Cloning of rabbit LCAT cDNA: increase in LCAT mRNA abundance in the liver of cholesterol-fed rabbits

Yukio Murata,[1,*] Eiichi Maeda,[*] Gen Yoshino,[†] and Masato Kasuga[*]

Second Department of Internal Medicine,[*] Kobe University School of Medicine, 7-5-1 Kusunoki-Cho, Chuo-ku, Kobe 650, Japan, and Department of Laboratory Medicine,[†] Toho University School of Medicine, Tokyo 143, Japan

Abstract Structure of rabbit lecithin:cholesterol acyltransferase (LCAT) and molecular basis for the effects of cholesterol feeding on LCAT expression were investigated by cloning and sequencing LCAT cDNA from rabbit. The rabbit and human sequences are 91% identical at the nucleotide level and 93% identical at the amino acid level. The interfacial substrate active site, asparagine-linked glycosylation sites, and sites at which rare mutations cause human familial LCAT deficiency are all highly conserved in the rabbit protein. The apparent molecular mass of rabbit LCAT, as determined by immunoblot analysis, was approximately equal to that of human LCAT. Rabbits showed 2.6- and 5.5-fold increases in serum LCAT activity 3 and 6 weeks, respectively, after switching to a cholesterol-enriched diet. Northern blot analysis revealed that the abundance of LCAT mRNA in liver increased 1.6- and 2.8-fold after 3 and 6 weeks, respectively, of cholesterol feeding. The marked temporal relation between the increase in serum LCAT activity and the liver LCAT mRNA abundance suggest that the regulation of LCAT activity in vivo may be primarily determined by changes in the amount of LCAT mRNA.—**Murata, Y., E. Maeda, G. Yoshino, and M. Kasuga.** Cloning of rabbit LCAT cDNA: increase in LCAT mRNA abundance in the liver of cholesterol-fed rabbits. *J. Lipid Res.* 1996. **37:** 1616–1622.

Supplementary key words LCAT • cholesterol reverse transport system • LCAT cDNA • nucleotide sequence • LCAT activity • cholesterol feeding

The plasma enzyme, lecithin:cholesterol acyltransferase (LCAT) plays an important role in the process of reverse cholesterol transport in humans by promoting cholesteryl ester formation (1). Cholesterol esterification by LCAT contributes to the formation of the hydrophobic lipid core of high density lipoprotein (HDL) particles and helps to maintain the gradient in cholesterol concentration between cell membranes and plasma that is important for cholesterol efflux from peripheral tissues (2, 3). This efflux is the first step in the transport of excess cholesterol from extrahepatic cells to the liver. Although LCAT is a key enzyme in this process, serum LCAT level and activity do not always reflect the suppression of atherosclerosis, and the factors that regulate plasma LCAT activity in vivo remain unclear.

We attempted to investigate the lipoprotein metabolism in an animal model with increased LCAT activity and to shed light on the role of LCAT in reverse cholesterol transport. Although the LCAT transgenic mouse can be a good tool to examine the role of LCAT in lipoprotein metabolism in vivo (4–6), rodents are thought not to be suitable animal models for studying cholesterol reverse transport because of their low cholesteryl ester transfer protein (CETP) activity, another important component of this system.

On the other hand, the rabbit seems to be a more suitable animal because of its high CETP activity and high sensitivity to cholesterol feeding. Structure and properties of rabbit LCAT have not been fully described. The nucleotide sequences of human, mouse, rat, baboon, and chicken LCAT cDNA have already been determined (7–11). In published reports, similarities with human LCAT at both the nucleotide and amino acid levels have been observed.

We now present the nucleotide and deduced amino acid sequences of rabbit LCAT cDNA, and examine excess cholesterol loading in the rabbit as a regulatory factor of LCAT activity in vivo. Simultaneous determination of rabbit serum LCAT activity and liver LCAT mRNA abundance after cholesterol feeding enabled us

Abbreviations: LCAT, lecithin:cholesterol acyltransferase; HDL, high density lipoproteins; CETP, cholesteryl ester transfer protein; apoA-I, apolipoprotein A-I; VLDL, very low density lipoproteins; LPL, lipoprotein lipase; HTGL, hepatic triglyceride lipase.

[1]To whom correspondence should be addressed.

to further study the crucial role of LCAT in the reverse cholesterol transport system.

MATERIAL AND METHODS

Cloning of LCAT cDNA

Screening of rabbit liver cDNA library in λgt10 with a full-length human LCAT cDNA (kindly provided by Dr. J. Mclean) yielded three positive cDNA clones. DNA from positive phage was isolated by standard procedures (7, 12) and subcloned into the M13 phage vector for DNA sequencing by dideoxy chain termination (13). All regions were sequenced at least twice.

