Lipoproteins have attracted wide interest clinically because they are major risk factors for cardiovascular disease (CVD) in humans. For example, high levels of LDL cholesterol are a major risk factor for myocardial infarction and sudden death (7). In contrast, low levels of HDL cholesterol strongly associate with an increased risk of CVD (8), whereas high levels associate with longevity (9). LDL and HDL are the major carriers of cholesterol in plasma and serum, accounting for the strong relationship between cholesterol levels and CVD.

Apolipoprotein (apo)B-100 is the major protein structural component of VLDL and LDL, whereas apoA-I serves that function in HDL. Chylomicrons contain apoB-48, a truncated form of apoB-100. Clinical studies suggest that levels of apoB-100 and apoA-I may be better predictors of CVD risk than LDL and HDL cholesterol levels (10).

Other protein components of LDL and HDL play important roles in lipid metabolism, energy homeostasis, and inflammation.

Most studies of lipoproteins have focused on single proteins. Proteomics, a global approach to understanding protein expression, regulation, and function, transcends analysis of individual components. One of its major tools is mass spectrometry (MS) (11, 12), which can detect and quantify hundreds or even thousands of proteins in one sample. MS measures molecular mass and therefore can also detect and characterize posttranslational modifications of proteins (13). Its ability to identify disease-related biomarkers is also a powerful advantage (14).

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Recent mass spectrometric studies have revealed that lipoproteins carry a diverse array of previously unsuspected roles in lipid metabolism, energy homeostasis, and inflammation.

Lipoproteins play a central role in extracellular lipid transport in species ranging from insects to mammals (1–3). As couriers of lipids, they transport triglycerides derived from the diet to peripheral tissues for energy metabolism. They also are important for the bidirectional movement of cholesterol between the liver and peripheral tissues. However, lipoproteins are implicated in pathways distinct from lipid metabolism. For example, inflammation markedly alters lipoprotein metabolism (4), and recent studies implicate lipoproteins as important mediators of the immune response and host defense mechanisms (5, 6).
proteins. For example, shotgun proteomics has implicated regulation of the complement pathway and proteolysis in HDL’s cardioprotective effects (15). This review highlights lipoproteomics, using MS to study lipoprotein-associated proteins and their biology. We first provide brief overviews of lipoprotein physiology and MS. Then we summarize lipoproteomic studies, focusing on recent insights they have provided into lipoprotein function and biology. We also discuss the limitations of our current approaches as well as important technical and conceptual issues that should be addressed in future studies.

APOLIPOPROTEINS ARE OF CENTRAL IMPORTANCE IN LIPOPROTEIN BIOLOGY

Lipoproteins are classically considered to be spherical particles containing a hydrophobic core of neutral lipid (cholesteryl ester and triglycerides) and a surface rich in amphipathic proteins, phospholipids, and free cholesterol (1–3, 16). These surface components solubilize the hydrophobic lipid core. In mammals, including humans, there are two major classes of lipoproteins: those containing apoB (chylomicrons, LDL, VLDL) and those containing apoA-I (HDL). The metabolism of each lipoprotein class is dictated by its major structural protein (16). For instance, apoB-100 is required for binding LDL to the LDL receptor, which plays the essential role in uptake of LDL from the circulation by peripheral cells and the liver (1). In contrast, the truncated form of apoB (apoB-48) present in chylomicrons lacks the LDL binding domain. Thus, remnant particles derived from chylomicrons by lipolysis of core lipids are cleared from the liver by pathways distinct from that of the LDL receptor.

Enterocytes, which line the small intestine, assemble chylomicrons from triglycerides, phospholipids, and cholesterol derived from the diet and then secrete the nascent lipoprotein into the blood (Fig. 1). Chylomicrons are the largest lipoproteins in humans (16). Together with VLDL, which is synthesized by the liver, these triglyceride-rich lipoproteins undergo lipolysis in muscle and adipose tissue to provide fatty acids for energy metabolism. ApoB-48 and apoB-100 serve as structural supports for chylomicrons and VLDL, respectively, permitting the formation of a large, hydrophobic core (17). ApoC-II, another protein carried by triglyceride-rich lipoproteins that contain apoB, is a cofactor for lipoprotein lipase, which hydrolyzes triglycerides in the particles to free fatty acids (18). Deficiency of either protein results in profound disturbances in lipid metabolism, demonstrating the pivotal role apolipoproteins play in lipoprotein physiology.

