Proteomics of Apolipoproteins and Associated Proteins From Plasma High-Density Lipoproteins

Pia Davidsson, Johannes Hulthe, Björn Fagerberg, Germán Camejo

Abstract—Proteomics studies have extended the list of identified apolipoproteins and associated proteins present in HDL and its subclasses. These proteins appear to cluster around specific functions related to lipid metabolism, inflammation, the immune system, hormone-binding, hemostasis, and antioxidant properties. Small studies suggest that there are substantial differences between the HDL proteome from cardiovascular disease patients and that from controls. Furthermore, dyslipidemia therapy shifts the HDL proteome from patients toward the profile observed in healthy controls. In addition, the proteome of HDL and LDL from patients with insulin resistance and peripheral atherosclerosis show significant differences with that of matched healthy controls. The proteome of HDL and LDL density subclasses have apolipoproteins and associated proteins profiles that suggest subclass-specific functions. However, proteomics studies of lipoproteins are few and small and should be interpreted with caution. Nonetheless rapid technical progress in proteomic platforms suggest that soon analysis time will be reduced and precise measurement of identified proteins will be possible. This, combined with controlled purification steps of HDL and its subclasses should provide further information about proteins involved in the particles postulated spectrum of functions, including those believed to be atheroprotective. (Arterioscler Thromb Vasc Biol. 2010;30:156-163.)

Key Words: HDL proteomics-apolipoproteins and associated proteins-lipoproteins functions

The metabolic fate and function of plasma lipoproteins in health and disease are in large part controlled by interactions of apolipoproteins in the particles surface with cell membranes receptors, enzymes, and lipid-transport proteins. The classical structural model of lipoproteins is that of microemulsions with a surface monolayer made of amphipathic phospholipids (PL), free cholesterol (C), and apolipoproteins surrounding a central core containing non-polar lipids, mainly triglycerides (TG) and cholesterol esters (CE).1 Kumpula et al2 recently proposed a new model derived from composition analyses and optimizing the spatial distribution of TG, C, and CE. The model suggest that substantial amounts of these neutral lipids are located at the surface monolayer, specially in small HDL subclasses.2 This new model could better explain how surface apolipoproteins and associated proteins can interact with surface lipids. And furthermore, it helps to understand how enzymes and lipid-transport proteins can have access to CE and TG that were thought to be in the particles core in the classical representation. Lipoproteins are reasonable stable molecular aggregates that transport relative large amounts of lipids in blood and extracellular fluid and that allow their lipids exchange between lipoprotein classes and with cells. The term and concept of apolipoproteins is an extension of that used to describe the protein of enzymes with cofactors, the apo-enzymes. The Greek prefix “apo” means “away from” or “separate from.” Thus any protein associated with lipids or a lipoprotein particle can be considered an apolipoprotein that is part of a holo-particle. However, in this review the term apolipoprotein refers only to the “classical apolipoproteins” (apoB100, apoAl, apoAII, apoE, apoC, apoD, and apoF). Other plasma proteins, including enzymes and lipid-transport proteins, can exist in association with lipoproteins and are in equilibrium with non–lipoprotein-associated forms. We will use the term “associated proteins” for these plasma proteins that remain bound to lipoproteins after their isolation. ApoB100, present in VLDL, IDL, and LDL, and apoB-48 present in chylomicrons and TG-rich particles, are large nonexchangeable apolipoproteins. The rest of the apolipoproteins, apoAI, apoAII, apoE, and the apoCs, found in all lipoprotein classes are considered to be exchangeable.3 The apolipoproteins in HDL are all of this type and have a domain structure with amphipatic α-helices (A-type) that orient themselves with their nonpolar, lipid-binding, surfaces interacting with polar and nonpolar regions of the particles surface lipids, whereas the opposite surfaces, rich in...
Large HDL
(low surface tension)

