Apolipoprotein A-Containing Lipoprotein Particles: Physiological Role, Quantification, and Clinical Significance

J. C. Fruchart and G. Ailhaud

High-density lipoprotein (HDL) particles are made up of two major populations of particles, differing in composition and metabolism. Both contain apolipoprotein (apo) A-I but only one contains apo A-II. Lipoprotein particles that contain only apo A-I (LpA-I particles) can increase cellular cholesterol efflux from cultured cells in vitro. LpA-I:A-II particles, however, do not increase cholesterol efflux. LpA-I:A-II can be determined directly with an enzyme-linked differential antibody immunosorbent assay. LpA-I is determined by differential electrophoresis: in the presence of a large excess of anti-apo A-II, LpA-I:A-II particles are retained in one peak, whereas LpA-I migrates as a second peak. Both lipoprotein forms of apo A-I-containing particles are present mainly in HDL, but the relative proportion of LpA-I is greater in HDL2 than in HDL3. Concentrations of LpA-I in plasma samples from normolipemic subjects average ~10% higher in women than in men. The lower apo A-I concentrations in patients with significant coronary artery disease reflect a decrease in the LpA-I particles. Data obtained in octogenarians also support the possibility that LpA-I might represent the anti-atherogenic fraction of HDL. Moreover, the concentration of LpA-I in children of parents with premature coronary heart disease was lower than that of a control group without any family history of this disease. Nutrients and hypolipidemic drugs seem to affect the two kinds of particles differently.

Additional Keyphrases: sex- and age-related effects

Epidemiological and clinical studies showing an association between decreased concentrations of high-density-lipoprotein (HDL) cholesterol and increased risk of premature coronary artery disease (CAD) (1, 2), have generated interest in the mechanism through which HDL prevents atherosclerosis.

Human HDL consists of a collection of particles differing in size, density, and apolipoprotein content (3).

On the basis of their hydrated density, two main subfractions have been identified: HDL2 and HDL3 (4); each of these subfractions can be further fractionated into discrete subclasses by different techniques (5, 6).

Some authors have suggested that the low HDL concentrations observed in CAD were mainly due to a decrease of HDL2 and particularly of the subfraction HDL2β (7).

HDL metabolism has not yet been fully characterized, and no specific functions have been assigned to the HDL subfractions, which are defined by physical ultracentrifugal flotation criteria. HDL particles apparently are produced in the liver and the intestine (8) and are continuously being converted in plasma (3, 5). Moreover, surface material derived from the catabolism of triglyceride (TG)-rich lipoproteins appears to contribute to their synthesis (9). Several studies have shown that HDL can promote reverse cholesterol transport, a chain of metabolic reactions that remove excess cholesterol from peripheral tissues and transport cholesterol, directly or indirectly, with other lipoproteins to the liver (10, 11). This process involves several steps. Interactions between HDL3 and HDL-binding sites are generally believed to facilitate egress of cholesterol from cells to the plasma lipoproteins (12, 13) and particularly to a small pre-β-migrating HDL (14). This incorporation of free cholesterol into acceptor lipoproteins, facilitating its esterification by lecithin:cholesterol acyltransferase (LCAT, phosphatidylcholine-sterol acyltransferase, EC 2.3.1.43) and cholesteryl ester transfer protein (CETP), mediates transfer to very-low-density lipoproteins and exchange for TG (15, 16).

In this complex pathway, reverse conversion of HDL2 and HDL3 takes place. Supply of surface material converts small, dense HDL3 to HDL2, whereas the action of hepatic lipase (EC 3.1.1.3) on TG-rich HDL2 causes reverse conversion of HDL2 to HDL3 (17).

LCAT deficiency leads to an increase of HDL3 in plasma (18); CETP deficiency induces an increase of HDL and particularly of cholesteryl ester-rich HDL2 (19); hepatic lipase deficiency results in the accumulation of TG-rich HDL2 (20).

