A nontransferable peptide analog of a plasma apolipoprotein diacyl lipid-associating peptide (diLAP) incorporates into model reassembled high density lipoproteins (R-HDL). In whole plasma in vitro, diLAP irreversibly transfers to native rat HDL₁ and human HDL₃, but not to rat HDL₂ or human HDL₄. The rate of transfer is dependent on the physical state of the lipid in the R-HDL. Exogenous cholesterol promotes the formation of larger HDL. When diLAP-labeled R-HDL were injected into rats, the diLAP that initially associated with HDL₂ transferred to HDL₁ over a period of 48 h. The rate of clearance of diLAP-labeled HDL was slower than that of apoA-I. The liver was the preferred site for diLAP-labeled HDL₁ uptake. In contrast, diLAP-labeled HDL₃ were associated with liver, ovaries, and adrenal glands, with the adrenal glands exhibiting the highest specific association. DiLAP was not found in the kidneys. These data show that 1) rat HDL is cleared more slowly than rat apoA-I; 2) HDL is removed from the plasma compartment as a particle; 3) there are tissue-specific differences in the removal of rat HDL₁ and HDL₃; 4) HDL₃ is a precursor to HDL₁; and 5) cholesterol and the activity of lecithin:cholesterol acyltransferase are essential to HDL remodeling.

Plasma lipoproteins, which are composed of a variety of lipids and proteins, are remodeled by spontaneous and protein-mediated processes to produce mature particles that transport lipids among various extravascular compartments. Lipoprotein interconversions are an important but poorly defined aspect of the remodeling process. One of the best understood examples of lipoprotein interconversion is the lipolytic cascade that begins with very low density lipoproteins (VLDL). This process has been relatively easy to study because there are no reliable markers for the processing of HDL₁, even though it is clear that substantial remodeling of this relatively long-lived particle occurs. Indirect evidence of the interconversion of HDL subfractions has been derived from studying the changes associated with lipolysis. These studies showed that postprandial lipemia leads to triglyceride-rich HDL₃ that are converted to HDL₂ (Patsch et al., 1984, 1987). Moreover, the in vitro studies showed that postprandial HDL₂ from hyperlipemic subjects can be converted to HDL₃ (Patsch et al., 1987), a process requiring apoA-I transfer among different particles.

HDL exhibit different rates of degradation according to the exchangeability of the marker employed to monitor plasma decay kinetics. Cholesterol and phospholipids exchange too rapidly to be used as a marker for HDL (Stein and Stein, 1966; Phillips et al., 1987); cholesteryl esters, triglycerides, and phosphatidylcholines are transferred between lipoprotein subclasses by specific protein factors (Jarnagin et al., 1987; Tall, 1986; Barter et al., 1987). Cells in culture also selectively remove cholesteryl esters from HDL (Pittman et al., 1987). Nevertheless, when apoA-I is used as a marker for HDL, the lifetimes obtained are on the order of 4–5 days in normal human subjects (Shepherd et al., 1978; Schaefer et al., 1986). These values should be considered a lower limit for the lifetime of HDL₁ because studies in rat clearly show that the liver is the major site for the removal of lipids and apoproteins, whereas the kidney is an important site for apoprotein catabolism (Glass et al., 1983; Ponsin et al., 1986). This and other evidence based upon the very rapid transfer of apoC proteins and acylated apolipoprotein analogs (McKeone et al., 1988; Hickson-Bick et al., 1988) suggest that the soluble apoproteins are poor markers for the identity and metabolic fate of a lipoprotein particle.

In vivo studies of synthetic apolipopptides have shown that the fraction of plasma peptide that is degraded in the kidneys is a predictable function of its affinity for HDL (Ponsin et al., 1984, 1985, 1986; Ponsin and Pownall, 1985). The results suggested the possibility of synthesizing an apolipopptide that was sufficiently hydrophobic to prevent its transfer between lipoproteins within the timeframe of lipoprotein remodeling. This paper describes the synthesis, characterization, and in vitro and in vivo testing of a nontransferable apolipopptide (diLAP) (Fig. 1), which is an analog of a
previously described peptide that binds phospholipids and activates LCAT (Pownall et al., 1980; Ponsin et al., 1984, 1986).