Small HDL
(high surface tension)

adsorption

remodeling

ejection

exchangeable apolipoproteins

associated proteins

Figure 1. Diagram of the possible changes induced by remodeling and surface pressure alterations in lipoproteins with exchangeable apolipoproteins and associated proteins based in the models evaluated in Wang et al.6,7 Decrease in the particle volumes reduce the surface area and can increase the surface pressure inducing desorption of loosely bound proteins. The change in curvature radius can induce exposure of new regions of the apolipoproteins altering the surface mosaic to which associated proteins are bound.

charged amino acids side chains, are in contact with the aqueous environment. Class Y and class G* a-helices, also present in exchangeable apolipoproteins, interact poorly with lipids but apparently can associate with proteins present in the aqueous environment. It should be stressed that the lipoprotein outer surface, to which the apolipoproteins and associated proteins are bound, is not a static structure. The degree of exposure to the aqueous environment of polar segments of apolipoproteins and the surface polar region of lipids is controlled by surface pressure that changes with the particle radius of curvature or size within each lipoprotein class.4,5 Therefore, desorption of associated proteins with increased surface exposure of segments of exchangeable and nonexchangeable apolipoproteins can occur when the particles become smaller by remodeling induced by lipolysis and lipid exchange occurring in circulation. These changes in the particles surface, caused by increase radius of curvature and surface pressure, can alter the proteome of lipoprotein subclasses. This is an important notion for the theme of this review, and Figure 1 is a diagram of possible alterations in binding of associated proteins and exchangeable apolipoproteins induced by lipoprotein remodeling and changes in their surface lateral pressure.

The HDL class of particles is structurally and metabolically heterogeneous. This is caused by its diverse metabolic origin and by the continuous remodeling of the particles by lipolytic enzymes, lipid transporters, and furthermore by lipid and apolipoprotein exchange with other circulating lipoproteins and tissues.1,6,7 The amount and type of apolipoproteins in isolated HDL can be modulated by alterations of lipid metabolism, activation of the innate immune system, and by the lipoprotein isolation procedure used.8,9 Already in 1986 it was found that HDL isolated during the acute phase response have profound changes in its associated proteins and apolipoprotein composition.10 This HDL is enriched in C-reactive protein (CRP), secretory phospholipase A2-IIa (sPLA2-IIa), serum amyloid A (SAA), and cholesterol ester transfer protein (CETP). Such remodeling has been suggested to preserve the acceptor role of HDL particles in cholesterol efflux from cells, but it may, at the same time, compromise other functions of HDL.6,11,12 In addition, HDL with higher levels of associated hemoglobin (Hb) was found in coronary heart disease patients than in the particles of healthy controls. Ex vivo in mice this Hb-loaded HDL was shown to decrease the nitric oxide–mediated endothelium-dependent vasorelaxation.13 Subjects with increased number of the metabolic syndrome components, which cause well-characterized changes in HDL particle size distribution, show a decreasing trend in the activity of lipoprotein-associated phospholipase A2.14 These results suggest that a more complete picture of the relations between HDL functions in physiological and pathological situations and changes in its protein complement may be obtained by unbiased evaluation of the particles entire set of apolipoproteins and associated proteins. Such knowledge could also suggest additional functions of HDL.

Participation of specific apolipoproteins profiles in lipoprotein structure and function, as well as its association with cardiovascular and metabolic disease, received a remarkable impulse with the pioneering studies of Alaupovic and collaborators.15–17 In these studies the main tools were sequential immunochemical procedures for isolation of particles with specific apolipoprotein composition, and their results led to the concept of apolipoprotein-defined lipoprotein families. It is feasible to extend immunodetection to most lipoprotein-associated proteins. This however implies biased preselection of the antibodies to be used and multiple assays. Thus, there is a clear need for methods that could measure and identify and quantify most of the proteins of lipoprotein particles with relative speed. The development of proteomic platforms has led to progress in such directions using isolated plasma lipoproteins and their subclasses. In the present review we will discuss results of several of these studies, especially those obtained with human plasma HDL.