In contrast to the triglyceride-rich chylomicrons and VLDL particles, the major lipids in HDL and LDL are cholesterol and cholesteryl esters (3). LDL is largely derived from circulating VLDL by the action of lipases on triglycerides (18). ApoB-100 provides structural support for VLDL and LDL (17). It also is the ligand for the LDL receptor on liver and peripheral tissue (1, 19). Modified forms of apoB-containing particles, such as oxidized LDL, promote the conversion of artery wall macrophages into cholesteryl ester-laden foam cells, the hallmark of the atherosclerotic lesion (20).

ApoA-I functions as the scaffold for HDL assembly (21). Unlike apoB, apoA-I is associated with very little lipid when it is secreted from hepatocytes and enterocytes. In this poorly lipidated state, it serves as the ligand for ABCA1, which exports phospholipid and cholesterol into nascent HDL particles, promoting their maturation (22, 23). HDL is protein-rich compared with other lipoproteins and contains many other apolipoproteins. Its apolipoproteins, in concert with other proteins like phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP), collaborate with apoA-I to orchestrate the exchange of lipids between lipoprotein particles (24). ApoA-I also has a central role in removing cholesterol from macrophage foam cells, making a key contribution to the cardioprotective effects of HDL (25).

Apolipoproteins are often classified as nonexchangeable and exchangeable and each class has distinct structural features. ApoB exhibits both amphipathic
α-helical and β-pleated sheet structure (26). Each chylo-
micron, VLDL, or LDL particle contains 1 mol of apoB, which is not exchanged between different particles. In contrast, apoA-I exists largely as an amphipathic α-helical protein (21). Each circulating HDL particle contains 2–4 mol of apoA-I that can move between lipoprotein particles. A wide variety of other apolipoproteins (apoCs, apoE, etc.) are found on lipoproteins containing apoB and apoA-I, though in smaller amounts than in the major structural proteins. Lipoproteins also contain low-abundance proteins that generally do not exhibit the typical structural features of apolipoproteins. Certain of these, such as PLTP, CETP, and LCAT, have important roles in lipid metabolism. However, over the past decade, there has been an explosion of interest in minor protein components of lipoproteins that have functions distinct from lipid metabolism. For example, paraoxonase and clusterin, which are cotransported with HDL in plasma, have been proposed to have antioxidant and anti-inflammatory properties (4, 5, 27, 28). Importantly, levels of these proteins are markedly altered in humans with CVD and in mice that are susceptible to atherosclerosis (27, 28). Loss of anti-inflammatory and antioxidant proteins, perhaps in concert with gain of proinflammatory proteins, may thus be another key contributor to the antiatherogenic effects of HDL.

LIPOPROTEOMICS: MASS SPECTROMETRIC ANALYSIS OF LIPOPROTEINS

There are three general approaches to identifying pro-
teins in complex mixtures (reviewed in Refs. 29–31). The first separates proteins based on isoelectric point (pI) and molecular size, using two-dimensional (2D) gel electrophoresis (32). Protein spots are visualized by staining and extracted from the gel. Following proteolytic digestion, peptides are analyzed with MS and/or MS/MS. This approach can be useful for quantifying proteomes of limited complexity that contain proteins that are relatively abundant. 2D gel electrophoresis is also a powerful strategy for identifying different isoforms or posttranslational modifications of the same protein. However, this analytical strategy also suffers from many limitations; for example: i) it can be difficult to reproducibly analyze multiple samples; ii) extraction and analysis of multiple protein spots by MS is laborious; iii) individual gel spots generally contain more than one protein, which complicates protein quantification; and iv) large, hydrophobic proteins, such as apoB, are unable to enter gels (33–39), making it difficult to quantify the contributions of individual proteins to the overall composition of lipoprotein particles.