**EXPERIMENTAL PROCEDURES**

**Materials—** ApoA-I and apoE were isolated from normal human plasma as previously described (Pownall et al., 1978; Rall et al., 1986). 1,2-Dimyristoyl-sn-glycero-3-phosphorylcholine (DMPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphorylcholine (POPC) were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL). Fatty acid-free rat serum albumin and cholesterol were obtained from Sigma Chemical Co. [125]Iodide was from Amersham Corp. Female Sprague-Dawley rats (180-220 g) were used for the in vivo experiments. DiLAP was prepared by a modification of the procedure of Ponsin et al. (1984). The 15-residue peptide was synthesized by conventional solid-phase methods on an Applied Biosystems Model 430 peptide synthesizer. While attached to the resin, 2, hexadecylcyclohexanoic acid was coupled to the amino-terminal end of the peptide chain in the presence of dicyclohexylcarbodiimide and N,N-dimethylaminopyridine; 2-hexadecylcyclohexanoic acid was synthesized via macromer synthesis followed by decarboxylation. The peptide was cleaved and deprotected with HF and purified by high performance liquid chromatography.

**Methods—** ApoA-I and albumin were labeled by the chloramine-T method (Greenwood et al., 1983). Because of its low solubility in water, the peptide was first dissolved in 2-propanol, and then the solution was added to the labeling buffer. The peptide or protein was used within 2 days of labeling. The protein or peptide was separated from the labeled by gel filtration. R-HDL were prepared by the cholate deproteinization method (Greenwood et al., 1984). The 15-residue peptide was synthesized by conventional methods, and deprotected with HF and purified by high performance liquid chromatography.

**In Vivo Association of 125I-Labeled Peptide or Protein with Organs—** Rats under ether anesthesia were injected via the femoral vein with 1,2-Dimyristoyl-sn-glycero-3-phosphorylcholine (POPC) before it was gently stirred; and over a period of 48 h, samples were removed, and the beads were pelleted, washed at 4°C, and counted. Similar studies of peptide transfer from latex beads to rat HDL were also performed. A trace of 125I-DiLAP was added to the POPC before it was used to coat the latex beads. The beads were mixed with a 10-fold excess of rat HDL and stored at 37°C for 48 h, during which aliquots were collected, and the radioactivity that remained in the pellet after washing at 4°C was counted. The half-times for transfer were estimated from an exponential plot of the fraction of counts remaining with the latex beads as a function of time.

**In Vivo Transfer Kinetics—** The kinetics of desorption of 125I-diLAP from HDL, were determined according to Retzinger et al. (1985) and McKeone et al. (1988). Rat HDL, were labeled by adding 125I-DiLAP labeled R-HDL to plasma. The mixture of beads and HDL, was centrifuged at 37°C for 4 h, and the remaining with the latex beads as a function of time.

**RESULTS**

**Clearance Kinetics—** The measurements of the kinetics of transfer of 125I-diLAP from HDL, to latex beads at 37°C (data not shown) were fitted to an exponential curve from which a transfer half-time of 4 days was calculated. Analysis of these data showed that over a period of 48 h, 28% of the 125I-diLAP transferred from the HDL, to the beads. When donor and acceptor identities were reversed, 8% of the peptide was transferred within 48 h, and a half-time of >2 weeks was obtained. These data indicate that 125I-diLAP transfers between hydrophobic surfaces. However, within the timeframe of our in vivo studies that last up to 48 h, this peptide can be used as a nontransferrable marker of HDL.

To determine how fast the label disappeared from the plasma compartment and what transformations of the HDL were associated with the labeled HDL, the behavior of the 125I-diLAP injected into rats was followed in two ways. The 125I-diLAP-labeled R-HDL was injected into rats, and samples were collected and split into two parts. One part was counted to calculate the kinetics of peptide turnover, and the other was applied to gradient gels to follow the distribution of the labeled peptide among HDL subfractions. Although the turnover kinetics of the peptide were nonexponential (Fig. 24), we determined a half-time of ~12 h, a value that is larger than that observed previously with apoA-I and other exchangeable peptides were used (Glass et al., 1985; Ponsin et al., 1986). Fig. 3 is a plot of the distribution of 125I-DiLAP among HDL subfractions as a function of time. Before injection, a single peak of radioactivity was found near the migration distance of HDL1. Between 2 and 5 h, a shoulder in the radioactivity appeared; and after 20 h, a distinct peak corresponding to the migration distance of HDL1 emerged. After 48 h, nearly all of the remaining radioactivity was associated with the fraction that migrates as HDL1. Knowing the distri-
High Density Lipoprotein Turnover