Recent studies with selected immunosorption techniques designed to isolate subpopulations of lipoproteins have shown that HDL cannot be regarded as homogeneous particles containing apolipoproteins (apo) A-I and A-II. The distribution of particles within HDL is heterogeneous not only with respect to their hydrated density.
but also in relation to their apolipoprotein composition (3, 21, 22). It is now recognized that HDL contains at least two types of apo A-I-containing lipoprotein particles, which might have different metabolic functions (23, 24) and clinical significance (25). One species contains as the main protein components both apo A-I and A-II (LpA-I:A-II); in the other species, apo A-II is absent (LpA-I). The definition of such lipoprotein particles by their apolipoprotein content has been proposed to be more appropriate for studying the chemical complexity and metabolic functions of the plasma lipoprotein system.

Our purpose in this review is to describe the recent progress made in isolation, characterization, quantification, and determination of the clinical significance of LpA-I and LpA-I:A-II.

Isolation and Composition of LpA-I and LpA-I:A-II

LpA-I and LpA-I:A-II are currently purified from total plasma by sequential immunoaffinity chromatography (22, 26, 27). Some authors do not find any difference in lipid composition between LpA-I and LpA-I:A-II (22), but others have claimed that the percentage of TG and the cholesteryl ester/total cholesterol ratio are lower in LpA-I than in LpA-I:A-II (27–29). The lipid/protein ratio appears to be higher in LpA-I than in LpA-I:A-II (28). The molar ratio of apo A-I to apo A-II in LpA-I:A-II is 1.5. Small quantities of apo A-IV, Cs, D, E are found in both fractions (30, 31).

Of considerable significance is the finding that proteins stimulating reverse cholesterol transport (LCAT, CETP) and other proteins such as apo J are mainly present in LpA-I and not in LpA-I:A-II (23, 30, 32).

Physiological Role in LpA-I and LpA-I:A-II

Our understanding with respect to the metabolism of apo A-I-containing particles is limited. However, Brewer et al. (28) have shown recently that both apolipoprotein particles are synthesized by the liver but that LpA-I particles are produced by the intestine only. The metabolic interrelationship between the two sub-populations is not well established, but apparently LpA-I particles are catabolized at a faster rate than are LpA-I:A-II particles (33). One of the key questions is whether LpA-I and LpA-I:A-II have different physiological roles (34).

To gain some insights into the mechanisms by which cholesterol movement takes place in peripheral cells, researchers have used cultured adipose cells as a model. Indeed, the fact that adipose tissue has the ability to accumulate, store, and, when needed, mobilize a large pool of unesterified cholesterol means that these cells meet the requirement for the study of reverse cholesterol transport (29). In studies of cholesterol efflux mediated by apo A-I-containing particles in mouse adipose cells, after cholesterol preloading with low-density lipoproteins, long-term exposure to LpA-I particles promoted cholesterol efflux, whereas no efflux was observed in the presence of LpA-I:A-II (35). These results, which emphasize that LpA-I:A-II particles behave as a distinct metabolic entity, have been confirmed in other studies (36, 37).

The ligands that recognize the cell surface HDL-binding sites have been identified as apo A-I, apo A-IV, and apo A-II (38, 39). We proposed (39) that apo A-I and apo A-IV play the role of agonists and apo A-II that of antagonist of cholesterol efflux. Slotte et al. (40) suggested that adding HDL to human fibroblasts or bovine endothelial cells induces a protein kinase C-dependent translocation of cholesterol from intracellular membranes to the cell surface. We recently demonstrated (37) that cholesterol efflux from adipose cells is coupled to diacylglycerol production and protein kinase C activation. The fact that the binding of apo A-I/dimyristoylphosphatidylcholine complexes, but not that of apo A-II/dimyristoylphosphatidylcholine complexes, produces diacylglycerol strongly supports the role of apo A-II as an antagonist in the production of cholesterol efflux. In support of this interpretation, we recently showed (35) that LpA-I:A-II can inhibit the LpA-I-promoted cholesterol efflux of cholesterol-preloaded adipose cells.