The second approach uses matrix assisted laser desor-
tion/ionization-time of flight-MS (MALDI-TOF-MS) to iden-
tify intact proteins (40–46). Because the mass of an intact protein offers limited diagnostic information, this method is generally limited to identifying known proteins. It also is best applied to the detection of relatively small proteins (typically less than 35 kDa). Protein quantification by MALDI-TOF-MS can also be problematic, and this approach also suffers from a limited dynamic range. Thus, MALDI-
TOF-MS is typically limited to detecting relatively abundant proteins that are already known to exist in lipoproteins (such as apoC, apoA, and apoE and their isoforms).

The third approach, termed shotgun proteomics (47), uses liquid chromatography (LC) in concert with electro-
spray ionization and tandem MS analysis (LC-ESI-MS/MS) to identify peptides. The peptide digest is separated by LC, introduced into the gas phase by ESI, and analyzed by tandem MS (15, 48, 49). To identify the proteins in the original mixture, the MS/MS spectrum collected for each peptide is searched against a database of all the theoretical spectra for a digest of the relevant genome. This method is powerful and can detect more than a thousand proteins and their posttranslational modifications in a single sample. It also is readily applied to the analysis of large numbers of samples, which is critical for clinical and translational studies. Data-base searching can be time-consuming and the analyses expensive, however (47), and protein quantifica-
tion by this method is generally semi-quantitative.

Identification of proteins by MS/MS generally involves the analysis of peptide digests (29–31). Proteins that asso-
ociate with lipids contain sizable hydrophobic portions that are stabilized by strong secondary structural features and these regions can resist complete proteolytic digestion (50, 51). In addition, the lipids in lipoproteins make protein digestion and mass spectrometric analysis challeng-
ing. However, recent technical advances have addressed many of these issues (reviewed in Ref. 51).

It is important to note that the method used to isolate lipoproteins significantly affects the protein content of the resulting particles (52). Most proteomic studies have re-
lied on ultracentrifugation, which is convenient because particle classes are defined by density and ultracentrifugation is widely used for clinical studies. However, the typical isolation involves high concentrations of KBr, a potent chaotropic agent, which can dissociate proteins from lipo-
proteins (53, 54). Substituting D2O/sucrose for KBr may overcome these limitations (39).

Methods involving size exclusion chromatography can enrich for specific size classes of lipoprotein, but can result in contamination by nonlipoprotein proteins, especially when serum or plasma is fractionated. Immunoaffinity chromatography has potential advantages because it should more readily preserve lipoprotein-associated pro-
teins (15, 36), but it classifies lipoproteins on the basis of a specific protein component (e.g., apoA-I or apoB).

EXPANDING THE LIPOPROTEOME

The proteins that have been identified to date by pro-
teomics analysis of lipoproteins are listed in Table 1. Significantly more proteins have been detected in human HDL than in LDL or VLDL, however, only HDL has been analyzed by shotgun proteomics (15, 48, 49). More complete proteomes for LDL and VLDL might be defined by similar analyses. At this time, no proteomic studies of chylo-
microns have been reported.
TABLE 1. Proteins detected in lipoproteins by mass spectrometry