"le (hws)

FIG. 2. Clearance kinetics for 125I-diLAP-labeled R-HDL in rat. Samples were injected into a femoral vein at \( t = 0 \), and the blood (~100 \( \mu l \)) was periodically collected from the contralateral femoral vein. A, serum decay curve for 125I-diLAP-labeled R-HDL in rat; B, distribution of 125I-diLAP among HDL subfractions as a function of time after injection expressed as a percent of collected counts; C, distribution of 125I-diLAP among HDL subfractions as a percent of injected counts; D, ratio of counts in HDL-subfractions to those found in HDLc. Each point is the average of values from three rats.

Distribution of 125I-diLAP between HDL subfractions (Fig. 3) and the serum clearance time (Fig. 2), it is possible to calculate the rate of clearance of HDL2 and HDL3 and plot the kinetics of conversion of the HDL in several ways. Fig. 2B shows that the total peptide radioactivity shifted from HDL2 to HDL3 over the 48-h interval. At the same time, the fraction of counts appearing as HDL2 declined and that associated with HDL3 initially grew and then also declined (Fig. 2C). Finally, Fig. 2D shows that the ratio of 125I-diLAP associated with HDL2 and HDL3 increased from nil to >4 over the same time interval.

Organ Distribution of R-HDL Containing ApoA-I and 125I-DiLAP—Studies of 125I-apoA-I-labeled HDL2 directly injected into the animals showed that nearly all of the radioactivity was associated with liver, kidney, adreanal glands, and ovaries (Fig. 4). These results were similar to earlier reports (Glass et al., 1983; Ponsin et al., 1986). To differentiate the organ distribution of HDL2 and HDL3, the difference in the kinetics of clearance of the two 125I-diLAP-labeled subfractions cited above was used. Rats injected with 125I-diLAP-labeled R-HDL were exsanguinated after 2 or 48 h. The plasma, which contained essentially 125I-diLAP-labeled HDL2 or HDL3, respectively, was reinfused into another set of rats, and the association with various tissues was determined. In both cases, virtually no 125I-diLAP was found in renal tissue. The highest specific association of 125I-diLAP-labeled HDL2 was found with adreanal glands followed by ovaries and liver, which exhibited about the same specific association. Association of 125I-diLAP-labeled HDL3 with adreanal glands and ovaries was much lower than that of 125I-diLAP-labeled HDL2 and was comparable to that with liver.

In Vitro Transformations of Plasma HDL—Additional experiments were conducted in vitro to determine the mechanism by which the 125I-diLAP-labeled HDL2 was converted to HDL3. 125I-diLAP-labeled R-HDL was incubated with rat plasma in vitro at 37°C (Fig. 5, A–D), and the electrophoretic migration of the 125I-diLAP was followed as a function of incubation time in both the presence and absence of exogenous cholesterol, which was added to the mixture at \( t = 0 \) by

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FIG. 3. Representative electrophoretic behavior of 125I-diLAP as function of time after injection into rats. Samples were collected at various times and stored on ice until they were transferred to individual lanes of 2-16% polyacrylamide gels, which were sliced and counted. Arrows in the upper left panel indicate the locations of R-HDL2, HDL3, and HDLc.

FIG. 4. Association of 125I-apoA-I and 125I-diLAP with organs 10 min after injection. ☐, apoA-I; □, 125I-diLAP-labeled HDL2; ■, 125I-diLAP-labeled HDL3.

FIG. 5. Transformation of 125I-diLAP-labeled R-HDL by rat plasma in vitro. A, 125I-diLAP was mixed with plasma at 37°C; and samples were collected periodically, stored on ice, and analyzed by polyacrylamide gel electrophoresis (4-30% acrylamide) at 4°C, at the end of 30 h. B, same as A with the addition of cholesterol. The arrows in A mark the migration distance for R-HDL and HDLc.
injection as an ethanol solution. At the earliest time point (5 min), there was already a bimodal distribution of the 125I-diLAP, with most of it co-migrating with HDLz. Between 3 and 30 h, the distribution of 125I-diLAP radioactivity with HDL2 was virtually unchanged. This behavior differed from that of apoE and apoA-I labeled R-HDL, the 125I-diLAP first appeared in HDL3 and was then shifted to HDL2. After 24 h, most of the label was recovered in HDL2.