Analysis of tissue distribution of LCAT mRNA

The tissue distribution of rabbit LCAT mRNA was investigated by Northern blot analysis. Total RNA was extracted from liver, kidney, spleen, adrenal glands, and testis of a Japanese white rabbit with the use of guanidine thiocyanate and was further purified by CsCl gradient ultracentrifugation (14). RNA (20 μg) was transferred to a nitrocellulose filter and hybridized with ^{32}P-labeled rabbit LCAT cDNA. The filter was subjected to a final wash at 55°C in 1× standard saline citrate containing 0.1% SDS. Hybridization signals were detected with an image analyzer (Fujix Bioimaging Analyser, BAS2000) for quantitation of specific mRNA abundance. Differences in the amount of applied RNA were corrected for by scanning the signals obtained with a β-actin cDNA probe.

Preparation of antibodies to rabbit LCAT

Polyclonal antibodies to a synthetic COOH-terminal peptide of rabbit LCAT were prepared in goats. A peptide corresponding to the COOH-terminal 18 amino acids of the mature LCAT with an added NH_2-terminal cysteine was coupled to keyhole limpet hemocyanin. The conjugate was dissolved in phosphate-buffered saline, emulsified with Freund's complete adjuvant, and injected into goats. The goats were administered booster injections of the antigen emulsified in Freund's incomplete adjuvant at 2-week intervals.

Determination of rabbit LCAT activity

Rabbit LCAT activity was determined with a proteoliposome substrate composed of rabbit apolipoprotein A-I (apoA-I), phosphatidylcholine, and [^{3}H]cholesterol (unesterified). ApoA-I was prepared essentially as described by Scanu, Lim, and Edelstein (15). Briefly, HDL was separated by ultracentrifugation from fresh normolipemic rabbit plasma, depleted of lipid, and fractionated by gel filtration chromatography on a Superdex-200 column (Pharmacia). The major peak (peak 3) fraction was dialyzed and lyophilized, and its purity was determined by SDS-polyacrylamide gel electrophoresis. Single-bilayer phosphatidylcholine-cholesterol vesicles were prepared according to the method of Batzri and Korn (16). The rate of [^{3}H]cholesterol esterification was measured as described (17). LCAT activity was measured in a total volume of 125 μl during incubation for 90 min at 37°C, and data are expressed as nmols of free cholesterol consumed per milliliter per hour.

Plasma LCAT level estimation

LCAT mass was estimated by Western blot analysis using a purified polyclonal antibody prepared as described above. Blot images were quantified by using NIH image.

Analysis of LCAT mRNA abundance in liver, LCAT activity, and LCAT mass in serum of cholesterol-fed rabbits

Male Japanese white rabbits with body mass of 2.0–2.5 kg were fed regular rabbit chow before the study and then were maintained on the same chow supplemented with 0.5% cholesterol. Blood was collected from five rabbits after a 24-h fast both before and 3 and 6 weeks after the start of the experimental diet. The serum concentration of total cholesterol was determined enzymatically and that of HDL-cholesterol by $MgCl_2$ phosphotungstate precipitation. Serum LCAT activity and LCAT level were estimated as described above. Three rabbits were killed under phenobarbital anesthesia at each time point, total RNA was isolated from the liver, and LCAT mRNA abundance was determined by Northern blot analysis as described above. Statistical analysis data are presented as means ± SD. The statistical significance of differences between time points was determined by Student's *t* test or the Wilcoxon rank sum test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Isolation and sequence of rabbit LCAT cDNA

Three clones were recovered from a rabbit liver cDNA library by screening with a full-length human LCAT cDNA probe and were partially sequenced. Homology with the human LCAT cDNA sequence revealed that one clone included a complete 1.5-kb LCAT cDNA at the internal *EcoR*I site. This clone contained an open reading frame that encodes a 440-amino acid protein (**Fig. 1**). The nucleotide sequence of rabbit LCAT cDNA is 90.8% identical to that of human LCAT cDNA; the amino acid sequences of the predicted proteins are 93% identical. The sequence identity between human and

