Human Lecithin:Cholesterol Acyltransferase Deficiency
In Vivo Kinetics of Low-Density Lipoprotein and Lipoprotein-X

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Objectives—Lecithin:cholesterol acyltransferase deficiency (LCAT-def) is characterized by low levels of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) and the accumulation of lipoprotein-X (LpX). Despite the low HDL, atherosclerosis is uncommon in LCAT-def. The decreased LDL would be a possible explanation but the underlying mechanism is not clear. In addition, the mechanism(s) for LpX accumulation is not known. The aim of the present study is to elucidate the mechanism(s) responsible for the low LDL and determine the plasma kinetics of LpX in LCAT-def.

Methods and Results—We conducted a radiotracer study in LCAT-def (n=2) and normal controls (n=10) and a stable isotope study in one patient and other controls (n=7). LCAT-def LDL was catabolized faster than control LDL in the control subjects as well as in LCAT-def patients. Control LDL was catabolized faster in LCAT-def patients than the controls. The production rate of LDL apolipoprotein B-100 was normal in LCAT-def. The increased LDL apoB-100 catabolism was confirmed by a stable isotope study. LpX was catabolized more slowly in LCAT-def.

Conclusions—The decreased LDL in LCAT-def is attributable to an increased catabolism caused by a rapid catabolism of abnormal LDL and an upregulation of LDL receptor pathway. The decreased catabolism of LpX contributes to its accumulation in LCAT-def. (Arterioscler Thromb Vasc Biol. 2006;26:1370-1375.)

Key Words: lecithin:cholesterol acyltransferase ■ low-density lipoprotein ■ lipoprotein-X ■ kinetics ■ stable isotope

lecithin:cholesterol acyltransferase (LCAT) is an enzyme that esterifies free cholesterol (FC) into cholesteryl ester (CE) in high-density lipoprotein (HDL). Human LCAT deficiency (LCAT-def) is characterized by corneal opacity, anemia, and proteinuria,1 with low levels of HDL and LDL and the accumulation of lipoprotein-X (LpX). In LCAT-def, HDL is markedly decreased, together with abnormalities in size and lipid composition.2 Most of the HDL in LCAT-def is small and discoidal,3 a characteristic resembling newly synthesized HDL.4,5 In the absence of LCAT, newly synthesized HDL cannot esterify FC to CE in its core so that HDL cannot develop into its mature stable forms of either HDL, or HDLc.6 This process of HDL maturation is important for reverse cholesterol transport, a hypothesis that HDL transports CE from peripheral atherosclerotic lesions to the liver. This hypothesis is based on epidemiological studies that indicated an inverse correlation between the HDL cholesterol (HDL-C) levels and the prevalence of coronary heart disease (CHD) as an independent anti-atherogenic factor for CHD.7 It is therefore interesting to note that despite a markedly low level of HDL-C and a loss of the HDL maturation process, CHD is not a common complication in LCAT-def patients.8,9 Gylling10 reported the levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were decreased in LCAT-def. The accompanying low level of LDL is perhaps the reasonable explanation for the low incidence of CHD in LCAT-def. Studies dealing with abnormalities in LDL lipid composition in LCAT-def reported that LCAT-def LDL (L-LDL) has higher amounts of triglyceride (TG), phospholipids (PL), and FC, and an extremely low amount of CE.11 In plasma, lipoprotein lipase (LPL) and hepatic lipase (HL) catalyze the lipolysis of TG in TG-rich lipoproteins such as chyromicron and very-low-density lipoprotein (VLDL). L-LDL rich in TG may be also affected by LPL and HL, although the influence of these enzymes on normal LDL (N-LDL) is considered minimal. This suggests L-LDL has a different catabolism from N-LDL. In addition, it is considered that there may be an upregulation of LDL receptor (LDL-R) because L-LDL poor in CE and rich in TG may cause a decrease in the cholesterol level in the liver.
LpX is unique lipoprotein characterized by a balloon-like structure constructed of PL, FC, apolipoprotein (apo) A-I, and albumin. LpX only appears in LCAT-def and cholestatic liver disease such as primary biliary cirrhosis, but the mechanism of LpX accumulation in these diseases is not clear.

