Studies on Cholesterol Ester Formation and Hydrolysis in Liver Disease: A Selective Review

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Plasma cholesterol esters are formed within the circulation by lecithin-cholesterol acyltransferase (LCAT), an enzyme produced by the liver. Patients with hepatocellular disease have low plasma LCAT activity. This largely accounts for the decreased levels of cholesterol esters observed in such patients and appears due to impaired hepatic production of the enzyme. In contrast, activity of the LCAT reaction in patients with cholestasis seems variable and is the subject of controversy, largely because the influence of abnormal cholestatic lipoproteins on the reaction requires further clarification.

Human liver contains a lysosomal cholesterol ester hydrolase (CEH) which may play an important role in hepatic cholesterol homeostasis. In patients with liver damage there is no concrete evidence of circulating CEH activity, but recent studies show elevated activity of hydrolase within the liver itself in acute hepatitis. Hepatic activity of another lysosomal enzyme, acid phosphatase, is not increased, suggesting that high CEH in hepatic liver does not simply reflect a general increase in lysosomal enzymes. The pathogenesis and significance of altered CEH activity in liver disease require further study.

It is a particular pleasure for me to participate in this Festschrift honoring Professor Gerald Klatskin, as he has had a major influence on my life. Among other things, Dr. Klatskin stimulated my interest in lipid metabolism when I was a Fellow in his Liver Study Unit at Yale a decade ago. Because of this, I have chosen to discuss selected aspects of cholesterol ester metabolism in human liver disease. Emphasis will be on contributions in which I have been personally involved.

THE LCAT REACTION AND LIVER DISEASE

In healthy individuals about two-thirds of the serum cholesterol exists in esterified form. It was long assumed that these cholesterol esters were formed directly by the liver, but in a series of brilliant experiments in the 1960s Glomset showed that they were in fact formed primarily in the circulation [1]. The basis for this concept dates back to 1935, when Sperry noted that plasma or serum standing at 37°C develops a progressive increase in cholesterol ester concentration with no change in total cholesterol [2]. Glomset's work 30 years later showed that this plasma esterification reaction is due to a circulating enzyme, lecithin-cholesterol acyltransferase (LCAT) [1,3,4]:

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LCAT
Lechithin + Free Cholesterol———Cholesterol Ester + Lysolecithin

During the past decade the biochemistry of this reaction has been studied in great detail. Study of the reaction was stimulated by the 1967 discovery by Norum and Gjone of a few patients in Scandinavia with familial LCAT deficiency [5]. Subsequent thorough investigation of this rare disorder has greatly advanced knowledge of normal lipoprotein metabolism [4,6,7]. The LCAT reaction is extraordinarily complex and has already been the subject of two international symposia, the first in Oslo in 1973 and the second just recently in London in May 1978. Reviews of the subject have been provided by Glomset [3,4] and by the proceedings of the two symposia [6,8].

LCAT is present in the infranatant fraction of serum subjected to ultracentrifugation at density 1.21 [9]. Early investigations suggested that the enzyme reacts with both “nascent” high density lipoproteins (HDL) and low density lipoproteins (LDL) as substrate, primarily the former [1,3]. A HDL protein, later identified as apo-AI, was shown to act as co-factor for the reaction by Fielding et al. in 1972 [10]. Lipids in very low density lipoproteins (VLDL) were thought to be only indirectly influenced by LCAT via non-enzymatic lipid exchange among lipoprotein fractions [3]. These views may need to be modified, in view of evidence presented at the recent London symposium. Studies with partially purified enzyme and various lipoprotein fractions, with serum from patients with Tangier disease (who lack HDL), and with apoproteins such as apo-AI and apo-C, have yielded complex and conflicting data on the biochemistry of the LCAT reaction, particularly the preferred substrate and the role of apoprotein co-factors [8]. Moreover, the physiological role of the reaction is not certain [8], although present evidence suggests that it plays an important part in triglyceride turnover and in prevention of plasma accumulation of “excess” free cholesterol derived from the surface of chylomicrons and VLDL [4,7].

LCAT in Parenchymal Liver Disease

My own interest in LCAT is clinical and centers around its activity in patients with liver disease. It has long been known that the ratio of esterified to free serum cholesterol is decreased in hepatobiliary disorders. In parenchymal liver disease this abnormality was traditionally ascribed to decreased ester synthesis by the damaged liver. With clarification of the LCAT reaction in the late 1960s, however, it became appropriate to examine whether low ester levels in liver disease could in fact be due to impaired formation within the circulation. Indeed, this possibility had actually been hinted at in the older literature [11,12], long before the nature of the cholesterol esterification reaction was understood.