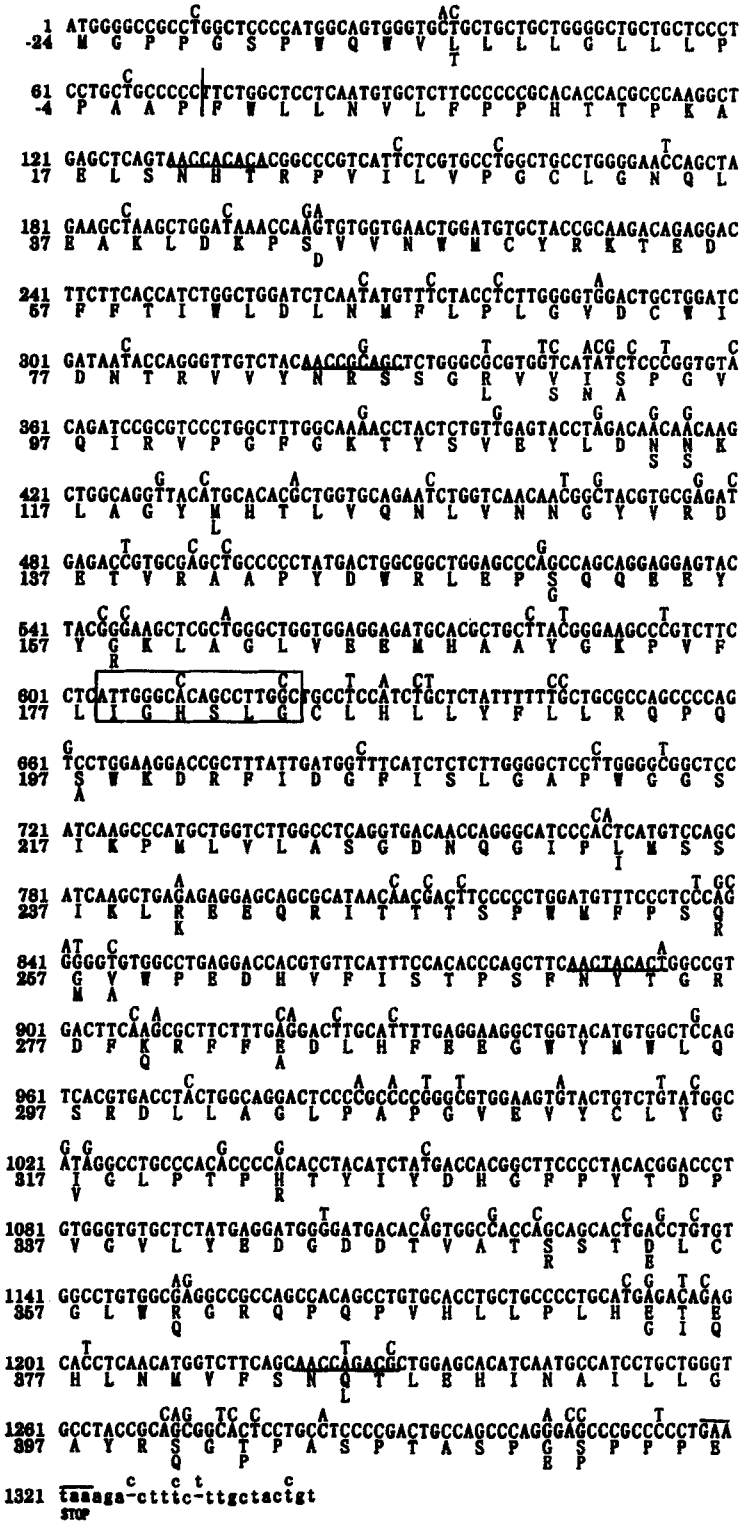

Fig. 1. Rabbit LCAT cDNA nucleotide and predicted amino acid sequences aligned with the corresponding human sequences. The amino acid sequence is numbered according to the mature protein. The nucleotide substitutions in human LCAT cDNA are aligned above the rabbit sequence, and amino acid substitutions are shown below. The putative interfacial substrate active site is boxed. Four potential Asn-linked glycosylation sites are underlined. The end of the signal peptide is marked by a vertical line, and the polyadenylation site is overlined. The GenBank/EMBL/DDBJ accession number for this rabbit LCAT cDNA sequence is D13668.

rabbit LCAT is higher than that between human and mouse LCAT, which is 84.5 and 86.4% at the nucleotide and amino acid levels, respectively (8).