Methodological Considerations in Proteomic Studies of Proteins Present in Lipoproteins

Depletion of Contaminant Proteins

The current proteomic approach to study the entire protein complement of individual lipoproteins classes requires depletion of other abundant plasma proteins that, if present, may interfere with the analysis. Depletion steps should preserve proteins and polypeptides that are part of the circulating lipoproteins. This obviously is a difficult task because only nonexchangeable apolipoproteins, like apoB100 and apoB48, are certain to remain as part of the particles after purification. Although the exchangeable apolipoproteins (apoAI, apoAII, apoE, and apoC) content of HDL and LDL remains reasonable reproducible after commonly used depletion procedures, this may not be the case for the content of associated proteins because their affinity with the particles could be weaker than that of...
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fractionation. This procedure and its modifications are by

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led to the key publication of Havel, Eder, and Bragdon

isolation and analysis.

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in circulation. Therefore we have to accept that the protein

proteins that are not bound to the particles when they are

class or subclass should be depleted of other plasma

lipid and protein composition. Furthermore the lipoprotein

class or subclass should be depleted of other plasma

proteins. Methods developed for lipoprotein class

isolation in the last decades, based on density (ultracen-

trifugation), charge (electrophoresis), size (exclusion chro-

matography), and specific precipitation, or combinations

of them are all in principle designed to separate lipoprotein

classes and subclasses from each other and from other

plasma proteins. Thus the resulting lipoprotein preparation

should be reasonably homogeneous in terms of their

physicochemical properties as density, size, charge and

lipid and protein composition. Furthermore the lipoprotein

class or subclass should be depleted of other plasma

proteins that are not bound to the particles when they are

in circulation. Therefore we have to accept that the protein

composition of lipoprotein classes is an operational defi-
nition qualified by the methods used for the particles

isolation and analysis.

The original work of de Lalla, Gofman, and Lindgren

led to the key publication of Havel, Eder, and Bragdon

describing how sequential ultracentrifugation of human

plasma or serum in solutions of neutral salts (KBr, NaI)
can be used to obtain defined VLDL, LDL, and HDL

fractions. This procedure and its modifications are by

far the most commonly used for isolation of lipoprotein

classes and subclasses in proteomic studies. However
during ultracentrifugation in neutral salts, in its sequential

version, or in density gradient alternatives, LDL and HDL

are exposed to ionic strengths 5 to 20 times above those of

human plasma and lymph. This is a condition that could

alter the lipoprotein protein complement by dissociation

of molecules bound by charge–charge interactions. The

possibilities that ultracentrifugation at high salt concentra-
tions can modify the exchangeable apolipoprotein and associ-
ated protein composition of lipoproteins has been recog-
nized and documented by several investigators (for a
reviews see Kane and Kunitake and Skinner). This
suggests that the association, at the lipoprotein surface-
monolayer, of polar and nonpolar lipids with amphipathic
apolipoproteins segments may be affected by high ionic
strength. This may also be the case for some of the
associated proteins that could be more loosely bound than
apolipoproteins.

To diminish possible stripping of proteins by high ionic

strength our laboratory developed differential and isopyc-

nic gradient ultracentrifugal procedures in buffers of D2O

and sucrose that maintain the lipoproteins at physiological

ionic strength and pH. These solutions allow isolation of

homogenous lipoprotein classes with reproducible content

of apolipoproteins, associated proteins, and lipid content

and composition. Recently we compared the protein

component number and relative content of human lipopro-
tins isolated with the classical KBr method or the proce-
dure using D2O/sucrose iso-ionic buffers. For this com-
parison a proteomic approach was used with the aid of