Dr. Bob Scheig and I decided to examine this question. We first modified available techniques to develop a clinical LCAT assay based on the ability of test serum to esterify \(^{14}\)C-cholesterol-labeled substrate, then applied the assay to healthy subjects and patients with a variety of hepatic disorders [13]. Results showed that serum LCAT activity was indeed diminished in most patients with hepatocellular disease. Moreover, the degree of LCAT impairment generally paralleled the severity of hepatic dysfunction, and the percent cholesterol esters in the serum correlated with the patient's LCAT status [13]. Similar observations were made independently by Gjone and Norum in Norway [14], and other authors have subsequently confirmed these findings [15–17]. There is now general agreement that impaired LCAT activity is an important factor in the pathogenesis of serum lipid derangements in patients...
with hepatocellular disease, although the complex lipoprotein disturbances in these patients cannot be entirely explained by this abnormality [18,19].

Attention was next directed to the pathogenesis of the low LCAT activity. Although deficient production of LCAT by damaged liver seems the most likely cause, several other explanations are theoretically possible. First, circulating LCAT inhibitors, for example bile salts [1], could be present in these patients' sera. We studied this possibility in a series of cross-incubation experiments, but found that serum from patients with liver disease did not inhibit the LCAT activity of normal serum [13]. Other authors have carried out similar studies and also have found that circulating LCAT inhibitors are not the cause of the low cholesterol esterification in liver disease [15]. Second, there could be a deficiency of LCAT activators in hepatic dysfunction. This possibility, however, has also been excluded by cross-incubation studies with normal serum [15].

A third possibility is the presence of abnormal substrate lipoproteins in the patients' sera, rather than a deficiency of LCAT itself. This explanation is not so readily excluded, because autologous lipoproteins are present in any patient's serum being assayed. This technical difficulty can be partly circumvented, however, by incubating relatively small amounts of test serum with relatively large amounts of substrate serum. We did this in a group of patients with hepatocellular disease, in each case assaying the patient's serum simultaneously with both autologous substrate and normal substrate [20]. The source of substrate had little influence on the assay, indicating that decreased cholesterol esterification was in fact not due primarily to abnormal substrate lipoproteins [20]. Blomhoff et al. reached the same conclusion when they also considered this question [17].

Fourth, cholesterol ester hydrolase could be present in the circulation. Human serum does not normally possess hydrolytic activity, but Stokke [21,22] has described a lysosomal cholesterol ester hydrolase (CEH) in human liver, discussed in more detail below. This enzyme could theoretically be released into the circulation by damaged liver cells, a possibility raised by Jones et al. [23]. If so, the "net cholesterol esterifying activity" of serum [23] would reflect an interaction between LCAT and CEH rather than LCAT alone; CEH would oppose the action of LCAT and thereby contribute to the observed decrease in serum cholesterol esterification. A few years ago we investigated this possibility by simultaneously assaying both LCAT and net esterifying activity, and also by searching directly for circulating hydrolase with a specifically developed radioassay [24]. Results yielded no evidence of circulating CEH activity, even in patients with profound hepatocellular dysfunction, and therefore provided strong evidence against Jones' hypothesis [24].

From these and other results it appears most likely that diminished esterifying activity in liver disease is due directly to impaired synthesis or release of LCAT by the damaged liver [19,20]. Experiments with the isolated perfused rat liver support this explanation. Both I and Boyer [25] and Osuga and Portman [26] showed that the isolated liver released LCAT into the perfusate progressively. This proved that the enzyme is produced by the liver, and thereby provided a logical basis for the diminished LCAT activity in hepatocellular disease.

**LCAT in Cholestatic Liver Disease**

The situation in cholestatic (obstructive) disorders appears more complex and remains controversial. Like parenchymal liver disease, biliary obstruction is also associated with a low ratio of esterified to free serum cholesterol, but this is usually due primarily to an increase in free cholesterol rather than a decrease in cholesterol
esters. The pathogenesis of the hypercholesterolemia is complex and has been reviewed by McIntyre et al. [27]. Pioneer studies by Seidel and his associates [28,29] established that serum from patients with cholestasis contains an abnormal lipoprotein, known as lipoprotein-X (LP-X), which can largely account for the in vivo lipid characteristics both morphologically and chemically. Under the electron microscope it appears as disc-shaped vesicles 300–700 angstroms in diameter which tend to aggregate [30,31]. Compared to normal LDL, LP-X contains much higher amounts of phospholipid and free cholesterol, smaller amounts of esterified cholesterol and triglyceride, and less protein [28,30].