The hexapeptide, Ile-Gly-His-Ser[181]-Leu-Gly, which is thought to be the catalytic center of LCAT, is preserved in the rabbit protein. Three of four potential Asn-linked

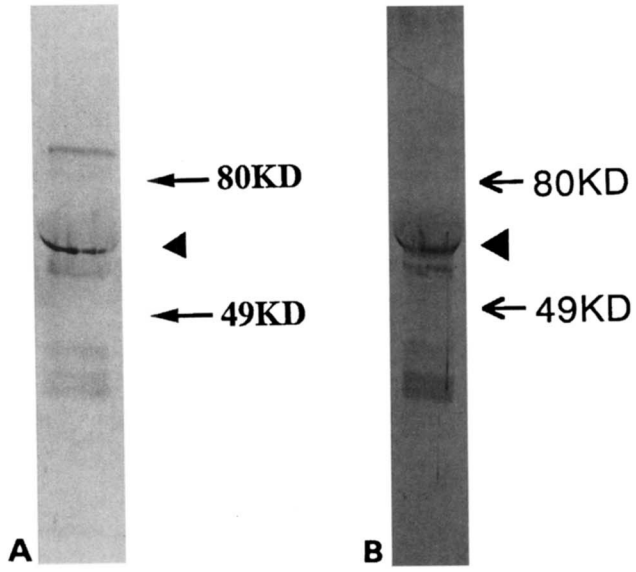

Fig. 2. Immunoblot analysis of LCAT in rabbit and human serum with antibodies prepared against a synthetic COOH-terminal peptide of the rabbit protein. Rabbit (A) and human (B) serum was fractionated in a 10% polyacrylamide gel containing 0.1% SDS. Arrowhead indicates LCAT. The positions of molecular mass standards are shown in kilodaltons.

glycosylation sites (Asn-X-Ser, Asn-X-Thr) (18) in rabbit LCAT are identical to those in the human protein, whereas the fourth site contains a substitution in the middle residue. Met^{293}, which we previously showed, was converted to Ile in a Japanese patient with LCAT deficiency (19) and is also conserved in the rabbit protein. Six cystein residues in positions 31, 50, 74, 184, 313, 356 were all conserved in rabbit protein.

Secondary structure and helical hydrophobic moment plots for rabbit LCAT, calculated according to Chou and Fasmen (20) and Kyte and Doolittle (21), respectively, were essentially matched to those of human LCAT (18). The apparent molecular mass (kDa) of rabbit plasma LCAT, as estimated by immunoblot analysis with antibodies prepared against a COOH-terminal peptide, was approximately 63 kDa, which is equal to that of the human protein (**Fig. 2A**). These antibodies can crossreact with human LCAT (Fig. 2B).

Tissue distribution of LCAT mRNA

Northern blot analysis of various rabbit tissues with a rabbit LCAT cDNA probe revealed the presence of LCAT mRNA only in the liver, not in adrenal, spleen, kidney, or testis (**Fig. 3**).

Effects of cholesterol feeding

Rabbits fed a cholesterol-enriched diet showed a significant increase in serum cholesterol concentration both 3 and 6 weeks into the diet (**Table 1**). Serum HDL-cholesterol tended to be increased at 3 weeks, but was significantly decreased after 6 weeks on the diet.

Serum LCAT activity was significantly increased 2.6-fold after 3 weeks, and 5.5-fold after 6 weeks of cholesterol feeding (Table 1 and **Fig. 4**).

Cholesterol feeding resulted in an increase in the amount of LCAT mRNA in the liver from 1.0 arbitrary unit before to 1.6 ± 0.4 after 3 weeks and 2.8 ± 0.8 after 6 weeks (**Fig. 5**). The increase in LCAT mRNA in liver paralleled that of LCAT activity in serum (Fig. 4).

DISCUSSION

The process of reverse cholesterol transport comprises several steps, including cholesterol efflux from cell membranes and incorporation into HDL particles, HDL maturation mediated by LCAT, cholesteryl ester transport from HDL to very low density lipoprotein (VLDL), and receptor-mediated uptake of both VLDL and low density lipoprotein (LDL) particles by the liver (2, 3). Although LCAT is an important component in this process, its role on antiatherogenic effect is still unclear (22). Increased LCAT activity seems to be an antiatherogenic factor as overexpression of human LCAT gene in transgenic mice has been reported to show a reduction in atherogenic lipoproteins and an increase in antiatherogenic lipoproteins (5). But an increase in LCAT activity is not a synonym for the regression of atherosclerosis, and a decrease does not necessarily mean progression. Heterozygous individuals with familial LCAT deficiency do not always show clinical manifestations of atherosclerosis, including coronary heart disease, though von Eckardstein et al. (23)

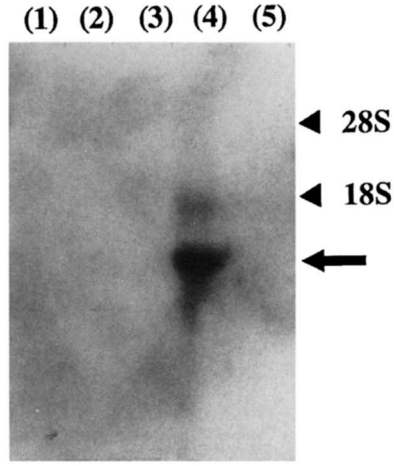

Fig. 3. Tissue distribution of LCAT mRNA. Total RNA (20 μg) from various rabbit tissues was subjected to Northern blot analysis with a LCAT cDNA probe. Lanes: 1, adrenal; 2, spleen; 3, kidney; 4, liver; 5, testis.