| Protein                          | VLDL | LDL       | HDL       |
|----------------------------------|------|-----------|-----------|
| Actin                            | 1a   | 3a,4,5,6,7,8,9,10,12 |           |
| Albumin                          | 1a,3b| 3c         | 5,6,12    |
| Alpha-1-acid glycoprotein        |      |           | 5         |
| Alpha-1-antitrypsin inhibitor    | 1a 4 | 5,6,7,9,12 |           |
| Alpha-1B-glycoprotein            | 1a   | 6,7       |           |
| Alpha-1-microglobulin/bikunin    | 6    |           |           |
| Alpha-2-antiplasmin              | 6    |           |           |
| Alpha-2HS-glycoprotein           | 1a   | 5,6       |           |
| Alpha-2-macroglobulin            | 5,6  | 12        |           |
| Alpha-amylase (salivary)         | 9    |           |           |
| Angiotensinogen                  | 6    |           |           |
| Apolipoprotein(a)                |      |           |           |
| ApoA-I                           | 1    | 3,4,5,6,7,8,9,10,12 |           |
| ApoA-II                          | 1    | 5,6,8,9,10,12 |           |
| ApoA-IV                          | 1    | 3c         | 6,7,8,9,10,12 |
| ApoB                             | 1    | 3d         | 6,12      |
| ApoC-I                           | 1    | 3d         | 5,6,9,12  |
| ApoC-II                          | 1    | 3a,4       | 6,8,9,12  |
| ApoC-III                         | 1    | 3a,4       | 5,6,7,8,9,10,12 |
| ApoC-IV                          | 1    |            | 6,12      |
| ApoD                             | 1    |            | 5,6,8,10,12 |
| ApoE                             | 1    | 3a,4       | 5,6,8,9,10,12 |
| ApoF                             | 1    |            | 5,6,12    |
| ApoH                             | 1    |            | 6         |
| ApoJ (Clusterin)                 | 3a   | 5,6,8,12   |           |
| ApoA-I                           | 1    | 6,7,8,9,12 |           |
| ApoM                             | 1    | 3c         | 6,8,9,12  |
| C4b binding protein              | 6    |            | 10        |
| Calgranulin A                    | 3d   |            |           |
| CETP                             | 1    |            | 6,10      |
| Complement C3                    | 1    |            | 6,8       |
| Complement C4                    | 1    |            | 6         |
| Complement C9                    | 1    |            | 6         |
| Fibrinogen                       | 1a   | 3d         | 6,7,10,12 |
| Fibronectin                      | 3b   |            |           |
| Haptoglobin-related protein      | 1    |            | 5,6,12    |
| Haptoglobin                      | 3d   | 8,10       |           |
| Hemoglobin                       | 1    |            | 11        |
| Hemopexin                        | 1a   | 6          |           |
| Immunoglobulin Mu                | 1a   | 3d         |           |
| Inter-tissue inhibitor chain H4  | 6    |            | 6,10      |
| Kininogen                        | 1    |            | 6         |
| LCAT                             | 1    |            | 6,10      |
| LPS binding protein              | 1    |            | 7         |
| Lysozyme C                       | 3d   |            |           |
| Paraoxonase 1                    | 1    | 5,6,8,12   |           |
| Paraoxonase 3                    | 1    | 6,12       |           |
| Platelet activating factor-acetyl| 1    |            | 12        |
| hydrolase                        | 1    |            | 12        |
| Platelet basic protein           | 1    |            | 12        |
| PLTP                             | 1    |            | 6,12      |
| Prenylcysteine lyase             | 6    |            | 1         |
| Prothrombin                      | 1    |            | 6         |
| Retinol binding protein          | 1    |            | 6         |
| SAA1/2                           | 1    | 3a,4       | 6,9,12    |
| SAA4                             | 3a   | 5,6,9,12   |           |
| SerpinF1                         | 1    |            | 6         |
| Transferrin                      | 1    |            | 6         |
| Transthyretin                    | 1    |            | 6,7,8,12  |
| Vitamin D binding globulin       | 1a   | 5,6        |           |
| Vitronectin                      | 1a   | 6          |           |

Studies include: 1. Mancone, 2007 (37); 2. Bondarenko, 1999 (40); 3. Karlsson, 2005 (34); 4. Stahlman, 2008 (39); 5. Heller, 2005 (44); 6. Vaisar, 2007 (15); 7. Hortin, 2006 (69); 8. Rezaee, 2006 (38); 9. Karlsson, 2005 (35); 10. Kunitake, 1994 (36); 11. Watanabe, 2007 (46); 12. Davidson, 2009 (64).

Proteins detected in ultracentrifuge-purified VLDL and described by the authors as contaminants.

Proteins detected in ultracentrifuge-purified LDL and not in LDL prepared by size exclusion chromatography.

Proteins detected in LDL prepared by size exclusion chromatography and not in ultracentrifuged LDL.

Proteins detected in LDL prepared by size exclusion chromatography and ultracentrifugation.

The most important message from proteomic analysis is simple: each lipoprotein class contains many proteins involved in processes distinct from lipid metabolism. For example, LDL contains lysozyme C, which can kill invading pathogens (34). This observation may be physiologically relevant, because apoB itself was recently shown to modulate the virulence of Staphylococcus aureus in vivo (55).

The HDL lipoproteome

HDL is anti-inflammatory (27), which may contribute to its atheroprotective capacity. Potential mechanisms include stimulation of nitric oxide secretion by endothelial cells (56), inhibition of neutrophil degranulation (57), regulation of the formation of the complement membrane attack complex (58, 59), and reduction of lipid peroxides (60).