The aim of the present study is to elucidate the mechanism(s) responsible for the decreased LDL level and to determine the plasma kinetics of LpX in LCAT-def. We conducted a radiotracer study in 2 LCAT-def patients and a stable isotope study in a LCAT-def patient, which allowed us to analyze the conversion from VLDL to LDL by endogenously labeling VLDL apoB-100. The current study combines these approaches to investigate the mechanism underlying the low levels of LDL in LCAT-def and can therefore provide new insight into the low risk of premature CHD in this disorder despite a markedly decreased HDL-C.

Methods and Materials

Subjects

Two patients with classical LCAT-def, a 51-year-old man and a 31-year-old man, and 17 normal volunteers (9 male and 8 female, 23.0±5.2 years old, mean±SD) participated in this study. The older patient (patient 1) had corneal opacity and moderate proteinuria but was otherwise healthy and using no medications. Patient 1 had classical LCAT-def and had been described as a compound heterozygote of LCAT with mutations in tyr418→stop and tyr156→asn.12 The younger patient (patient 2) had corneal opacity with moderate to severe proteinuria. He had moderate proteinuria during the radiotracer study and nephrotic range proteinuria at the time of the stable isotope study. Patient 2 had classical LCAT-def and had been previously described but his mutation has not been analyzed.13

Normal volunteers were selected by blood and physical examination from a pool recruited by advertisement. Their plasma TC, TG, and HDL-C levels were normal and they were free from diabetes mellitus, kidney disease, liver disease, and anemia. Ten of 17 volunteers participated in the radiotracer study (controls A). The remaining 7 took part in the stable isotope study (controls B; previously reported by Ikewaki et al).14

The patients and the volunteers were hospitalized in the metabolic wards of the National Institutes of Health Clinical Center, Bethesda, Md. They were all placed on the same metabolic diet, which contained 37% of total calories as fat, 47% as carbohydrate, 16% as protein, and 200 mg of cholesterol per 1000 kcal. All subjects gave their written informed consents to participate in this study. The protocol of this study was approved by the Institutional Review Board of the National Heart, Lung, and Blood Institute (NHLBI) at National Institutes of Health.

LDL and LpX Kinetic Studies With Radiotracers

The LDL fraction (d=1.030 to 1.050 g/mL) was isolated by sequential ultracentrifugation (in a Beckman 60.1 Ti rotor at 54,000 rpm at 4°C) from blood samples taken from the LCAT-def patients and from the normal controls 6 days before readministration to the subjects. The LDL fractions from the 2 patients and from the control subjects were labeled with 125I and 131I, respectively, using an iodine monochloride method.15 This radiolabeled LDL fraction from LCAT-def patients contained both L-LDL and LpX. The labeled LDL from each source was vigorously dialyzed against phosphate-buffered saline (PBS) containing 1 mMol/L EDTA, and then filtered and tested for pyrogenticity and sterility before being injected into the subjects. The radiolabeled patient and control LDL were then pooled separately in preparation for administration to the study subjects.

Both pooled LDL fractions were injected (intravenously) into the subjects at 8:00 am after 12 hours of fasting. The blood samples were collected in vacuum tubes containing EDTA-Na2 (0.1%) at 10 minutes after the injection and at frequent intervals over the succeeding 14 days. The samples were immediately chilled on ice and the plasma was separated by centrifugation. Sodium azide and aprotinin were added to the plasma at a final concentration of 0.05% (wt/vol) and 200 U/mL, respectively. The separated plasma was stored at 4°C for further analyses. The radioactive activity in 4 mL of plasma obtained at each time point was counted using a gamma counter (Packard Cobra) 14 days after the injection of radiolabeled LDL. All subjects started to take 900 mg of potassium iodide in divided doses for 4 days starting 1 day before the study and 300 mg in divided doses for the remaining 17 days.

