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 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. 2009;29:00-00.)

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). Kumpula et al 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. 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, apoAI, 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. 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 charged amino acids side chains, are in contact with the aqueous environment. Class Y and class G* α-helices, also present in exchangeable apolipoproteins, interact poorly with lipids but apparently can associate with proteins.
from cells, but it may, at the same time, compromise other functions of HDL. 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. 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. 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. 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 (apoA1, 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 apolipoproteins. Methods developed for lipoprotein class isolation in the last decades, based on density (ultracentrifugation), charge (electrophoresis), size (exclusion chromatography), 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 definition 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.18,19 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 concentrations can modify the exchangeable apolipoprotein and associated protein composition of lipoproteins has been recognized and documented by several investigators (for a reviews see Kane and Kunitake8 and Skinner20). 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.21,22

To diminish possible stripping of proteins by high ionic strength our laboratory developed differential and isopycnic 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.23–25 Recently we compared the protein component number and relative content of human lipoproteins isolated with the classical KBr method or the procedure using D2O/sucrose iso-ionic buffers.25 For this comparison 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 surprisingly, 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 <1.019 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, chromatography, immunologic, and mass spectrometry-based analyses without need for buffer exchange or concentration 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 differences in particles of similar size and density should be valid. Nonetheless, we should keep in mind that differences 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.25 In proteomic studies alternatives to the use of high-salts ultracentrifugation, like immuno-adsorption, have been explored for purification of HDL preparations.26,27 In these schemes affinity chromatography with immobilized antibodies against apo AI and apoAII are used to trap HDL and after extensive wash with

### 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>Protein %</th>
<th>Peaks No.</th>
<th>Protein %</th>
<th>Peaks No.</th>
<th>Protein %</th>
<th>Peaks No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>KBr</td>
<td>10.60±1.03</td>
<td>29 (14%)</td>
<td>19.40±0.79</td>
<td>33 (6%)</td>
<td>47.58±0.72</td>
<td>35 (13%)</td>
</tr>
<tr>
<td>D2O/Suc</td>
<td>10.08±0.98</td>
<td>29 (11%)</td>
<td>22.60±1.00</td>
<td>42 (12%)</td>
<td>50.04±0.77</td>
<td>49 (9%)</td>
</tr>
<tr>
<td>ns</td>
<td>ns</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Lipoproteins were isolated by sequential ultracentrifugation in KBr and D2O/sucrose buffers (see24,25). Protein percent content was calculated by adding the protein values measured spectrophotometrically to those of total lipids evaluated gravimetrically in lipid extracts: (protein+lipids)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.29
physiological saline the antigen-antibody complex is dis-sociated either with 1 mol/L acetic acid or 3 mol/L isothiocyanate. Homogeneous HDL fractions have been obtained in these experiments. However, no data were reported about potential depletion of associated lipoproteins by the elution with molar concentrations of acetic acid or isothiocyanate. Based on the above discussion, we conclude that there is still need for innovative use of techniques that could allow isolation of lipoprotein classes and subclasses without modifying physiological pH and ionic strength and without exposure to potential protein stripping forces.

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 equal concentrations of protein samples labeled with phoretic (DIGE) technology, which allows coseparation of competitive HDL fractions have been obtained in these experiments. However, no data were reported about potential depletion of associated lipoproteins by the elution with molar concentrations of acetic acid or isothiocyanate. Based on the above discussion, we conclude that there is still need for innovative use of techniques that could allow isolation of lipoprotein classes and subclasses without modifying physiological pH and ionic strength and without exposure to potential protein stripping forces.

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 equal concentrations of protein samples labeled with phoretic (DIGE) technology, which allows coseparation of competitive HDL fractions have been obtained in these experiments. However, no data were reported about potential depletion of associated lipoproteins by the elution with molar concentrations of acetic acid or isothiocyanate. Based on the above discussion, we conclude that there is still need for innovative use of techniques that could allow isolation of lipoprotein classes and subclasses without modifying physiological pH and ionic strength and without exposure to potential protein stripping forces.

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 equal concentrations of protein samples labeled with phoretic (DIGE) technology, which allows coseparation of competitive HDL fractions have been obtained in these experiments. However, no data were reported about potential depletion of associated lipoproteins by the elution with molar concentrations of acetic acid or isothiocyanate. Based on the above discussion, we conclude that there is still need for innovative use of techniques that could allow isolation of lipoprotein classes and subclasses without modifying physiological pH and ionic strength and without exposure to potential protein stripping forces.