When human rather than rat plasma was used, the 125I-diLAP was first recovered in the HDL2 subfraction, but was gradually transferred to HDLz as incubation time was increased. These data show that the peptide is initially associated with HDLz but after 24 h, most of the label is found in the larger HDLz particles. No difference in the kinetics of label transfer from HDL3 to HDL2 was observed when apoE was substituted for the apoA-I in the R-HDL.

Initial Association of 125I-DiLAP with Plasma HDL—Since we observed a rapid association of 125I-diLAP-labeled R-HDL with plasma HDL, we attempted to study this process at lower temperatures to better define its energetics and mechanism. These data are shown in Fig. 7, which contains plots of the rates of transfer of 125I-diLAP from labeled R-HDL to HDLz. With R-HDL composed of either apoA-I or apoE with POPC or DMPC, the rate increased with increasing temperature. The differences are more apparent in Arrhenius plots (Fig. 8). With R-HDL that contains POPC and apoA-I or apoE, these plots were linear, with activation energies and rate constants that are given in Table I. In contrast, when POPC was replaced with DMPC, nonlinear plots having two line segments that intersect at the gel-to-liquid crystalline transition temperature (Tc) of DMPC were observed; this behavior was found with R-HDL composed of either apoA-I or apoE.

**DISCUSSION**

In humans, HDL are thought to be critical in reverse cholesterol transport from peripheral tissues to the liver (Phil...
lips et al., 1987; Schwartz et al., 1982; Koo et al., 1985). Therefore, a better understanding of HDL remodeling within the plasma compartment is important in developing quantitative models of in vivo cholesterol metabolism. HDL are highly polymorphic (Patsch and Patsch, 1986); and the metabolic relationships between HDL subfractions, although poorly defined, remain an important component of cholesterol transport. The major difficulty in determining whether a given subfraction of HDL is a product or precursor of other fractions is that all of the components of HDL exchange freely via either spontaneous or protein-mediated pathways (Tall et al., 1986; Phillips et al., 1987; McKeone et al., 1988; Shepherd et al., 1978). Using a series of synthetic acylated apolipoproteins, we showed that their hydrophobicity was the major determinant of their affinity for HDL (Ponsin et al., 1984, 1986; Hickson-Bick et al., 1988; McKeone et al., 1988). According to two criteria, association with HDL and activation of plasma LCAT, these peptides retained many of the properties of apoA-I, but contained hydrophobic proline groups that could be changed for a given experiment according to the required affinity for a lipoprotein surface. On the basis of these observations and a model (Ponsin et al., 1985) for peptide association with HDL, we synthesized a new diacyl peptide that is very hydrophobic and is predicted to be practically nontransferable among lipoprotein subclasses. The hydrophobicity of diLAP is sufficiently large to obviate any significant transfer during a 2-day study.

**Transformation of HDL Particles in Human Plasma**—When 

\[ {^{251}}-\text{diLAP} \] labeled R-HDL were incubated with human plasma at 37 °C, the radioactivity corresponding to 

\[ {^{251}}-\text{diLAP} \] was transferred into the HDL, subfraction faster than the discs and HDL could be separated. This was followed by a time-dependent transfer of the radioactivity into HDL, subfractions. Gradient gel electrophoresis indicated that this process proceeded in two distinct steps characterized by HDL having different physical properties. In the first step, the particles containing 

\[ {^{251}}-\text{diLAP} \] exhibited a density corresponding to that of HDL, but were not fully remodeled to the size of mature HDL,. During the second step, the dimensions of the particle containing 

\[ {^{251}}-\text{diLAP} \] continued to increase in size by acquiring additional lipid. On the basis of one widely used model (Edelstein et al., 1979; Shen et al., 1977), this was likely to be mostly core or neutral lipid. After 24 h, most of the 

\[ {^{251}}-\text{diLAP} \] appeared in a density and size range corresponding to that of mature HDL,. The two differently sized particles in the density range of HDL, have been characterized in great detail by Blanche et al. (1981), who designated the small and large fractions as HDL\textsubscript{large} and HDL\textsubscript{small}, respectively. Based on this designation, our data suggest that HDL, are converted to HDL, with HDL, being an intermediate.