Four hundred μL of the plasma collected from the subjects at each time point was injected into fast performance liquid chromatography (fast protein liquid [FPLC]) for the separation of 125I-L-LDL and 131I-LpX. FPLC was performed using 2 Superose 6 columns connected in series, eluted with PBS/0.02% EDTA at a flow rate of 0.5 mL/min, and 500 μL was collected in each aliquot. Sixty fractions were collected and the radioactive activity of each fraction was counted. Because LpX and LDL were distributed in fractions 10 to 15 and 22 to 35, respectively, the radioactivities of LpX and LDL were calculated by the following formula:

\[
\text{Plasma radioactivity of LpX} = \left( \frac{\text{radioactivity in plasma}}{\text{total radioactivity of fractions 10 to 15}} \right) \times \left( \text{total radioactivity of all FPLC fractions} \right)
\]

\[
\text{Plasma radioactivity of LDL} = \left( \frac{\text{radioactivity in plasma}}{\text{total radioactivity of fractions 22 to 35}} \right) \times \left( \text{total radioactivity of all FPLC fractions} \right)
\]

In this calculation, the radioactivities of 4 mL of plasma at the corresponding time points were used.

Kinetic analyses were performed with SAAM II (SAAM Institute Inc, Seattle, Wash). Plasma decay curves were drawn by dividing the plasma radioactivity at each time point by that of the 10-minute time point. The residence times (RT) were calculated from the curve under the multi-exponential curve fitting. The fractional catabolic rates (FCR) were obtained as a reciprocal of RT. The production rates (PR) of LDL apoB were determined using the formula:

\[
\text{PR} = (\text{LDL apoB level}) \times (\text{plasma volume}) \times \text{FCR/(body weight)}
\]

ApoB-100 Kinetic Study With a Stable Isotope

A kinetic study with a stable isotope was performed in patient 2. The detailed protocol for sample processing has been previously reported.14,16 In brief, after a 12-hour fast, 100 μCi phenylalanine (99% 13C; Cambridge Isotope Laboratories, Woburn, Mass) was administered to the study subject as a priming bolus of 600 μg/kg, immediately followed by a constant infusion of 12 μg/kg per minute for up to 12 hours. Blood samples (20 mL) were obtained at 10 minutes, 1, 2, 3, 4, 5, 6, and every 2 hours until the end of infusion, and then at 18, 24, 36, 48, and 72 hours. During the infusion, meals were served in equal small portions every 2 hours. The plasma was immediately separated by centrifugation. VLDL, LDL, and LpX were isolated by sequential ultracentrifugation, dialyzed against 10 mmol/L ammonium bicarbonate, lyophilized, and delipidated. ApoB-100 was isolated by preparative gradient SDS-PAGE (5% to 15%). ApoB-100 bands were cut from gels, subjected to hydrolysis in 6N HCl at 110°C for 24 hours. The protein hydrolysates were lyophilized and free amino acids were purified by cation exchange chromatography (AG-50W-X8; Bio-Rad Laboratories, Richmond, Calif). The recovered free amino acids were derivatized to the N-heptafluorobutyryl isobutyryl esters, dissolved in ethyl acetate, and then analyzed by gas chromatography-mass spectrometry in the chemical ionization mode, using isobutane as the reagent gas. Selective ion monitoring at 418 m/z for unlabelled phenylalanine and 424 m/z for 13Cphenylalanine was used to determine the isotope ratio. Each sample was analyzed at least 2 times. Acquired data were converted to tracer/tracee ratios using the method of Cobelli et al.17 The previously reported multicompartamental model16 built on SAAMII was
TABLE 1. Body Mass Index and Lipid Levels of LCAT-Deficient Patients and the Controls