surface-enhanced laser desorption ionization-time of flight

mass spectrometry (SELDI-TOF-MS) and 2-dimensional

gel electrophoresis (2-DE). Interestingly, but not surpris-

ingly, the D2O/sucrose or KBr buffers produced VLDL

samples with similar number of protein components (there

is no major differences in ionic strength at d <0.019

g/mL), but LDL and HDL isolated with D2O/sucrose

showed significantly higher number of peptides/proteins in

the low molecular weight area, 2 to 10 kDa (Table). The

results suggest that high salts in the KBr procedure can
deplete, especially HDL, of proteins that are normally

associated at physiological ionic conditions. The D2O/
sucrose procedure, in addition, has the advantage that

lipoproteins can be used directly for electrophoretic, chro-
matography, immunologic, and mass spectrometry-based

analyses without need for buffer exchange or concentra-
tion steps. The D2O/sucrose method apparently avoids the high
ionic strength-induced (KBr, NaI) dissociation of proteins

that are mainly attached by charge-charge associations.

However, it does not solve the potential problem of
centrifugal shear-stress dissociation. It should be said,

nonetheless, that in efforts to compare the proteome of

lipoprotein particles isolated from subjects with metabolic
alteration with those of healthy controls, as long as the

same isolation procedure is used, the documented differ-
ences in particles of similar size and density should be
valid. Nonetheless, we should keep in mind that differ-
ences in size distribution and lipid composition at the

surface monolayer, as those present in lipoproteins from

healthy controls and in subjects with dyslipidemia of

insulin resistance, may lead to particles with dissimilar

affinity for exchangeable proteins. In proteomic studies

alternatives to the use of high-salts ultracentrifugation, like

Table. Percentage Protein Content and No. of Peptide/Protein Peaks (SELDI-TOF-MS) Measured in Lipoprotein Fractions Isolated From Six Samples of the Same Plasma Pool Using Ultracentrifugation in KBr and D2O/Sucrose Buffers

<table>
<thead>
<tr>
<th>Method</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>KBr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein %</td>
<td>10.60±1.03</td>
<td>19.40±0.79</td>
<td>47.58±0.72</td>
</tr>
<tr>
<td>Peaks No.</td>
<td>29 (14%)</td>
<td>33 (6%)</td>
<td>35 (13%)</td>
</tr>
<tr>
<td>D2O/Suc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein %</td>
<td>10.08±0.98</td>
<td>22.60±1.00</td>
<td>50.04±0.77</td>
</tr>
<tr>
<td>Peaks No.</td>
<td>33 (6%)</td>
<td>42 (12%)</td>
<td>49 (9%)</td>
</tr>
<tr>
<td>ns</td>
<td></td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>ns</td>
<td></td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Lipoproteins were isolated by sequential ultracentrifugation in KBr and D2O/sucrose buffers (see). Protein percent content was calculated by adding the protein values measured spectrophotometrically to those of total lipids evaluated gravimetrically in lipid extracts: \( \text{protein} / \left( \text{protein} + \text{lipids} \right) \times 100 \). The values are expressed as means±SEM. The No. of peptide/protein peaks was measured in the m/z range 2000 to 10 000 of the SELDI-TOF-MS profiles. The figures in parentheses are the percent coefficient of variation for the No. of peaks: (SD/mean)×100. ns indicates not significant.

Taken with permission from Ståhlman et al.
Proteomic Platforms Used for Evaluation of the HDL Proteome