**Transformation of HDL Particles in Rat Plasma**—In contrast to human plasma, rat plasma contains little or no detectable HDL, (Oschry and Eisenberg, 1982). The majority of rat HDL appears in the HDL, density range, while a minor part resides in a larger particle (HDL\textsubscript{L}) that is relatively enriched in apoE. After rats were injected with 

\[ {^{125}}-\text{diLAP} \]-labeled R-HDL, the radioactivity was immediately recovered in particles having the dimensions of HDL,. As time passed, an increasing fraction of the label appeared in the fractions corresponding to HDL, and at the end of 48 h, nearly all of the label in the plasma was found in HDL, suggesting that HDL, is not a precursor to HDL,. The calculated plasma decay curve of HDL, corresponded to a half-life of \( \sim 7 \) h.

Plasma HDL, may diminish over a period of time through two or more routes; these include 1) the conversion of HDL, to HDL, within the plasma compartment and 2) the direct clearance of HDL, via transport to extravascular compartments. These two metabolic pathways are characterized by the rate constants \( k_1 \) and \( k_2 \), respectively; the sum of these two rate constants is the apparent rate constant that may be calculated from 

\[ k_{app} = k_1 + k_2 = 0.693/112 = 0.693/7 \text{ h} = 0.1 \text{ h}^{-1}. \]

Previous studies in the rat using either labeled apoA-I or HDL containing 

\[ {^{125}}-\text{diLAP} \] labeled synthetic apolipoproteins have shown that the plasma clearance of HDL is associated with a half-time of \( \sim 9 \) h (Glass et al., 1983; Ponsin et al., 1986), which corresponds to a clearance rate constant of 0.08 h\(^{-1}\). Both the apoA-I and the synthetic peptides used in previous studies readily exchanged between HDL particles. Since nearly all of the labeled apoA-I or synthetic peptide is associated with HDL, the clearance rate constant of apoA-I is similar (if not identical) to \( k_1 \). Thus, we can estimate that the rate constant \( k_2 \) for the conversion of HDL, to HDL, is 0.02 h\(^{-1}\). From this, we infer that the transformation of HDL, into HDL, in normal rats represents a minor pathway in the catabolism of HDL and that the major pathway is the direct removal of HDL,.

The total half-time for the disappearance of 

\[ {^{125}}-\text{diLAP} \] is about one-third longer than that of 

\[ {^{125}}-\text{apoA-I} \]. We attribute the lower lifetime to the absence of renal filtration of diLAP. A major pathway for the removal of apoA-I from the plasma compartment is through uptake and degradation in the kidneys. This pathway is not used for the uptake of cholesteryl esters, which are not soluble enough to form free monomers in the aqueous phase (Glass et al., 1983). Similarly, the diLAP does not transfer to the aqueous phase. Therefore, this pathway is not expected to contribute to the disappearance of 

\[ {^{125}}-\text{diLAP} \] in HDL, and 

\[ {^{125}}-\text{diLAP} \] is removed only as a component of an HDL particle. In the absence of this additional catabolic route, the plasma half-time of 

\[ {^{125}}-\text{diLAP} \] and the HDL particles with which it is associated is 12 h in rat.

Our data are the first unambiguous evidence that HDL is removed from plasma as a particle. The difference in the rates of removal of the various components of HDL from plasma is characteristic of a dynamic particle containing many chemical species that readily transfer to or exchange with other lipoproteins and cells that are in contact with the vascular compartment. Only measurements with a nonexchangeable species would accurately reflect the clearance kinetics for the entire particle. This route is presumed to occur via a specific receptor (Graham and Oram, 1987), and our data suggest that this occurs primarily in the liver, adrenal glands, and ovaries. The fractions of HDL, and HDL, that are targeted to these three tissue sites are different, and this implies that there are some tissue-specific factors that modulate the behavior of the HDL receptor. When labeled model HDL discs were incubated in normal plasma in vitro, only HDL, were formed; as previously reported by Gavish et al. (1987), no HDL, were found. However, when excess cholesterol was added to the incubation medium, HDL, were converted into much larger particles, suggesting that the formation of HDL, may depend upon a source of additional cholesterol. Thus, factors that regulate the amount of cholesterol available for the formation of the larger particles could also determine which tissue sites will remove HDL from plasma.