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 equal concentrations of protein samples labeled with phoretic (DIGE) technology, which allows coseparation of competitive HDL fractions have been obtained in these experiments. However, no data were reported about potential depletion of associated lipoproteins by the elution with molar concentrations of acetic acid or isothiocyanate. Based on the above discussion, we conclude that there is still need for innovative use of techniques that could allow isolation of lipoprotein classes and subclasses without modifying physiological pH and ionic strength and without exposure to potential protein stripping forces.

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 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. Rezzae and collaborators 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,
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. 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. 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 dyslipemias and with that of patients with ACVD. 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.

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, 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 HDL3 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 electrospray 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. 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. 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. Moreover, many investigators believe that the effect of niacin on reduction of CAD risk is related to its effects on HDL. The number of identified HDL3 proteins in the study by Green et al was 27; 7 less than in the previous study from the same laboratory. The authors attributed this difference to a dissimilar MS procedure used (LC-ESI-MS/MS versus LC-Fourier transform-MS/MS), which is 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 apoJ 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 apoJ 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 subclasses with the same density range, significant differences in associated proteins and apolipoproteins exist between patients and controls.24,25 The values are means±SE relative intensity units of duplicate analyses of 10 patients and 10 controls using the same amount of plasma and lipoprotein protein. These changes appear significantly associated with the affinity of the particles for human arterial proteoglycans. This property is believed to be a key initial step in the entrapment of LDL in the arterial wall contributing to atherogenesis. One of the most interesting results in this study was that even when comparing sLDL 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 D2O/sucrose buffers with physiological ionic strength that yield lipoproteins with a higher content of associated proteins and apolipoproteins. Recently we extended the use such buffers to the NCEP definition of the metabolic syndrome and were evaluated in lipoproteins and plasma from healthy controls (controls, empty bars) and patients with type 2 diabetes and peripheral atherosclerosis fitting the metabolic syndrome definition of the NCEP (patients, patterned bars). The peptide/protein peaks were evaluated using SELDI-TOF-MS and identified by MALDI-TOF/TOF of the hydrolyzates as described in the text and in references. The values are means±SE relative intensity units of duplicate analyses of 10 patients and 10 controls using the same amount of plasma and lipoprotein protein.

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. Overproduction of hepatic VLDL, increase generation of small dense LDL (sLDL), and reduction of circulating levels of apoAI-containing HDL particles are its main characteristics. Mechanistic studies indicate that the HDL subclass distribution and composition in insulin resistance and type 2 diabetes are frequently altered and these changes compromise its capacity to meditate cholesterol efflux and possible other antiatherosclerotic functions. Using SELDI-TOF-MS, our laboratory found that, compared to healthy matched controls, the small dense LDL (sLDL) 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, apoCI, and apoE. Furthermore,
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 apoCIII 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 McGillicuddy et al54 provided elegant evidence in humans and mice indicating that acute phase-HDL enriched in SAA induced by acute endotoxemia have impairment capacity to remove cholesterol from macrophages. This alteration is associated with the increase in SAA and decrease in apoAI of the acute-phase HDL.

**Conclusions**

The classical apolipoproteins have structural functions and provide recognition signals that control the interactions of lipoproteins with cells and tissues. All lipoproteins, in addition, contain surface-associated proteins that also appear to participate in their multiple functions. Recent proteomic studies have extended the number of identified associated proteins and show that HDL and LDL subclasses have unique profiles that may modulate specific functions. Abnormalities of some apolipoproteins, as decrease of apoAI in HDL and increase of apoB100 and apoCIII in LDL, have been well documented to be associated with risk of ACVD. The discussed proteomics studies suggest that alterations of many apolipoproteins and multiple associated proteins also may be present in patients with coronary atherosclerosis and dyslipidemias and in subjects with peripheral atherosclerosis and the dyslipidemia of insulin resistance. In addition, recent data suggest that a proteomic approach can be useful for evaluation of dyslipidemia treatments because it can give functional information beyond that based on changes of lipids and lipoprotein levels. However, proteomic studies of HDL and LDL in controls and patients with metabolic 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 proteome 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**

7. deGoma EM, deGoma RL, Rader DJ. Beyond high-density lipoprotein cholesterol levels: Evaluating high-density lipoprotein function as influenced by novel therapeutic approaches. *Am Coll Cardiol. 2008;51:2199.*


Proteomics of Apolipoproteins and Associated Proteins From Plasma High-Density Lipoproteins
Pia Davidsson, Johannes Hulthe, Björn Fagerberg and Germán Camejo

Arterioscler Thromb Vasc Biol. published online September 24, 2009;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/early/2009/09/24/ATVBAHA.108.179317.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/