Qualitative and quantitative evaluations of HDL proteins have been reported with the use of 1-D/2-DE in combination with proteolytic fragmentation, peptide separation by high performance liquid chromatography (HPLC), and protein identification with double MS in tandem (MS/MS) or with matrix-assisted laser desorption/ionization–time flight mass spectrometry (MALDI-TOF-MS). Modifications of these approaches to reduce their limitations for identification and quantification of protein mixtures are under continuous development, and some of these possible improvements were recently reviewed. Several of the new techniques are intended to overcome the limitations in reproducibility and long time required for proteomic evaluations using 2-DE. The new differential in-gel electrophoretic (DIGE) technology, which allows coseparation of equal concentrations of protein samples labeled with cyanine dyes (Cy2 or Cy5) and use of an internal standard stained with a dye with different spectra (Cy3) in the same 2-DE experiment, is a promising approach. The ideal solution could be a protein fractionation procedure with quantitative recovery of the separated protein, or peptide fragments, with possibilities for external or internal calibration of the signal-response to mass. This has led to searches for other methods, like direct evaluation of protein digests separated by 2D-LC or directly analyzed by MS/MS. The application of some of these procedures to HDL was recently evaluated by Heller et al. It should be noted, however, that quantification of individual proteins or peptides from complex mixtures or proteolytic digests by MS/MS is still difficult because the most abundant components of proteins/peptides mixtures limit ionization of the less abundant ones. In addition, as pointed out by Duncan et al., in complex proteomes several proteins can be precursors of the same peptide and as a result, depending on the peptide(s) selected, the precursor protein levels may be significantly underestimated. However, in many cases, rather than trying to establish absolute content of all individual components, we are interested in comparing lipoprotein proteomes associated with specific metabolic alterations or pathologies with those of lipoproteins from healthy individuals. Within this less ambitious aim, sensitivity, reproducibility, small sample volumes, and the possibility for analysis of several samples in reasonable time take precedence over absolute values. SELDI TOF-MS, or ProteinChip technology, appears to be convenient for comparisons of more than a few samples because it can handle several individual analyses per week and requires minute amounts of the protein mixtures. However, protein identities are not revealed in the process because the analytes cannot be subjected to digestion or tandem mass spectrometry. SELDI-TOF-MS can be regarded as a peptide/protein profiling technique requiring downstream analysis, digestion, and identification of analytes after the quantification step, and its advantages and limitations have been recently reviewed. Other promising efforts aimed to improve quantitative proteome evaluations include isotope labeling of peptide mixtures before MS analyses. Enzyme-catalyzed $^{18}$O$_2$-labeling appears appropriate for complex mixtures as long as the hardware and software used can distinguish differences in mass of 4 Da (for a recent review see Fenselau and Yao). Other methods of improving the quantitative evaluation of proteomic studies are based on new computational tools used after the online LC-MS peaks are detected or the signals from stable isotope labeling are collected.

The HDL Proteome in Health and Disease

In the last decades HDL has acquired an extensive functions catalogue that includes participation in lipid metabolism, atheroprotective effects, and partaking in the innate immune response. In many of the early HDL proteomic studies the main aim was to identify proteins that could provide clues about the diverse postulated function of this lipoprotein. On the other hand, more recent studies have compared the lipoprotein proteome of healthy controls with that from patients suffering atherosclerotic cardiovascular disease (ACVD) or other metabolic alterations in which changes in HDL levels or subclasses distribution have been previously documented. Earlier studies were conducted with application of 2-DE, in combination with matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS). These results show that in HDL were present, besides the classical apolipoproteins: apoAI (6 isoforms), apoAII, apoAIV (6 isoforms), apoE (6 isoforms), apoM (2 isoforms), apoCII, apoCIII (3 isoforms), other proteins as SAA (2 isoforms), α-amylase, and α-1-antitrypsin. Interestingly, although the methods used in these ground-breaking studies are considered semiquantitative, the authors already reported differences in the distribution of apolipoprotein and associated proteins between the large HDL2 and the small HDL3 subclasses that have been confirmed by more recent studies.

