA.111.224360
39. Srivastava NK, Yadav R, Mukherjee S, Pal L, Sinha N. Abnormal lipid metabolism in skeletal muscle tissue of patients with muscular dystrophy: *in vitro*, high-resolution NMR spectroscopy based observation in early phase of the disease. *Magn Reson Imaging.* 2017;38:163–173. doi: 10.1016/j.mri.2017.01.001
40. Sellers SL, Milad N, White Z, Pascoe C, Chan R, Payne GW, Seow C, Rossi F, Seidman MA, Bernatchez P. Increased nonHDL cholesterol levels cause muscle wasting and ambulatory dysfunction in the mouse model of LGMD2B. *J Lipid Res.* 2018;59:261–272. doi: 10.1194/jlr.M079459
41. Milad N, White Z, Tehrani AY, Sellers S, Rossi FMV, Bernatchez P. Increased plasma lipid levels exacerbate muscle pathology in the mdx mouse model of Duchenne muscular dystrophy. *Skelet Muscle.* 2017;7:19. doi: 10.1186/s13395-017-0135-9
42. Swertfeger DK, Li H, Rebholz S, Zhu X, Shah AS, Davidson WS, Lu LJ. Mapping atheroprotective functions and related proteins/lipoproteins in size fractionated human plasma. *Mol Cell Proteomics.* 2017;16:680–693. doi: 10.1074/mcp.M116.066290
43. Davidson WS, Thompson TB. The structure of apolipoprotein A-I in high density lipoproteins. *J Biol Chem.* 2007;282:22249–22253. doi: 10.1074/jbc.R700014200
44. Davidson WS, Silva RA, Chantepie S, Lagor WR, Chapman MJ, Kontush A. Proteomic analysis of defined HDL subpopulations reveals particle-specific protein clusters: relevance to antioxidative function. *Arterioscler Thromb Vasc Biol.* 2009;29:870–876. doi: 10.1161/ATVBAHA.109.186031

45. Vidova V, Spacil Z. A review on mass spectrometry-based quantitative proteomics: targeted and data independent acquisition. *Anal Chim Acta.* 2017;964:7–23. doi: 10.1016/j.aca.2017.01.059
46. Kodama S, Tanaka S, Saito K, Shu M, Sone Y, Onitake F, Suzuki E, Shimano H, Yamamoto S, Kondo K, et al. Effect of aerobic exercise training on serum levels of high-density lipoprotein cholesterol: a meta-analysis. *Arch Intern Med.* 2007;167:999–1008. doi: 10.1001/archinte.167.10.999
47. Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA, Bouchard C, Buchner D, Ettinger W, Heath GW, King AC. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *JAMA.* 1995;273:402–407. doi: 10.1001/jama.273.5.402
48. Sarzynski MA, Ruiz-Ramie JJ, Barber JL, Slentz CA, Apolzan JW, McGarrah RW, Harris MN, Church TS, Borja MS, He Y, et al. Effects of increasing exercise intensity and dose on multiple measures of HDL (High-Density Lipoprotein) function. *Arterioscler Thromb Vasc Biol.* 2018;38:943–952. doi: 10.1161/ATVBAHA.117.310307
49. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, Doyle RT Jr, Juliano RA, Jiao L, Granowitz C, et al; REDUCE-IT Investigators. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *N Engl J Med.* 2019;380:11–22. doi: 10.1056/NEJMoa1812792
50. Augustine AH, Lowenstein LM, Harris WS, Shearer GC, Block RC. Treatment with omega-3 fatty acid ethyl-ester alters fatty acid composition of lipoproteins in overweight or obese adults with insulin resistance. *Prostaglandins Leukot Essent Fatty Acids.* 2014;90:69–75. doi: 10.1016/j.plefa.2013.12.003
51. Shearer GC, Borkowski K, Puumala SL, Harris WS, Pedersen TL, Newman JW. Abnormal lipoprotein oxylipins in metabolic syndrome and partial correction by omega-3 fatty acids. *Prostaglandins Leukot Essent Fatty Acids.* 2018;128:1–10. doi: 10.1016/j.plefa.2017.10.006
52. Sawrey-Kubicek L, Zhu C, Bardagjy AS, Rhodes CH, Sacchi R, Randolph JM, Steinberg FM, Zivkovic AM. Whole egg consumption compared with yolk-free egg increases the cholesterol efflux capacity of high-density lipoproteins in overweight, postmenopausal women. *Am J Clin Nutr.* 2019;110:617–627. doi: 10.1093/ajcn/nqz088
53. Nijstad N, Gautier T, Briand F, Rader DJ, Tietge UJ. Biliary sterol secretion is required for functional *in vivo* reverse cholesterol transport in mice. *Gastroenterology.* 2011;140:1043–1051. doi: 10.1053/j.gastro.2010.11.055
54. de Boer JF, Schonewille M, Dikkers A, Koehorst M, Havinga R, Kuipers F, Tietge UJ, Groen AK. Transintestinal and biliary cholesterol secretion both contribute to macrophage reverse cholesterol transport in rats-brief report. *Arterioscler Thromb Vasc Biol.* 2017;37:643–646. doi: 10.1161/ATVBAHA.116.308558
55. Dikkers A, de Boer JF, Groen AK, Tietge UJ. Hepatic ABCG5/G8 overexpression substantially increases biliary cholesterol secretion but does not impact *in vivo* macrophage-to-feces RCT. *Atherosclerosis.* 2015;243:402–406. doi: 10.1016/j.atherosclerosis.2015.10.010
56. Mistry RH, Verkade HJ, Tietge UJ. Reverse cholesterol transport is increased in germ-free mice-brief report. *Arterioscler Thromb Vasc Biol.* 2017;37:419–422. doi: 10.1161/ATVBAHA.116.308306
57. Pamir N, Hutchins PM, Ronsein GE, Wei H, Tang C, Das R, Vaisar T, Plow E, Schuster V, Koschinsky ML, et al. Plasminogen promotes cholesterol efflux by the ABCA1 pathway. *JCI Insight.* 2017;2:e92176.