TABLE 1. Levels of serum cholesterol, HDL-cholesterol, and LCAT activity

Cholesterol Feeding	n	Total Cholesterol	HDL-Cholesterol	LCAT Activity
weeks		*mg/dl*		*nmol/ml/h*
0	5	51.3 ± 3.5	20.2 ± 2.0	37.8 ± 2.6
3	5	321.6 ± 40.6[a]	37.3 ± 21.6	99.7 ± 33.2[a]
6	5	1138.3 ± 233.7[a]	12.5 ± 1.1[a]	207.9 ± 73.3[a]

Data are expressed as means ± SD; n, number of samples.
[a]Significantly different from non-cholesterol feeding ($P < 0.05$).

reported that cholesterol efflux from fibroblast into plasma was reduced in LCAT deficiency. In addition to the role in reverse cholesterol transport, the mechanism by which plasma LCAT is regulated is also unclear. Although several conditions, such as obesity (24), nephrosis (25), liver disease (26), cold stress, a high-cholesterol diet (27), copper, and cigarette smoking (28) affect plasma LCAT activity, the basis for these effects requires further elucidation. The present study was therefore undertaken to investigate the molecular basis for the effects of cholesterol feeding on LCAT regulation in the rabbit, which is thought to be a suitable animal model for such a study because of its high CETP activity and high sensitivity to cholesterol feeding.

Prior to the examination of cholesterol feeding, we had to indicate the molecular similarities between human and rabbit LCAT. We have now shown that LCAT is highly conserved between rabbit and human at both the nucleotide and amino acid levels. The sequence conservation encompasses the enzyme active site, glycosylation sites, and sites at which mutations have been shown to cause human familial LCAT deficiency (19, 29, 30).

Both human and rabbit LCAT cDNA encode 440 amino acid residues plus 24 amino acid signal peptides. [Gly-X-Ser-X-Gly] is known to be identical to the amino acid sequence at the interfacial active site of lipase family (e.g., LPL, HTGL, and pancreatic lipase). The hexapeptide, Ile-Gly-His-Ser^{181}-Leu-Gly, which is the catalytic center of LCAT, is also conserved among five species: human, mouse, rat, baboon, and rabbit.

Both human and rabbit LCAT contains six cysteins. Cys^{50}, Cys^{74}, Cys^{313}, and Cys^{356}, known to form disulfide bridges in human enzyme (18), were conserved in rabbit protein. The free cystein residues in positions 31 and 184, which are essentially required for cholesteryl ester synthesis (18), are also conserved. Recently, the sequence of chicken LCAT cDNA, in which two free

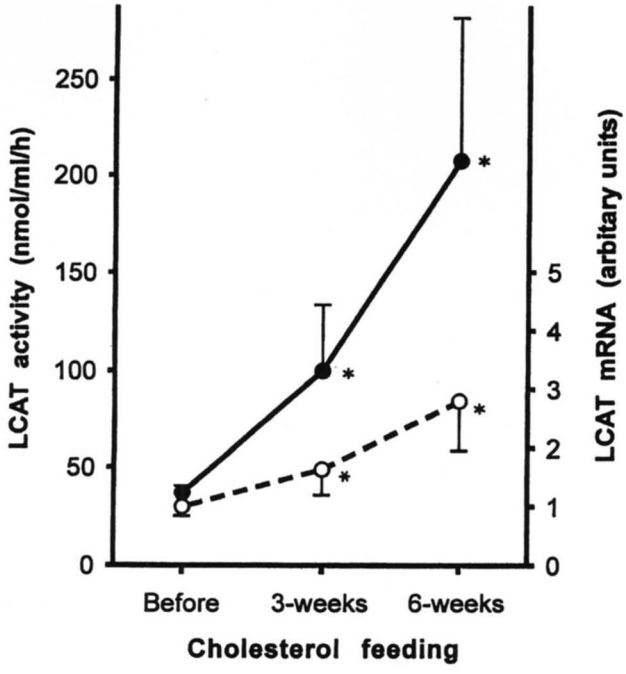

Fig. 4. Effects of cholesterol feeding on serum LCAT activity (●) and liver LCAT mRNA abundance (○) in rabbits. *$P < 0.05$ vs. before cholesterol feeding.