The status of the LCAT reaction in cholestasis is controversial. Initial reports noted variable esterification of serum cholesterol in biliary obstruction [11,12,15]. These studies were based on long in vitro incubations, however, which reflect the extent rather than the rate of esterification (substrate is rate-limiting to the LCAT reaction after a relatively short incubation time). Experiments involving briefer incubations with sensitive methods for measuring the small amount of cholesterol esterified, such as gas-liquid chromatography or use of trace amounts of radiolabeled cholesterol incorporated into substrate serum lipoproteins, are therefore preferable. Using the latter technique, I and Scheig initially found normal or high LCAT activity in a few patients with cholestasis who were part of our larger group of patients with liver disease [13]. In contrast, Gjone in Norway reported low LCAT activity in patients with obstructive jaundice [14,32]; he also used a radioassay but with some technical differences from our own. Subsequent studies by other authors also yielded variable and conflicting results (summarized in [20]).

To study further these discrepancies I simultaneously assayed LCAT activity by both our own method and the technique used by Gjone [20]. Results showed excellent correlation between the two methods, indicating that technical factors were not solely responsible for the disparate results. Instead, differences in the severity and duration of cholestasis, and thus of secondary hepatocellular damage, appeared to be the likely explanation [20]. In separate experiments, we carried out further studies in patients with cholestasis, using both an isotopic and a colorimetric LCAT assay [33]. Results confirmed our initial findings of normal or increased activity in autologous patient serum. However, by cross-incubating normal and patient sera we found that cholestatic serum regularly stimulated the LCAT reaction of normal serum. Thus substrate lipoproteins in cholestasis (perhaps LP-X) appear to have a major influence on the LCAT assay, unlike the usual situation in primary hepatocellular dysfunction [33].

These and other results suggest that the overall LCAT reaction in obstructive jaundice reflects a complex interaction among cholestatic lipoproteins, LCAT deficiency due to concomitant hepatocellular damage, and possibly other unknown factors [20,33,34]. Because of these variables, particularly the influence of cholestatic lipoproteins, LCAT activity per se in patients with cholestasis remains controversial.

A closely related and similarly controversial issue is whether LP-X itself serves as a substrate for the LCAT reaction. We were the first to suggest this possibility, in 1970 [13] and again in 1973 [33], based on our experimental findings plus speculation that the high content of free cholesterol and lecithin in LP-X should theoretically provide a particularly good substrate for LCAT. Wengeler and Seidel, however, concluded that LP-X is not in fact a LCAT substrate [35]. In contrast, Ritland and Gjone, using different experimental methods, reached the opposite conclusion [36]. Most recently, Patsch et al. reported that LP-X is indeed a substrate for purified LCAT [37]. It is apparent, however, that the issue is far from being settled.
The most important factor making uncertain the activity of LCAT in obstructive jaundice is the presence of cholestatic lipoproteins in the plasma or serum being tested. All clinically applicable LCAT assays to date, whether based on changes in cholesterol concentration in autologous plasma or on use of a standard non-autologous substrate, have been subject to this difficulty. However, a new LCAT assay devised within the past year by Alcindor and his colleagues in Paris may obviate this hitherto unavoidable problem. Their assay has been partly published in French [38] and was presented at the May 1978 LCAT symposium in London [39]. Through subsequent personal correspondence, Alcindor has kindly sent me details of the method (personal communication, June 1978).

The crux of Alcindor's technique is complete delipoproteinization of the test plasma in which LCAT is to be assayed. This is achieved by dextran sulphate-CaCl₂ precipitation in the presence of a stabilized emulsion of triglyceride and phospholipid [38,39]. Valid clinical application of this new assay, however, will require proof that the delipidation step does not concomitantly affect LCAT in the test plasma, and also that it efficiently removes LP-X and other abnormal lipoproteins equally as well as normal lipoproteins. We are planning experiments to assess these points. If successful, use of Alcindor's assay may settle some of the uncertainties about LCAT activity in patients with cholestasis.