We used LC-ESI-MS/MS to investigate the possibility that HDL carries proteins that might play a previously unsuspected role in its anti-inflammatory properties (15). Shotgun proteomics identified 48 proteins in HDL and HDL₃ (the dense fraction of HDL) isolated by ultracentrifugation from healthy controls and/or CVD subjects. This analytical approach provides a comprehensive view of the HDL proteome because we identified 22 of 23 HDL proteins with established roles in lipid metabolism.

We used Gene Ontology (GO) Consortium analysis to associate the complex array of HDL proteins to biological processes (15). Unexpectedly, there were more acute-phase-response proteins, whose plasma concentrations are altered markedly by acute inflammation, than proteins implicated in lipid metabolism. We also identified 13 proteins not previously known to reside in HDL, including complement factors C9 and C4A/C4B as well as the complement regulatory protein vitronectin. Pioneering studies of HDL protein complexes that kill protozoa (61, 62) led to the proposal that HDL serves as a platform for the assembly of proteins. Our detection of a series of proteins involved in complement activation is consistent with this hypothesis.

Proteolysis of structural proteins in atherosclerotic lesions is thought to play a critical role in plaque rupture, the major cause of myocardial infarction and sudden death in subjects with CVD (16). It is therefore of interest that we found a family of proteins in HDL that contain protease inhibitor domains (15). Serine protease inhibitors, termed serpins, are key regulators of numerous biological pathways involved in coagulation, matrix remodeling, and inflammation. These observations raise the possibility that HDL is cardioprotective, in part, by inhibiting proteolysis and plaque rupture.

Complement activation increases tissue damage in animal models of acute myocardial infarction. It is noteworthy that we identified multiple complement regulatory proteins in HDL, which may limit injury to cardiac cells and prevent activation of the coagulant cascade.

HDL subspecies contain distinct protein populations that associate with antioxidant effects

LDL oxidation is implicated in the unregulated accumulation of cholesterol by macrophages, a key event in
HDL has been proposed to inhibit LDL oxidation in vivo, which may contribute significantly to its cardioprotective effects (27). Systemic inflammation has been proposed to convert HDL to a dysfunctional form that loses these antiatherogenic effects. However, the underlying factors that render HDL dysfunctional remain poorly understood.

Proteins such as paraoxonase (PON) that are cotransported with HDL in plasma have been proposed to have antioxidant properties (27). To begin to investigate the role of specific subspecies in the antiatherogenic effects of HDL, Davidson et al. (64) used LC-ESI-MS/MS to define the protein composition of five HDL fractions isolated by isopycnic density gradient ultracentrifugation. In parallel studies, they evaluated the ability of the different HDL subspecies to inhibit LDL oxidation in vitro. Importantly, levels of three specific proteins (apoL, PON1, and PON3) were highly enriched in the dense subfractions of HDL. Moreover, the ability of HDL to inhibit LDL oxidation strongly associated with the HDL content of those proteins. These observations support the proposal that HDL is composed of distinct subpopulations of particles that have discreet biological properties. Understanding the role of specific proteins and HDL subspecies may lead to new diagnostic and therapeutic approaches to atherosclerosis.

The LDL/VLDL lipoproteome

We used GO analysis to associate the proteins detected in VLDL and LDL (Table 1) with biological process. When compared with the whole human genome using the database for annotation, visualization, and integrated discovery (DAVID) resource (65, 66), the only category of proteins in VLDL that was significantly enriched associated with lipid transport (p<1 × 10⁻¹²). LDL was enriched in proteins associated with both lipid transport (p<1 × 10⁻⁵) and the inflammatory response (p<1 × 10⁻³).

In contrast, our analysis revealed that the HDL proteome (15) was enriched in proteins associated with lipid transport (p<1 × 10⁻²¹), the inflammatory response (p<1 × 10⁻¹⁵), complement activation (p<1 × 10⁻⁶), the acute phase response (p<1 × 10⁻¹¹), and protease inhibition (p<1 × 10⁻¹¹). Thus, the available data suggest that the HDL proteome is much more diverse than that of other lipoproteins.