Rapid developments in LC MS/MS have extended in less than 2 years the number of identified proteins in HDL particles from 13 to 53, including all the known apolipoproteins except apoB100 and apoB48, and Rezzae and
collaborators\textsuperscript{26} indicated for the first time that the identified proteins in HDL could be separated into clusters whose members shared participation in defined functional areas. These areas were: lipid metabolism and transport, inflammatory markers, immune system and complement factors, growth factors, hormone-binding proteins, and proteins involved in hemostasis. Furthermore, it was found that the HDL surface monolayer serve as a binding matrix not only for intact apolipoproteins and associated proteins but also for a wide range of small peptides that were originally thought to be associated mostly with plasma albumin. In this study, using a combination of HPLC and mass spectrometry, more than 100 peptides in the range of 1000 to 5000 Da were detected. MS sequence analysis was used to assign the peptides to parent apolipoproteins that were identified as apoAI, apoAIV, apoCIII and apoL; in addition, other peptides were assigned to nonlipoprotein plasma proteins as fibrinogen, \textalpha_{1}-protease inhibitor, and transthyretin.\textsuperscript{36} The circulating life of these peptides appears to be extended by binding to albumin, HDL, and possibly to other lipoproteins. Hortin\textsuperscript{37} suggested that specific peptide signatures could have diagnostic values.

Recent data from Davidson et al\textsuperscript{38} supports the hypothesis that proteomics of HDL subpopulations may lead to a more comprehensive understanding of differential functions of individual particle subclasses. In these experiments HDL\textsubscript{2b-2a}, -3a, -3b, and HDL\textsubscript{3c} were isolated by isopycnic density gradient ultracentrifugation in neutral salts, and 28 different HDL-associated proteins and apolipoproteins were identified in each subclass using LC/ESI/MS and a quadropole TOF analyzer. The identified proteins appear to associate into 5 distinct clusters with different abundance of the individual proteins in the HDL subclasses. Furthermore, there are strong correlations between levels of the components in the clusters of individual subclasses and their potential antioxidant properties. The authors proposed that this is specially the case for the dense HDL\textsubscript{3} subclass that shows elevated content of the cluster containing paraoxonase (PON) 1/2, PON 3, and apoL-I. Moreover, this dense HDL\textsubscript{3} subclass appears to be the unique carrier of apoAIV, PON1, phospholipid transport protein (PLTP), apoJ, apoF, and apoL-I. This last protein is a component of the trypanosome lytic factor of human serum. In the other hand, the apparent preference of apoE, apoCII and apoCIII for the light HDL\textsubscript{2} subclass was proposed to be related to the known interaction of these particles with components of the LDL receptor family.\textsuperscript{38} In this study efforts were made to obtain internally consistent data calibrating the sample preparation and hardware with internal standards (see online material in reference\textsuperscript{38}). The results again indicate that quantitative proteome profiles may help to assign specific roles to subclasses of HDL in health and disease. One limitation of the HDL subclasses obtained by density gradient or differential ultracentrifugation is that they are still heterogeneous in terms of charge. Future studies should consider the possibility of introducing a charge-based subfractionation step before the proteomic analysis. Although this will certainly prolong the analysis, it may uncover further subclasses-specific associations.

**HDL Proteome in Coronary Artery Disease**

One of the driving forces behind proteomic studies is the prospect that unbiased comparison of protein profiles of physiological fluids, or those from functional biochemical aggregates, can provide more information than just that offered by evaluation of few of their individual protein components. This viewpoint also motivates proteomic studies of HDL, specially because the association between low levels of the particles with risk for ACVD and their apparently multiple functions. Most of the clinical and epidemiological studies supporting this association have measured HDL-cholesterol (HDL-C) and more recently evaluated the concept of the apoB/apoAI ratio.\textsuperscript{39} Nonetheless, despite the clinical usefulness of these parameters, there is currently an intense search for additional structural properties of HDL that could be additional biomarkers of ACVD risk. Based in the recent proteomic-based results discussed above, it is reasonable to expect that sets of functionally associated proteins can provide information about their participation in the spectrum of atheroprotective actions attributed to HDL.\textsuperscript{28,29,38} In the case of HDL, initial studies showed that it was feasible to obtain reproducible profiles of the proteins present in the particles using ultracentrifugation depletion steps and mainly 2-DE, and MS of peptides resulting from trypsination of individual spots. Naturally this led to studies in which the aim became to compare the HDL proteome of healthy subjects with that of particles from patients with dyslipidemias\textsuperscript{28,40} and with that of patients with ACVD.\textsuperscript{29} Some of the studies, in addition, have had the intention of documenting differences that may allow the assignment of specific proteins to functional defects of HDL. Although this looks as a straightforward and natural evolution of the field, there are caveats concerning the interpretation and potential clinical use of the results because of their complexity and their still semiquantitative status, as discussed by Reilly and Tall.\textsuperscript{41}