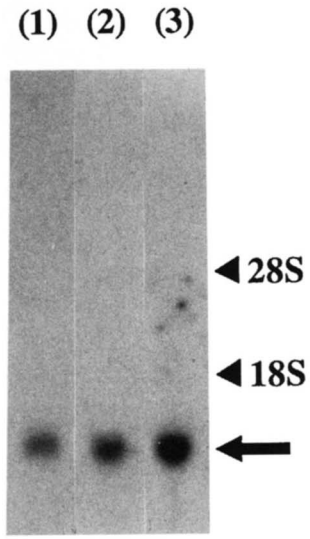

Fig. 5. Northern blot analysis of total RNA from rabbit liver with a rabbit LCAT cDNA probe before (lane 1) and after 3 weeks (lane 2) and 6 weeks (lane 3) of cholesterol feeding. Arrow indicates LCAT mRNA.

cysteins are not present, was reported by Hengstschlagen-Ottnad et al. (11). They show the possibility that these residues are not required for LCAT activity (11).

Deduced secondary structure and hydrophobicity, which are essential for activating this enzyme, are also highly conserved between rabbit and human LCAT. The catalytic region and several surrounding nucleotides (residues 175–192) could form an amphipathic α-helix, a structure implicated in lipid binding, in rabbit as well as human. This region may interact with the lipid interface containing the substrate of LCAT reaction.

The similarity in apparent molecular mass between the rabbit and human proteins, which we showed in this study, suggests that glycosidic linkages of rabbit LCAT are essentially identical to those of human LCAT. Both human and rabbit LCAT contains approx. 24% (w/w) sugar chains, the correct processing of which is important for the stability and activity of the enzyme, as Francone, Evangelista, and Fielding (31) and Qu et al. (32) have reported in the studies using site-directed mutagenesis. Both human (33) and rabbit LCAT mRNAs have been detected only in the liver, indicating that this organ is the main source of plasma LCAT. In contrast, rat LCAT mRNA is present in brain and testis (8). These results again suggest that the rabbit is indeed an appropriate animal model for investigating the reverse cholesterol transport system.

CETP activity and CETP mRNA abundance, as well as LCAT activity, have previously been shown to be increased in the rabbit by cholesterol feeding (27, 34, 35). We have now shown that the increase in plasma LCAT activity induced by cholesterol feeding is accompanied by comparable changes in LCAT mRNA abundance in liver. We used a proteoliposome containing rabbit apolipoprotein A-I as substrate and the volume of rabbit serum added to the reaction mixture was 0.5 μl or less, making it unlikely that our determination of LCAT activity was affected by rabbit serum HDL and free cholesterol. Thus, the measured activity likely reflects the mass of LCAT protein. Attempts to measure changes in LCAT mass by immunoblot analysis were unsuccessful.

The strong temporal relation between the increases in LCAT activity and LCAT mRNA suggests that the elevation of LCAT activity in vivo may be determined primarily by the abundance of LCAT mRNA as well as by CETP (34, 35). The change of LCAT activity is most likely due to the transcriptional events rather than translational and post-translational events. Increase in LCAT protein and LCAT activity after cholesterol feeding might reflect a self-defense mechanism against atherogenesis, and an increase in LCAT mRNA might also reflect a self-defense mechanism in the long term. Activation of LCAT with concomitant change in HDL mass and well-balanced CETP may be required for antiatherogenic action.

Our data on the effects of cholesterol feeding on serum cholesterol concentration and LCAT activity are similar to those of Tsopanakis et al. (27). However, their study showed that serum HDL started to decrease from the 3rd week of treatment, whereas we did not detect a significant decrease in HDL-cholesterol after 3 weeks of cholesterol feeding. One possible explanation for this discrepancy is that CETP activity may vary among rabbits at the 3rd week. This conjecture warrants further studies including determination of HDL subclasses after cholesterol feedings.

In summary, we have shown that the amino acid sequence of rabbit LCAT is highly homologous to that of the human protein. Furthermore, there was a marked relation between the increase in plasma LCAT activity and the increase in LCAT mRNA abundance in the liver of rabbits after cholesterol feeding, suggesting that the change in LCAT activity is likely attributable to transcriptional events. Our data should contribute to a better understanding of the role of LCAT in reverse cholesterol transport.

Supported in part by HMG-CoA Reductase Research Foundation, Ono Medical Research Foundation, and by a grant-in-aid for scientific research from the Japanese Ministry of Education, Science, and Culture (No. 03671153).

Manuscript received 3 April 1996 and in revised form 30 April 1996.