<table>
<thead>
<tr>
<th>Patient</th>
<th>Controls (mean±SD)</th>
<th>Controls (mean±SD)</th>
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<tr>
<td></td>
<td>A (n=10)</td>
<td>B (n=7)</td>
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<tr>
<td>BMI, kg/m²</td>
<td>23.1</td>
<td>25.5</td>
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<tr>
<td>TC, mg/dL</td>
<td>166</td>
<td>95</td>
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<tr>
<td>TG, mg/dL</td>
<td>496</td>
<td>287</td>
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<tr>
<td>HDL-C, mg/dL</td>
<td>4.0</td>
<td>8.0</td>
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<tr>
<td>LDL-C, mg/dL</td>
<td>63</td>
<td>30</td>
</tr>
<tr>
<td>apoA-I, mg/dL</td>
<td>42</td>
<td>46</td>
</tr>
<tr>
<td>apoB, mg/dL</td>
<td>62</td>
<td>44</td>
</tr>
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</table>

Results

Lipid Levels of LCAT-def Patients and Controls

The lipid levels of the 2 LCAT-def patients and controls A and B are shown in Table 1. The TG levels were higher in the two patients than the mean TG levels in the controls. The HDL-C levels in patients 1 and 2 (4.0 mg/dL and 8.0 mg/dL, respectively) were considerably lower than the mean of HDL-C levels in the controls. The apoA-I levels in the patients were much lower than the mean apoA-I levels in the controls. The LDL-C levels and the apoB levels in the patients were much lower than the mean levels in the controls.

Separation of L-LDL and LpX by FPLC

After the injection of radiolabeled LDL, plasma was obtained from the patients and the control subjects and injected into FPLC. The radioactivity of $^{125}$I distributed in 2 peaks in LCAT-def patients (Figure 1A), thus indicating the radiolabeled LDL fraction of LCAT-def to contain 2 particles which were different in size. The larger particles made the first peak in fractions 10 to 15 and the smaller particles made the second peak in fractions 22 to 35. Because fractions 22 to 35 correspond to those for LDL, the smaller and larger particles were thus considered to represent L-LDL and LpX, respectively. In contrast, the radioactivity of $^{131}$I revealed only a single peak corresponding to the LDL size in LCAT-def patients (Figure 1B). These distribution patterns of $^{125}$I and $^{131}$I were also observed when the plasma obtained from the controls was run on FPLC (Figure 1C and 1D). However, L-LDL was observed to be decreased in size in the control subjects at 6 and 24 hours (Figure 1C), whereas N-LDL remained in the same size (Figure 1D). This slight shift of particle size of L-LDL was observed in all of the control subjects. The radioactivities of LpX and L-LDL or N-LDL decreased without changing the relative distributions at 6 and 24 hours (Figure 1) and also throughout the study periods.

For the analysis of radiolabeled lipoproteins phosphoimage from the plasma of LCAT-def was injected into FPLC (Figure 2). The decay curves for the controls were calculated using Friedewald’s formula. The apolipoprotein levels were measured by immunoturbidimetry.

Other Analytical Methods

For the analysis of apolipoproteins, 4% to 20% gradient SDS-PAGE was performed. Immunoblotting against apoE was carried out by transferring protein to polyvinylidene difluoride microporous membrane (Immobilon-P) (Millipore Co, Bedford, Mass). After the transfer, the membrane was incubated with anti-apoE monoclonal antibody and then with anti mouse IgG antibody. The apoE band was visualized by peroxidase using an ABC kit (Vector Laboratory, Inc, Burlingame, Calif) with 4-chloro-1-naphthol. For the analysis of radiolabeled lipoproteins 4% to 20% native PAGE was performed. Proteins were transferred to the membrane and phosphoimage of the membrane was taken using PhosphorImager 445 SI (Molecular Dynamics, Sunnyvale, Calif).

The lipid levels were measured by the enzymatic method. HDL-C was measured by the precipitation method. The LDL-C level was calculated using Friedewald’s formula. The apolipoprotein levels were measured by immunoturbidimetry.
0.56 d^{-1}) and patient 2 (0.96 d^{-1} versus 0.48 d^{-1}) (Table 2). L-LDL was also catabolized faster than N-LDL in the control subjects (Figure 2, middle). The FCR for L-LDL was 0.69±0.11 d^{-1} and that for N-LDL was 0.39±0.07 d^{-1} (Table 2). To evaluate the LDL-R upregulation, N-LDL catabolism in the LCAT-def patients and controls were compared. As shown in the lower panel of Figure 2, the decay curve revealed N-LDL to be catabolized faster in the LCAT-def patients (circle and triangle for patients 1 and 2, respectively) than in the control subjects (square).