Most studies on HDL proteomes of patients with cardiovascular disease and controls share the limitation that samples from few individuals have been analyzed and that evolving methodological approaches have been used. Nonetheless, the results are promising because patients with ACVD studied show distinct characteristics in groups of associated proteins that are believed to be important for the particles atheroprotective properties. In the study by Vaisar et al,\textsuperscript{29} HDL was shown to carry apolipoproteins and proteins with functions in lipid metabolism, the acute phase response, complement regulation, and blood coagulation. Furthermore, a whole set of proteinases inhibitors was detected in both plasma HDL and in apoAI-containing particles isolated from human atherosclerotic lesions. Interestingly, in this study the proteome of the HDL\textsubscript{3} subclass from 6 controls and 7 patients with coronary artery disease (CAD) was compared, and a significant enrichment of apoCIV, PON1, complement C3, apoAIV,
and apoE, was observed in the patients. In this analysis, HDL and HDL3 were isolated by ultracentrifugation in neutral salts, and protein identification and relative quantification was done after trypsin digestion, peptide fractionation by HPLC, and peptide identification by electro-spray ionization-MS/MS. The interesting article describing these results contains also a balanced discussion of the difficulties of using MS for quantitative documentation of the protein complement of complex mixtures.\(^{29}\) In a recent study from the same laboratory the proteome of HDL3 from 6 CAD patients was compared before and after 1-year treatment with combined statin/niacin therapy.\(^{40}\) In addition, the proteome obtained after treatment was compared to the HDL protein profile from healthy controls. The results from the niacin-treated group are particularly interesting because this substance increases HDL-C by more than 16% and appears to do this mostly by rising HDL2-C but with modest effect on HDL3-C.\(^ {41}\) Moreover, many investigators believe that the effect of niacin on reduction of CAD risk is related to its effects on HDL.\(^ {42}\) The number of identified HDL3 proteins in the study by Green et al\(^ {40}\) was 27; 7 less than in the previous study from the same laboratory.\(^ {29}\) The authors attributed this difference to a dissimilar MS procedure used (LC-ESI-MS/MS versus LC-Fourier transform-MS/MS), which was more adapted to measure quantitative differences. Another observation was that the HDL3 of CAD patients is significantly enriched in apoE and apoCII and contain less apoA and phospholipid transport protein (PLTP) than the particles from controls. Niacin/statin treatment of the patients decreases HDL3 apoE to the levels of healthy controls. The authors proposed that an increase in apoE could accelerate hepatic removal of apoE-rich HDL3 and that this is responsible for its low plasma level in patients with CAD. Thus, decrease in apoE-rich HDL removal, caused by the niacin/statin treatment, may lead to the increase number of circulating particles. The treatment, in the other hand, raises apoA and PLTP in HDL3; a change that was suggested may improve the participation of HDL3 in reverse cholesterol transport (RCT). It would have been interesting to extend the above analyses to HDL2 because niacin appears to have larger effects in largest HDL particles.\(^ {42,43}\) A recent review\(^ {44}\) of the articles by Vaisar et al\(^ {29}\) and Green et al\(^ {40}\) discuss in more detail the possible origin of the differences observed.