Posttranslational modifications of proteins

Lipoprotein oxidation is implicated in atherogenesis (63). It is noteworthy that HDL isolated from humans with established CVD contains much higher levels of two oxidized amino acids, chlorotyrosine and nitrotyrosine, than does HDL of apparently healthy control subjects (70–72). Chlorotyrosine is a specific oxidation product of the heme protein myeloperoxidase (73) and macrophages in human atherosclerotic lesions express high levels of that enzyme (74). When specific tyrosine and methionine residues in apoA-I, the major protein in HDL, are oxidized by myeloperoxidase, it loses its ability to remove cholesterol from cells by the ABCA1 pathway (70, 75). This pathway may be important in generating dysfunctional HDL because the interaction of apoA-I with ABCA1 is critical in protecting macrophages from cholesterol accumulation in mouse models of hypercholesterolemia and accelerated atherosclerosis (25).

These observations suggest that the inflamed atherosclerotic lesion is one potential location where oxidative damage of proteins could be clinically important. In future studies, it will be of great interest to use targeted MS/MS
to determine if site-specific modifications of apoA-I characteristic of myeloperoxidase can be detected in HDL isolated from humans with established CVD.

TOWARD QUANTITATIVE AND TRANSLATIONAL LIPOPROTEOMICS

In addition to identifying the proteins present in complicated mixtures of proteins, proteomics can be used to estimate the relative abundance of proteins (reviewed in Refs. 29, 31, 76–78). In the case of 2D gels, the intensity of protein spots detected with certain dyes is proportional to the protein concentration in the sample. The intensity of each spot can be normalized using spiked proteins, an endogenous protein whose concentration is constant from sample to sample, or with chemical tags (79). However, this approach relies on the assumption that all proteins in the mixture enter the gel, and that each protein spot consists of a single protein species (80). Neither of these assumptions is true for LDL and VLDL, which contain apoB as their major structural protein. In particular, apoB is problematic because of its large size and hydrophobicity, which causes the protein to precipitate out of solution during isoelectric focusing. It is also difficult to standardize 2D gel electrophoresis to yield reproducible results with large numbers of samples.

MALDI-TOF-MS can provide relative quantification of intact proteins, particularly if the protein mixture is not complex and there are large differences in relative abundance. This approach was used to demonstrate that apoC-III is highly enriched in the small dense LDL of subjects with type 2 diabetes or carotid atherosclerosis when compared with the small dense LDL of normal control subjects (42). In the same study, apoE was significantly depleted from the same fraction. Both proteins have important biological roles and the changes in concentration in this fraction could turn out to have important clinical implications.

A variety of label-free methods have been developed for quantifying relative protein abundance by MS, including spectral counting, summing all the MS/MS spectra observed for peptides derived from a single protein in an LC-ESI-MS/MS analysis (81, 82). Because proteins that are more abundant in a sample have a higher probability of being identified during data-dependent MS/MS scanning, the number of tandem spectra acquired for individual peptides reflects protein abundance. Pioneering studies demonstrated that spectral counting quantified exogenously added proteins in yeast cell lysates over a dynamic range of two orders of magnitude (81). Quantitative changes in protein expression in cultured cells and biological fluids, as assessed by immunoblotting, correlated strongly with spectral counting (83, 84). Collectively, these observations provide strong evidence that spectral counting is a sampling statistic that can assess relative protein abundance in biological material.

We used spectral counting to demonstrate that HDL₃ isolated from the plasma of subjects with established CVD had significantly higher concentrations of apoE, complement C3, apoC-IV, PON-1, and apoA-IV than HDL₃ from normal controls (15). To confirm that this approach can estimate protein abundance semi-quantitatively, we measured apoE levels immunochemically in HDL₃ isolated from a different set of 64 subjects: 32 with established CVD and 32 age-matched controls. Levels of apoE were significantly higher in HDL₃ isolated from the CVD subjects. In striking contrast, the two groups had similar levels of apoA-I and apoA-II. Importantly, relative protein abundance, as assessed by MS and biochemical assays, was similar, supporting the proposal that spectral counting can assess relative protein abundance in lipoproteins.