REFERENCES

1. Fielding, C. J., and P. E. Fielding. 1981. Evidence for a lipoprotein carrier in human plasma catalyzing sterol efflux from cultured fibroblasts and its relationship to lecithin:cholesterol acyltransferase. *Proc. Natl. Acad. Sci. USA.* **78:** 3911–3914.
2. Fielding, C. J., and P. E. Fielding. 1982. Cholesterol transport between cells and body fluids: role of plasma lipoproteins and the plasma cholesterol esterification system. *Med. Clin. North Am.* **66:** 363–373.
3. Rothblat, G. H., F. H. Mahlberg, W. J. Johnson, and M. C. Phillips. 1992. Apolipoproteins, membrane cholesterol domains, and the regulation of cholesterol efflux. *J. Lipid Res.* **33:** 1091–1097.
4. Vaisman, B. L., H-G. Klein, M. Rouis, A. M. Berald, M. R. Kindt, G. D. Tally, S. M. Meyn, R. F. Hoyt, Jr., S. M. Marcovina, J. J. Albers, J. M. Hoeg, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1995. Overexpression of human lecithin:cholesterol acyltransferase leads to hyperalphalipoproteinemia in transgenic mice. *J. Biol. Chem.* **270:** 12269–12275.
5. Mehlum, A., B. Staels, N. Duverger, A. Tailleux, G. Castro, C. Fievet, G. Luc, J-C. Fruchart, G. Olivecrona, G. Skretting, J. Auwerx, and H. Prydz. 1995. Tissue-specific expression of the human gene for lecithin:cholesterol acyl-