### HDL Proteome in Insulin Resistance

The dyslipidemia of insulin resistance and type 2 diabetes is the product of unpaired insulin signaling in adipose tissue, liver, and skeletal muscle and it appears to be a major contributor to the excess risk for ACVD in these conditions.\(^ {45}\) Overproduction of hepatic VLDL, increase generation of small dense LDL (sdLDL), and reduction of circulating levels of apoAI-containing HDL particles are its main characteristics.\(^ {46}\) Mechanistic studies indicate that the HDL subclass distribution and composition in insulin resistance and type 2 diabetes are frequently altered and that these changes compromise its capacity to mediate cholesterol efflux and possible other antiatherosclerotic functions.\(^ {6,9,14,47}\) Using SELDI-TOF-MS, our laboratory found that, compared to healthy matched controls, the small dense LDL (sdLDL) of subjects with the metabolic syndrome and subclinical peripheral atherosclerosis have a significant higher content of all the apoCIII isoforms and a lower content of apoAI, apoCII, and apoE. Furthermore, these changes appear significantly associated with the affinity of the particles for human arterial proteoglycans.\(^ {24}\) This property is believed to be a key initial step in the entrapment of LDL in the arterial wall contributing to atherogenesis.\(^ {48–50}\) One of the most interesting results in this study was that even when comparing sdLDL subclasses with the same density range, significant differences in associated proteins and apolipoproteins exist between patients and controls. In these experiments we used LDL subclasses isolated with gradients of D\(_2\)O/sucrose buffers with physiological ionic strength that yield lipoproteins with a higher content of associated proteins and apolipoproteins.\(^ {24}\) Recently we extended the use such buffers to isolation of HDL (1.063 to 1.210 g/mL) that then was directly used for 2-DE and SELDI-TOF analyses.\(^ {25}\) We compared with this procedure the most prominent identified protein components of plasma and lipoproteins from 10 patients and 10 healthy matched controls. Patients and controls were not from the cohort of the Atherosclerosis and Insulin Resistance Study (AIR). The patients fulfilled the NCEP definition of the metabolic syndrome and were shown to have peripheral atherosclerosis by ultrasound evaluation of carotid and femoral intima-media thickness.\(^ {24}\) Equal amounts of plasma proteins or lipoprotein-proteins were applied to anionic (Q10) and cationic (CM10) protein chips (Ciphergen Biosystems) as described.\(^ {23,24}\) It can be observed in Figure 2 that application of the SELDI-TOF-MS technology to whole plasma provides limited information, in spite of the narrow dispersion of the intensity values (AU), being SAA and apoAI the only discriminators between plasma from patients and controls. However, Figure 2 illustrates the significant differences between patients and controls in most of the identified protein components in HDL and LDL (VLDL is not shown). In HDL from patients, a lower content of the isoform apoCII and higher content of all apoCIII isoforms and SAA-IV were the most significant differences between patients and controls. SAA on HDL has been found to markedly reduce the affinity of this lipoprotein for hepatocytes but it increases 3- to 4-fold the affinity for macrophages. This is believed may cause the clearing of HDL to be redirected from liver toward macrophages, a phenomenon that has been proposed to shift the particles from an antiatherogenic, antiinflammatory function to an atherogenic one.\(^ {51,52}\) In addition SAA in HDL appears to reduce the capacity of HDL subclasses for cholesterol efflux promotion (see Chait et al for a review)\(^ {53}\). Furthermore, recently McGillicudy et al\(^ {44}\) provided elegant evidence in humans and mice indicating that acute phase-HDL enriched in SAA induced by acute endotoxemia have impair capacity to remove cholesterol from macrophages.
disturbances have been limited in number and size because of the labor-intensive nature of the present proteomics platforms. Thus we need to wait for additional research to establish the general significance of the promising reported findings. More extensive comparative studies of the lipoproteins in health and disease will be possible with advances shortening analysis time and improving qualitative and quantitative evaluation. It can be anticipated that more extensive and precise proteomics of HDL and other lipoproteins will provide information leading to a better understanding of their multiple physiological functions and about abnormalities contributing to atherosclerosis.

Disclosures
None.

References
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