**Figure 2.** Kinetic analyses of radiolabeled LCAT-def LDL in an LCAT-def patient and a control and a comparison of normal LDL catabolism in the LCAT-def patients and controls. Upper, L-LDL (square) catabolised faster than N-LDL (circle) in LCAT-def patient 2. Middle, L-LDL (square) is catabolised faster than N-LDL (circle) in a control subject. Lower, N-LDL is catabolised faster in LCAT-def patients (circle and triangle for patients 1 and 2) than in the control subjects (square).

The metabolic channeling of apoB-100 in the LCAT-def patient 2 is summarized in the supplemental Table 1. In the control subjects, 44.4% of VLDL was directly removed, 51.7% was converted to IDL, and 36.7% was eventually converted to LDL. In contrast, in the LCAT-def patient, 83% of VLDL was directly removed from the plasma, resulting in less fractions of VLDL converted to IDL (14.7%) and LDL (17.2%). The rate constants of lipolytic conversion from VLDL to IDL and from IDL to LDL in the LCAT-def patient were 13.3 and 12.5 pools/d, respectively, which were not impaired in comparison to the corresponding average value of control subjects (6.5 and 7.3 pools/d).

**LpX Kinetic Study With a Radiotracer**

The kinetic curves of LpX were drawn using the radioactivities of the LpX fractions separated by FPLC (Figure 4). The decay curves for the controls were obtained using the mean values at each time point. Catabolism of LpX was slower in LCAT-def patients than the control subjects. The FCR of LpX was 0.56 d^{-1}and 0.48 d^{-1} in patients 1 and 2, respectively, whereas that was 1.16 d^{-1} in the controls.

**Discussion**

Lipid abnormality, especially a low level of HDL-C, is one of the most characteristic features of LCAT-def. However, despite a markedly low level of HDL-C, CHD is not a common complication in this disease. Not only is the HDL level decreased but also is its function; the cholesterol efflux is considered decreased. Thus, the accompanying low level of LDL would be a possible explanation for the CHD infrequency in LCAT-def. Accordingly, in the current study as well, both LCAT-def patients were free from atherosclerotic disease even though their HDL levels were extremely low. In addition, their
The mechanism underlying the low LDL level as a possible reason for the infrequent CHD in LCAT-def patients is of great interest but it has not been investigated. In the present study, to analyze the catabolism of L-LDL we injected both LCAT-def and normal controls and these findings were consistent with the previous report by Gylling.10

Figure 3. Tracer/tracee ratio curves for VLDL apoB-100 (circles), IDL apoB-100 (triangles), and LDL apoB-100 (squares) in the LCAT-def patient 2 (A) and a representative control subject (B). Data were fitted by the multicompartmental model using SAAMII.