CHOLESTEROL ESTER HYDROLASE IN HUMAN LIVER

Hepatic cholesterol esterifying and cholesterol ester hydrolysing enzymes were characterized long ago in the rat [40]. Virtually nothing was known about these enzyme systems in man, however, until the studies of Stokke a few years ago [21]. He found that, in striking contrast to rat liver, human liver does not contain cholesterol esterifying systems at a neutral pH even with the addition of various co-factors. A reversible cholesterol esterase, however, is present at acid pH. This enzyme has maximal hydrolysing activity at pH 4.5–5.0, with some tailing to neutral pH. The hydrolase requires no co-factors, is stimulated by low concentrations of the surfactant Triton X-100, and is inhibited by sulfhydryl-blocking agents [21]. Stokke subsequently established that this acid cholesterol ester hydrolase (CEH) is of lysosomal origin in the calf, and presumably also in man [22].

The physiological significance of this enzyme is as yet unknown, but theoretical considerations favor an important role in lipoprotein cholesterol ester metabolism. First, two rare diseases are known to be associated with deficient hepatic acid lipase activity, Wolman's disease and cholesterol ester storage disease [41,42]; in both conditions abnormal hepatic accumulation of cholesterol ester occurs. Second, in order to maintain constant ester levels in the circulation, tissue uptake and hydrolysis of plasma cholesterol esters must balance their formation. Since the liver is the major site of lipoprotein clearance from blood [7], it should logically also be the site of cholesterol ester hydrolysis. Third, cholesterol is excreted from the liver as lipoprotein free cholesterol, biliary excretion of free cholesterol, and conversion of free cholesterol to bile acids. Thus circulating cholesterol esters entering the liver presumably are hydrolysed to free cholesterol before further metabolism and turnover.

In addition to these theoretical points, recent experimental evidence suggests involvement of CEH in hepatic lipoprotein catabolism. Cooper recently showed that the perfused rat liver rapidly removes chylomicron remnants from the perfusate, followed by hydrolysis of the cholesterol ester [43]. Similarly, using rat hepatocyte monolayers, Floren and Nilsson observed an efficient degradation of cholesterol ester
in chylomycin remnants, with strong indirect evidence of lysosomal hydrolase involvement [44]. In addition, Stein et al. recently studied the catabolism of VLDL and LDL by rat liver, using chloroquine as an inhibitor of lysosomal enzymes, and concluded that acid hydrolases participate in hepatic lipoprotein catabolism [45].

All of these recent results support the idea that hepatic lysosomal hydrolases play a major role in the physiologic degradation of plasma lipoproteins. To extend this idea, the action of hepatic CEH on individual lipoprotein classes should be studied in man, since results obtained using experimental animals may be subject to complications arising from species differences in hepatic cholesterol esterifying enzymes [21]. Such studies will require fresh human liver tissue as enzyme source, with individual lipoprotein classes containing radiolabeled cholesterol ester as substrate. Our current experiments with CEH activity in human liver needle biopsies (see below) are well suited to this type of study, and we are planning studies to examine directly the role of individual lipoprotein classes as substrate for hepatic CEH in man.

**CEH in Liver Disease**

In theory, abnormal CEH activity in patients with primary hepatic disease might affect the balance between cholesterol ester formation and breakdown, and thereby contribute to lipid derangements. This could occur in at least two ways. First, hydrolase could “leak” out of damaged hepatocytes and enter the circulation, where it would oppose the esterifying action of LCAT [23]. As mentioned above, however, we were unable to detect evidence of circulating CEH activity even in patients with severe hepatic disease [24]. This does not entirely exclude the possibility that circulating hydrolase is present in the occasional patient, but certainly CEH does not usually contribute to the impaired serum cholesterol esterification observed in hepatic disease.

Second, altered hydrolytic activity within the liver could affect homeostasis between ester formation and hydrolysis. We have recently been studying this possibility. We first had to develop a clinically useful CEH assay that could be applied to portions of needle biopsy specimens. This was successfully accomplished by working out a sensitive radioassay modified from Stokke’s technique [21] and validated in a series of preliminary experiments on pH spectrum, kinetics, etc. Details of this work have just been published [46,47]. The assay enables CEH activity to be determined in about 2 mg of liver homogenate, and is therefore suitable for clinical studies on percutaneous needle biopsy specimens.