We recently used MS to test the hypothesis that aggressive lipid therapy with atorvastatin and niacin modifies the HDL proteome in humans with established CVD (48). To quantify changes in protein abundance, we employed two complementary methods, spectral counting and extracted ion chromatograms. Both methods are label-free (i.e., do not require introduction of a mass tag into the proteins), which makes them well-suited for analyzing HDL obtained in clinical studies. For extracted ion chromatograms, the ion current for a given peptide and charge state are extracted from the full scan mass spectrum and used to construct a chromatogram. The area under the curve then provides a quantitative measure of relative peptide abundance.

We used spectral counting as an initial screen to identify proteins that appeared to be differentially expressed and then used extracted ion chromatograms to quantify the relative abundance of these proteins in HDL₃ isolated from CVD subjects before and during treatment with atorvastatin and niacin (48). This approach offers two important advantages. First, extracted ion chromatograms estimate protein ratios more accurately than spectral counting (81). Second, it is possible to compare the extracted ion chromatogram ratios of multiple peptides detected from the same protein (48), which should increase confidence in the results.

Spectral counting, together with extracted ion chromatograms, identified four HDL₃ proteins whose relative abundance appeared to change as a result of treatment: apoE, apoJ, apoF, and PLTP (48). Levels of apoE fell, whereas levels of apoJ, apoF, and PLTP rose. We used two approaches to confirm that our mass spectrometric techniques accurately assessed changes in the relative abundance of proteins in HDL. First, when we analyzed HDL₃ isolated from a different set of 13 subjects, we observed a strong linear correlation between apoE levels as assessed by extracted ion chromatograms and biochemically. Second, we used an immunochemical approach to confirm that combination therapy with niacin and statin reduced levels of apoE in HDL₃ in an independent group of different subjects. Our observations indicate that when newly diagnosed CVD subjects are treated with combination therapy, the HDL₃ proteome is remodeled to more closely resemble that of HDL₃ of healthy control subjects (48).
CHALLENGES AND FUTURE DIRECTIONS

The elaborate cast of proteins in lipoprotein particles begs the question of how the proteins get there in the first place. For example, interactions between apoB and other lipoprotein-associated proteins during assembly of VLDL in the liver have not been detected (85, 86). Moreover, apoA-I is apparently secreted from the liver devoid of other proteins and lipid (87). Lipoprotein particles thus seem to acquire most of their nonstructural proteins while coursing the lymphatics, bloodstream, and tissues. Apolipoproteins are generally assumed to associate with lipoproteins via hydrophobic interactions, but studies of the trypanosome lytic factor strongly suggest that protein-protein interactions are also involved (61, 62). It will be of great interest to determine the molecular basis for assembling the protein cargo of lipoproteins.

Abundant blood proteins have been detected in isolated lipoproteins, raising concern about possible contamination (15, 33, 34, 36, 37). The best-studied of these putative contaminant proteins, albumin, plays a role in lipid metabolism via its fatty acid-binding sites (88). One group of investigators isolated albumin using seven different biochemical and immunochemical techniques (89). ApoA-I was readily detected in every preparation, suggesting that it normally interacts with albumin in blood. Other investigators found a relatively constant molar ratio of albumin to apoA-I in lipoproteins ranging from the density of HDL to that of VLDL (44). These observations raise the possibility that albumin interacts with apoA-I, and perhaps other lipoproteins, and that these interactions play previously unsuspected roles in lipoprotein function in vivo. In future studies, it will clearly be important to use complementary biochemical and functional studies to determine whether minor protein components of lipoproteins that are abundant in blood truly associate with the lipoproteins.

The availability of biomarkers that could reveal the presence of CVD in time to ward off a heart attack or stroke would have an enormous impact on health-care costs and public health, given that CVD is the leading cause of death in the industrialized world. However, the ability to identify subjects at increased risk for heart disease is limited. Our recent studies suggest that the HDL proteome may distinguish patients with CVD from healthy people (15). Moreover, we have shown that lipid lowering therapy remodels the HDL proteome in subjects with established CVD, yielding a protein composition that resembles that in apolipoprotein A-I free HDL3 particles (48). These studies raise the possibility that quantifying the lipoproteome could provide insights into the efficacy of lipid therapy and help identify agents with cardioprotective actions.

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