An impaired conversion from VLDL-IDL-LDL may be another possible explanation for the decreased LDL level in LCAT-def. LPL and HL are considered to play an important role in VLDL-IDL-LDL conversion. Blomhoff et al21 reported a decreased post-heparin plasma LPL activity in LCAT-def patients with no difference in the post-heparin plasma HL activity. Cholesteryl ester transfer protein and LCAT may also affect this process. Cholesteryl ester transfer protein transfers CE from HDL to VLDL and IDL and transfers TG in the reverse direction, thus helping the VLDL-IDL-LDL conversion by increasing the CE amount and decreasing the TG amount in VLDL and IDL. LCAT produces CE not only in HDL but also in LDL and enriches CE in LDL to the mature forms. These processes are believed to be involved in the conversion from VLDL to LDL and they may be impaired in LCAT-def. The stable isotope study in the present study allowed us to analyze the rate of synthesis of LDL apoB-100 while also enabling us to evaluate the VLDL to LDL conversion. From an analysis with a stable isotope, the LCAT-def patient showed a rapid catabolism rather than a decreased synthesis of LDL apoB-100. Furthermore, the PR of LDL apoB-100, as calculated from a radiotracer study, was almost the same level in LCAT-def and in controls (15.5 mg/kg per day) (Table 2). These findings indicate that an impaired conversion from VLDL to LDL is an unlikely reason for the decrease in LDL in LCAT-def. This view is also supported by the normal rate constants from small VLDL to IDL (13.3 versus 6.5 pools/d) and IDL to LDL (12.5 versus 7.3 pools/d) in the LCAT-def patient in comparison to the controls. Furthermore, as shown in supplemental Table I, fractions of VLDL directly cleared from the circulation was ~80%, 2-fold of the average in control subjects. Although not entirely clear, the upregulated LDL-R, together with the altered VLDL composition caused by the lack of LCAT, may contribute to an increased LDL uptake. Another notable observation in the stable isotope study is the increased VLDL apoB-100 production. This may explain hypertriglyceridemia observed in both LCAT-def patients. We speculate that VLDL apoB-100 production was increased by the compensatory increased protein synthesis in patient 2 with the nephrotic range proteinuria during the stable isotope study. However, in the present study the number of patients was limited and thus to clarify the exact reason of the increased production of VLDL apoB-100 further study is needed. The current observations obtained from the stable isotope study therefore indicates
that lipolytic reactions from VLDL to LDL primarily mediated by LPL and HL as well as the direct clearance of VLDL are not impaired under LCAT-def in vivo.

The FCR for LDL apoB was ≈50% greater as assessed in the stable isotope study than assessed by the radiotracer study (1.52/d versus 0.96/d). The exact reasons for this discrepancy are not clear at present. However, because these studies were conducted at different times and the degree of renal impairment was different between the 2 studies, the metabolic condition of the patient is considered to be different between the times of the 2 studies. In fact, the LDL pool sizes were observed to be different between the 2 studies (LDL apoB: 27.5 mg/dL versus 38.0 mg/dL) and the LDL apoB kinetics thus observed may not be the same. We proposed using a new isolation technique to separate the LpX particle from the LDL by isolating the particles by FPLC. LDL in the stable isotope studies was isolated by ultracentrifugation and apoB-100 isolated by SDS-PAGE possibly accounting for some of the difference. Although there was somewhat of a difference in the FCRs for LDL apoB obtained from the 2 studies, both values were increased compared with the mean values in controls. Thus, the accelerated catabolism of LDL in LCAT-def patients was evident regardless of the labeling methodologies.

The LDL fraction separated by ultracentrifugation from LCAT-def has been reported to contain LpX.11,22 Gel filtration allows for the separation of these particles. In the present study, the collected plasma was injected into FPLC and L-LDL was subsequently separated from LpX at each time point. No conversion was observed between the 2 particles. Major protein(s) was apoB-100 in LDL fraction and apoA-1 and albumin in LpX fraction. In contrast to the findings of Alaupovic et al.,23 no apoE was detected in LpX and the amount of apoC was also negligible. Therefore, neither protein transfer between LpX and LDL nor lipoprotein conversion is considered to occur, thus enabling us to follow these particles over a period of time using the FPLC separation technique. LpX only appears in LCAT-def and cholesteryl liver disease, although the mechanism of the LpX accumulation is not clear. In the present study a decreased catabolism of LpX was observed in LCAT-def in a comparison to that observed in normal controls and this slower catabolic rate is therefore considered to contribute to the accumulation of LpX in LCAT-def. However, to clarify this mechanism further study is needed.

In conclusion, the LDL decrease in LCAT-def is due to an increased LDL catabolism. Two separate mechanisms are considered to contribute to the rapid catabolism of LDL: namely, a rapid catabolism of abnormal LDL and an upregulation of LDL-R pathway. In addition, the catabolism of LpX is decreased in LCAT-def and this is considered to contribute to the LpX accumulation in this disease.

Acknowledgments

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References


Supplemental Table 1 Metabolic channeling of apoB-100 in the patient 2 with LCAT deficiency from stable isotope study

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<tr>
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<tr>
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