**In Vivo Organ Distribution Volumes of HDL Particles in Rat**—Our results permit us to distinguish the tissue sites where HDL, is localized from those of HDL,. As expected from previous studies (Ponsin et al., 1986; Glass et al., 1983), HDL, distributed into the steroidogenic tissues, liver, adrenal glands, and ovaries. HDL, accumulation was comparable to that of HDL, in the liver, but was reduced by 65–75% in
adrenal glands and ovaries. Although it is well known that HDL are the main source of cholesterol for steroidogenic tissues in rats, our data show that this property is restricted, for the most part, to the HDL₂ subfraction. Of importance, no ¹³⁵-I-DLAP was found in the kidneys. This observation has important implications for HDL metabolism. First, it confirms that the transport of HDL apoproteins into the kidney is largely due to the fraction that is lipid-free and in the aqueous part of the plasma compartment. Second, it supports our conclusion that this peptide is nontransferable and remains in solution only when bound to lipid surfaces.

**Initial Transformation of HDL Discs into HDL**—The R-HDL resembled the nascent HDL discs that were secreted by rat liver (Hamilton et al., 1976). When these labeled HDL-like discs were incubated at 37 °C in human or rat plasma or were injected into rats, the labeled peptide always appeared in the HDL for too short a time to permit isolation of any of the discs from the initial mixture. Instead, the labels were found in the major HDL subfractions of rat and man, HDL₂ and HDL₃, respectively. This was unexpected in view of the current concept that nascent HDL are transformed into spherical HDL by the successive effects of sequestering free cholesterol from other lipoprotein or tissue pools and action of the enzyme LCAT. Although this concept is based upon the high LCAT reactivity of nascent HDL, it does not require a priori that the observed morphological changes from discoidal to spherical HDL are necessarily driven only by LCAT activity; moreover, there is no evidence that this is the predominant mechanism in plasma in vivo. In view of the rapidity of the initial transformation relative to the LCAT reaction, it is unlikely that the in vivo HDL conversion process requires LCAT activity.

To distinguish spontaneous remodeling processes from those that are LCAT-driven, model HDL discs composed of DMPC or POPC were incubated with rat plasma as a function of temperature. When POPC was the phospholipid source, the temperature increment increased with temperature and gave linear Arrhenius plots. In contrast, when DMPC, which exhibits a gel-to-liquid crystalline transition temperature at 23.7 °C (Hinz and Sturtevant, 1972), was substituted for POPC in the same experiment, the Arrhenius plots were composed of two straight lines that intersected at the transition temperature of the lipid. These data support the concept that the initial transformation of the R-HDL (and perhaps nascent HDL) into spherical HDL is a purely physicochemical process. One possible mechanism is that fragments of nascent or model HDL fuse with the surface of the smallest pre-existing HDL particles, namely HDL₂ and HDL₃ in rat and human plasma, respectively. This would permit the particle surface to increase in the metabolic step that precedes the action of LCAT, which forms the cholesteryl ester-rich core.

On the basis of these studies, a model of HDL interconversions is proposed. The first step is the physicochemical association of R-HDL with HDL₂, a process that is rapid and dependent upon the physical state of the lipid in the R-HDL. This process might also emulate the association of transient HDL species, which derive their cholesterol from peripheral tissue, with a preformed HDL₂ (Francone et al., 1989) and lead to the formation of a larger HDL species. Concomitantly, there may be spontaneous monomolecular transfer of cholesterol from peripheral tissue to the HDL₂. A large fraction of the rat plasma HDL is removed as HDL₂, with the highest specific tissue association being found with ovaries and adrenal glands. However, the highest total association of HDL₂ with an organ was with liver. The association of the HDL₂ with liver cells may be mediated by an HDL-specific receptor (Graham and Oram, 1987), whereas uptake of HDL₁ is thought to involve B,E-receptors (Gordon et al., 1983; Eisenberg et al., 1984). Additional HDL remodeling is achieved by LCAT, which forms the end product of a cholesteryl ester-rich HDL. Future studies with diLAP should help determine how these processes occur in other mammalian species and what impact various drugs and diets have on them.

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