transferase in transgenic mice alters blood lipids, lipoproteins and lipases towards a less athrogenic profile. *Eur. J. Biochem.* **230:** 567–575.
6. Francone, O. L., E. L. Gong, D. S. Ng, C. J. Fielding, and E. M. Rubin. 1995. Expression of human lecithin:cholesterol acyltransferase in transgenic mice. *J. Clin. Invest.* **96:** 1440–1448.
7. Mclean, J., C. Fielding, D. Drayna, H. Dieplinger, B. Baer, W. Kohr, W. Henzel, and R. Lawn. 1986. Cloning and expression of human lecithin:cholesterol acyltransferase cDNA. *Proc. Natl. Acad. Sci. USA.* **83:** 2335–2339.
8. Warden, C. H., C. A. Langer, J. I. Gorden, B. A. Taylor, J. W. Mclean, and A. J. Lusis. 1989. Tissue-specific expression, developmental regulation, and chromosomal mapping of the lecithin:cholesterol acyltransferase gene. *J. Biol. Chem.* **264:** 21573–21581.
9. Meroni, G., N. Malgaretti, P. Magnaghi, and R. Taramelli. 1990. Nucleotide sequence of the cDNA for lecithin:cholesterol acyltransferase (LCAT) from the rat. *Nucleic Acids Res.* **18:** 5308.
10. Hixon, J. E., D. M. Driscall, S. Birnbaum, and M. L. Britten. 1993. Baboon lecithin:cholesterol acyltransferase: cDNA sequences of two alleles, evolution, and gene expression. *Gene.* **128:** 259–299.
11. Hengstschlager-Ottnad, E., K. Kuchler, and W. J. Schneider. 1995. Chicken lecithin:cholesterol acyltransferase; molecular characterization reveals unusual structure and expression pattern. *J. Biol. Chem.* **270:** 26139–26145.
12. Hyunh, T. V., R. A. Young, and R. W. Davis. 1985. A practical approach. *In* DNA Cloning Techniques. D. M. Glover, editor. IRL Press, Oxford. Vol. I: 49–78.
13. Messing, J., R. Crea, and P. H. Seeburg. 1981. A system for shotgun DNA sequencing. *Nucleic Acids Res.* **9:** 309–321.
14. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* **18:** 5294–5299.
15. Scanu, A. M., C. T. Lim, and C. Edelstein. 1972. On the subunit structure of the protein of human serum high density lipoprotein. *J. Biol. Chem.* **247:** 5850–5855.
16. Batzri, S., and E. D. Korn. 1973. Single bilayer liposomes prepared without sonication. *Biochim. Biophys. Acta.* **298:** 1015–1019.
17. Yamazaki, S., T. Mitsunaga, Y. Fujiwara, and T. Nisida. 1983. Interaction of lecithin:cholesterol acyltransferase with human plasma lipoproteins and with lecithin-cholesterol vesicles. *J. Biol. Chem.* **258:** 5847–5853.
18. Yang, C., D. Manoogian, Q. Pao, F-S. Lee, R. D. Knapp, A. M. Gotto, Jr., and H. J. Pownall. 1987. Lecithin:cholesterol acyltransferase. *J. Biol. Chem.* **262:** 3086–3091.
19. Maeda, E., Y. Naka, T. Matozaki, M. Sakuma, Y. Akanuma, G. Yoshino, and M. Kasuga. 1991. Lecithin:cholesterol acyltransferase deficiency with a missense mutation in exon 6 of the LCAT gene. *Biochem. Biophys. Res. Commun.* **178:** 460–466.
20. Chou, P. Y., and G. D. Fasman. 1978. Prediction of the secondary structure of proteins from their amino acid sequence. *Adv. Enzymol. Relat. Areas Mol. Biol.* **47:** 45–148.
21. Kyte, J., and R. F. Doolittle. 1982. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157:** 105–132.
22. Quintao, C. R. 1995. Is reverse cholesterol transport a misnomer for suggesting its role in the prevention of atheroma formation? *Atherosclerosis.* **116:** 1–14.
23. von Eckardstein, A., Y. Huang, S. Wu, H. Funke, G. Noseda, and G. Assmann. 1995. Reverse cholesterol transport in plasma of patients with different forms of familial HDL deficiency. *Arterioscler. Thromb. Vasc. Biol.* **15:** 691–703.
24. Weisweiler, P. 1987. Plasma lipoproteins and lipase and lecithin:cholesterol acyltransferase activities in obese subjects before and after weight reduction. *J. Clin. Endocrinol. Metab.* **65:** 969–973.
25. Subbaiah, P. V., and R. A. Rodby. 1994. Abnormal acyltransferase activities and accelerated cholesteryl ester transfer in patients with nephrotic syndrome. *Metabolism.* **43:** 1126–1133.
26. Bingle, C., S. Ghazi, J. S. Owen, and S. K. Srai. 1991. LCAT mRNA in liver disease. *Lancet.* **338:** 1531.
27. Tsopanakis, C., D. Kotsarellis, I. Dontas, P. Karayannacos, and G. Skalkeas. 1988. Effects of cold stress on serum lipids, lipoproteins, and the activity of lecithin:cholesterol acyltransferase in rabbits. *Biochem. Med. Metab. Biol.* **39:** 148–157.
28. Bielicki, J. K., M. R. McCall, J. J. M. van den Berg, F. A. Kuypers, and T. M. Forte. 1995. Copper and gas-phase cigarette smoke inhibit plasma lecithin:cholesterol acyltransferase activity by different mechanisms. *J. Lipid Res.* **36:** 322–331.
29. Kodama, T., Y. Akanuma, M. Okazaki, H. Aburatani, H. Itakura, K. Takahashi, M. Sakuma, F. Takaku, and I. Hara. 1983. Abnormalities in plasma lipoprotein in familial partial lecithin:cholesterol acyltransferase deficiency. *Biochim. Biophys. Acta.* **752:** 407–415.
30. Norum, K. R., and E. Gjone. 1967. Familial lecithin:cholesterol acyltransferase deficiency. Biochemical study of a newborn error of metabolism. *Scand. J. Clin. Lab. Invest.* **20:** 231–243.
31. Francone, O. L., L. Evangelista, and C. J. Fielding. 1993. Lecithin:cholesterol acyltransferase: effects of mutagenesis at N-linked oligosaccharide attachment sites on acyl acceptor specificity. *Biochim. Biophys. Acta.* **1166:** 301–304.
32. Qu, S-J., H-Z. Fan, F. Blanco Vaca, and H. J. Pownall. 1993. Effects of site-directed mutagenesis on the N-glycosylation sites of human lecithin:cholesterol acyltransferase. *Biochemistry.* **32:** 8732–8736.
33. Mclean, J., K. Wion, D. Drayna, C. Fielding, and R. Lawn. 1986. Human lecithin:cholesterol acyltransferase gene: complete gene sequence and sites of expression. *Nucleic Acids Res.* **14:** 9397–9406.
34. Warren, R. J., D. L. Ebert, P. J. Barter, and A. Mitchell. 1991. The regulation of hepatic lipase and cholesteryl ester transfer protein activity in the cholesterol-fed rabbit. *Biochim. Biophys. Acta.* **1086:** 354–358.
35. Quinet, E. M., L. B. Agellon, P. A. Kroon, Y. L. Marcel, Y-C. Lee, M. E. Whitelock, and A. R. Tall. 1990. Atherogenic diet increases cholesteryl ester transfer protein messenger RNA levels in rabbit liver. *J. Clin. Invest.* **85:** 357–363.