Quantitative Determination of LpA-I and LpA-I:A-II

Several methods have been developed for direct quantification of apo A-I-containing particles in human plasma: immunoprecipitation (41), two-phase electromunoassay (42), and enzyme-linked differential-antibody immunosorbent assay (28). These methods are well adapted for use in research laboratories but are time-consuming and inaccurate. The recent development of a differential electromunoassay allows the direct measurement of LpA-I. By using a large excess of anti-A-II antibodies, LpA-I:A-II particles are retained in one peak and LpA-I migrates as a second peak (43). This new system can provide specific and reproducible determinations of LpA-I in human plasma.

Clinical Significance of LpA-I and LpA-I:A-II Measurements

Coronary Artery Disease. Concentrations of LpA-I but not LpA-I:A-II were lower in normolipemic patients with angiographically documented CAD (44) than in a group of asymptomatic subjects and a group of patients with arteriographically normal coronary arteries (25). However, in a similar study where the patient group had higher TG concentrations than the control subjects, we found that both LpA-I and LpA-I:A-II were diminished in CAD patients to a similar degree [one can postulate that the decrease in LpA-I:A-II particles in hypertriglyceridemic patients may be related to the decreased formation of such particles during lipolysis (45, 46)]. A recent study of patients before coronary bypass surgery concluded that their concentrations of both LpA-I and LpA-I:A-II were lower than in control subjects (46). However, discrimination analysis indicated that LpA-I was the more powerful discriminating factor in this hypertriglyceridemic population.

A case control study of apo A-I-containing particles has been performed in three populations with contrasting risks for CAD (ECTIM Study) (47). Male patients
with myocardial infarction and control subjects were recruited in two French centers (Strasbourg and Toulouse) and a Northern Irish center (Belfast). The standardized mortality rates in Belfast, Strasbourg, and Toulouse were respectively 348, 102, and 78 per 100 000 for the tested population. LpA-I and LpA-I:A-II concentrations were lower in the patients than in the control subjects, but the difference in LpA-I concentrations between the three populations was statistically significant: LpA-I for the controls and the myocardial infarction cases was much lower in Belfast than in the French centers. In this study, the multivariate analysis suggested that the LpA-I/HDL cholesterol ratio was very significant.

Recently it has been also observed that the concentration of LpA-I (but not of LpA-I:A-II) in children whose parents have a premature form of CAD is lower than in a control group with no familial history of CAD (Amouyel, Isorez, Bard, Goldman, Zylberberg, and Fruchart, ms. submitted). The relevance of these observations requires further investigation.

Longevity. Assuming that octogenarians, who have survived periods of life during which the incidence of CAD is very high, should have several protective factors against CAD, we compared in octogenarians and younger control subjects (ages 30–50 years) the concentrations of apo A-I-containing particles: LpA-I was significantly increased and LpA-I:A-II was clearly lower in the octogenarians (48). Brewer et al. (28) investigated LpA-I and LpA-I:A-II in a kindred with hyperalphalipoproteinemia and decreased risk of CAD. The selective increase in LpA-I in the 60-year-old putative homozygote proband with a family history of longevity supports the concept that these particles may represent the anti-atherogenic fraction of HDL.

Dyslioproteinemias. Some dyslioproteinemias have specific profiles of apo A-I particles. For instance, type III dyslioproteinemia, a disorder with accelerated atherosclerosis, is characterized by a decrease of LpA-I and an increase of LpA-I:A-II (49, 50). Non-insulin-dependent diabetes mellitus is characterized by a specific decrease in LpA-I (51). The HDL decrease observed in patients with chronic renal failure who have to undergo hemodialysis is mainly due to a decrease in LpA-I:A-II (52).

Diet. Diet also modifies LpA-I concentration. In a study of the effect on LpA-I of a high polyunsaturated/saturated fatty acid ratio (53), a ratio value of 1.1 led to a decrease in LpA-I in comparison with a ratio of 0.2. Chronic alcohol consumption was shown to lead to an increase in LpA-I:A-II, whereas LpA-I decreased (54); moderate alcohol intake, however, increased both types of these particles (W. Valmaki et al., Arteriosclerosis, in press). The interpretation of such changes is difficult because the decrease in LpA-I may be due to overcatabolism or undersynthesis. Only kinetic studies can answer this crucial question, and the clinical importance of this effect may depend on this mechanism.