We then applied our CEH assay to a series of 69 patients undergoing diagnostic liver biopsy for usual clinical indications [46]. In brief, we found that CEH activity was more than threefold higher in liver homogenates from patients with acute hepatitis than in histologically normal livers ($p < 0.01$). In contrast, hepatic CEH activity was not significantly altered in alcoholic liver disease, obstructive jaundice, or a group of miscellaneous liver disorders. Several pieces of evidence suggested that the increased hydrolase activity in acute hepatitis was directly related to liver cell necrosis. For example, CEH values correlated well with SGOT levels ($r = 0.84$) and returned to normal as hepatitis resolved. However, we found no apparent relationship to serum levels of either free or esterified cholesterol, nor to serum LCAT activity determined concomitantly by radioassay [46].

Most recently we have turned our attention to the pathogenesis of the high CEH activity in patients with hepatitis. The first question was whether the observed abnormality reflected a nonspecific general increase in lysosomal activity; alterna-
tively, CEH could be disproportionately elevated. The former possibility seems reasonable, as viral hepatitis is associated with an increased size and number of hepatocyte lysosomes [48]. To investigate this, in 41 patients we concomitantly measured hepatic activity of CEH and of acid phosphatase, a standard lysosomal marker enzyme [47]. Results showed that, unlike CEH, acid phosphatase activity is not significantly increased in liver biopsy homogenates from patients with acute hepatitis [47]. This disparity between the activity of the two lysosomal enzymes suggests that high CEH activity is not simply a reflection of general enhanced lysosomal activity.

One possible explanation for the discrepancy between CEH and acid phosphatase activities is that the two enzymes have different solubilities. It is well established that about 70 percent of total acid phosphatase activity is soluble when lysosomes are disrupted [49] and that soluble lysosomal enzymes quickly disappear into the circulation when released into the cytoplasm [50]. CEH might therefore be more tightly bound to lysosomal membranes than acid phosphatase, or, if released, more easily adsorbed by other subcellular fractions.

In recent months we have been directly comparing hepatic CEH activity with that of other lysosomal enzymes of differing solubilities—specifically, β-galactosidase, known to be highly soluble [50,51], and N-acetyl-β-glucosaminidase, which in contrast is known to have a high affinity for subcellular fractions [51,52]. For further comparison, acid esterase and cathepsin D, two other lysosomal enzymes, have also been assayed. This was done by scaling down established methodologies with only minor modifications, which has enabled us to use the small amounts of homogenate we obtain from human percutaneous biopsy specimens. All assays are done after alternately freezing and thawing the homogenates several times to ensure rupture of lysosomes and hence measurement of total enzyme activity. Results to date have revealed that CEH activity quite closely parallels activity of each of the above enzymes, with correlation coefficients of 0.77 to 0.90 (unpublished observations). This argues against differences in solubility as an explanation for the high CEH activity in hepatic liver.

An important, as yet untested possibility, is that the hydrolase is mainly derived not from hepatocytes, but rather from Kupffer cell hyperplasia and/or mononuclear cell infiltration (these two findings are important histologic features of acute hepatitis). Although there are no direct data on this point, four recent pieces of evidence in the literature make the postulate seem reasonable. First, CEH activity has been detected in blood monocytes [53]. Second, Drevon et al. have shown that non-parenchymal cells of rat liver (largely Kupffer cells) contain acid CEH activity approximately equivalent to that of parenchymal cells [54]. Third, in a recent preliminary communication Ganguly et al. have reported increased lysosomal enzyme release in blood monocytes from patients with chronic liver disease, inflammatory bowel disease, and rheumatoid arthritis [55]. Finally, Van Berkel et al. have very recently reported studies on the in vitro degradation of I2-labeled LDL and HDL by liver homogenates, and found that a proportion of the rat’s hepatic capacity for lipoprotein degradation is localized in non-parenchymal cells [56]. These observations suggest a possible link between non-parenchymal cell function and our own observations on increased hepatic CEH activity in human hepatitis.

CONCLUDING COMMENTS

Cholesterol ester formation and hydrolysis in liver disease is a complex subject, and I have touched on only selected aspects in the above survey. Yet even within the
narrow confines of my discussion it is apparent that much has yet to be learned. For example, how many of the complex lipoprotein derangements associated with hepatic disease can be ascribed to impaired LCAT activity? What is the clinical importance of LCAT deficiency in parenchymal liver disease? How do cholestatic lipoproteins influence the LCAT reaction, and does LP-X act as substrate for LCAT, either directly or indirectly? Is LCAT activity per se low, normal, or high in cholestasis? What is the role of hepatic lysosomal CEH in normal lipoprotein catabolism? Are hepatocytes or non-parenchymal cells the primary source of CEH in man? Why is hepatic CEH activity increased in acute hepatitis, and what effect does this have on cholesterol ester homeostasis?

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