**Effects of drugs on apo A-I particles.** Considering the preliminary results obtained in clinical and epidemiological studies, it is obviously interesting to study the effect of drug therapy on lipoprotein particles as defined by their apolipoprotein composition. Two main questions may be raised concerning the effect of drugs: Do compounds with various mechanisms of action lead to different effects on apo A-containing particles? Is there any relationship between the pharmacological modulation of a particular lipoprotein family and the change in cardiovascular morbidity and mortality? We now have additional information to more accurately answer the first question, but further investigation is certainly needed to answer the second one.

Atmeh et al. (42) showed that LpA-I may be increased by the use of nicotinic acid, whereas probucol leads to a decrease in this particle. In contrast, nicotinic acid decreased LpA-I:A-II, and probucol had no major effect on its concentration. We have recently shown (Table 1) that fenofibrate decreases LpA-I and increases LpA-I:A-II, whereas hydroxymethylglutaryl-CoA reductase inhibitors (simvastatin and pravastatin), as well as cholestyramine, increase both particles (55–58). Also, we recently found (unpublished data) that estradiol may increase the LpA-I concentration in postmenopausal women without altering the LpA-I:A-II concentration. Because it has been suggested that LpA-I may represent the particle that is involved in cholesterol efflux from peripheral cells, we speculate that the increasing effect on LpA-I may potentiate the beneficial cardiovascular effect of low-density lipoprotein cholesterol reduction also seen with these compounds. Inversely, the decreasing effect obtained with fenofibrate might be considered as a potentially harmful effect. However, kinetic studies are necessary to determine whether the increasing effect of the hydroxymethylglutaryl-CoA reductase inhibitors and cholestyramine is due to oversynthesis or undercatabolism and whether the decreasing effect of fenofibrate is due to undersynthesis or overcatabolism. The clinical importance of the effect of drugs on LpA-I may depend on these findings.

The introduction of new immunological methods that allow the separation of various apo A-I-containing lipo-

**Table 1. Changes in Apo A-I-Containing Lipoprotein Particles under Different Therapies**

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Concno, mg/L</th>
<th>Base</th>
<th>Week 9(8)</th>
<th>Week 10(12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LpA-I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simvastatin</td>
<td>440</td>
<td>+25*</td>
<td>+56*</td>
<td></td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>440</td>
<td>−128*</td>
<td>−151*</td>
<td></td>
</tr>
<tr>
<td>Cholestyramine</td>
<td>450</td>
<td>+217*</td>
<td>+151*</td>
<td></td>
</tr>
<tr>
<td>Pravastatin</td>
<td>430</td>
<td>+130*</td>
<td>(48)</td>
<td></td>
</tr>
<tr>
<td>LpA-I:A-II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simvastatin</td>
<td>860</td>
<td>+54</td>
<td>+18</td>
<td></td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>780</td>
<td>+139*</td>
<td>+223*</td>
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<tr>
<td>Cholestyramine</td>
<td>930</td>
<td>+118*</td>
<td>+412*</td>
<td></td>
</tr>
<tr>
<td>Pravastatin</td>
<td>960</td>
<td>+249*</td>
<td>+326*</td>
<td></td>
</tr>
</tbody>
</table>

Significantly different from baseline: *P < 0.05, **P < 0.01 (Wilcoxon test).
protein particles reveals the existence of subpopulations with different lipid and apolipoprotein composition and different metabolic functions. Quantification of Lp-A-I and Lp-A-I-A-II might allow more accurate predictions of the risk of developing premature atherosclerosis. This approach might also provide a new basis for the classification of dyslipoproteinemias and the study of the effects of hypolipidemic drugs.

References
42. Atmeh RF, Shepherd J, Packard CJ. Subpopulations of apo-


