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PLASMA LIPID TRANSFER ACTIVITIES

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INTRODUCTION

A protein capable of exchanging cholesteryl ester (CE) between low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) obtained from

hypercholesterolemic rabbits was shown to be present in the $d > 1.25$ fraction of normal and hypercholesterolemic rabbit plasma (100). A similar activity was observed in human plasma (22, 57, 76, 77, 83, 85). Barter et al (16) published the first report that the $d > 1.21$ fraction of plasma contains a factor that accelerates the exchange of triglycerides among rabbit lipoproteins. Since then, several reviews (12, 36, 91, 95) have been published. In this article, we briefly review the purification and characterization of lipid transfer protein (LTP), as well as the methodology for measuring lipid transfer activity (LTA). In addition, we provide a more detailed review of the role of LTP in lipoprotein metabolism and the regulation of LTA.

PURIFICATION

LTP

The first purification of LTP from human plasma in our laboratory (76) was based on ammonium sulfate precipitation, ultracentrifugation at $d = 1.25$ g/ml, and chromatography on phenyl-Sepharose, carboxymethyl (CM)-cellulose, and concanavalin A–Sepharose. This procedure identified the LTP as a relatively large glycoprotein, which is present in plasma after the lipoproteins are removed by ultracentrifugation. At about the same time, Chajek & Fielding (22) found that an appreciable amount of LTA was bound to HDL, and they purified the LTP by an antibody-affinity step followed by concanavalin A–Sepharose chromatography. Rajaram et al (83) also achieved a partial purification of LTP in rabbit plasma. Subsequently, Morton & Zilversmit (70) improved the purification and observed two fractions with molecular weights of 58,000 and 66,000. The two fractions were equally effective in transferring various apolar lipids; triglyceride (TG) and cholesteryl ether transfer was accelerated approximately 30% less than that of CE. Also of interest was that various mercurial reagents inhibited TG transfer without affecting CE transfer. Abbey et al (1) purified a similar pair of human plasma LTPs and prepared an antibody to the higher-molecular-weight human LTP fraction. This antibody precipitated both LTP fractions, as well as rabbit LTP previously isolated by Abbey et al (3). Through the use of fast protein liquid chromatography, only the higher-molecular-weight fraction of LTP was observed by this group, and they suggested that the lower molecular weight fraction represented a degradation product.

In studies by Harmony and co-workers, LTP was initially purified as lipid transfer complexes with molecular weights similar to those above (56). Later, by using immunoaffinity purification (19), active LTPs were found with molecular weights as low as 3000 (20). Hesler et al (51) improved the purification of LTP by introducing the adsorption of LTP on a TG emulsion. They reported a molecular weight of 74,000 for CE transfer protein and

showed that the transfer activity was lessened by lipid peroxides (51). Jarnagin et al (59) reported a molecular weight of 74,000 for a CE transfer protein that also transferred phospholipid. Both Hesler et al (51) and Jarnagin et al (59) observed that the purified protein had little or no capacity to transfer TG. Differences in molecular weights reported by different groups may be due, in part, to the presence of neuraminidase, which is copurified with LTP (60). This enzyme, which releases sialic acid, is activated at 4°C unless EDTA or mercaptoethanol is present (60).

Albers et al (5) isolated LTP as a relatively heat-resistant protein that is capable of increasing the rate of cholesterol esterification in plasma when added together with purified lecithin:cholesterol acetyltransferase (LCAT). Furthermore, they demonstrated net transfer of CE and TG between lipoprotein fractions. Tollefson & Albers reviewed purification procedures and characterization of LTPs (95). Investigations of amino acid composition of LTP have shown it to be hydrophobic (59), perhaps even more so than any of the apolipoproteins (51).

Phospholipid Transfer Protein

Several laboratories have purified a LTP that preferentially transfers phospholipids (LTP-II) (5, 24, 92), but little is known about its properties or its role in lipid transport.

LTA Inhibitor

Morton & Zilversmit (69) observed that lipoprotein-deficient pig or rat plasma inhibited LTA of partially purified LTP. Similarly, the addition of human lipoprotein-deficient plasma to human LTP decreased the transfer of both CE and TG between lipoprotein fractions in a standard assay. The inhibitor of LTA was separated from LTP on a phenyl-Sepharose or a CM-cellulose column. A subsequent study (86) showed the inhibitor to be a sialoglycoprotein with a molecular weight of 32,000. Nishide et al (73) purified the inhibitor and reported a molecular weight of 29,000. It was present in a subfraction of high-density lipoprotein (HDL) and inhibited the transfer of CE, TG, and phospholipid. Removal of the inhibitor by an immunoaffinity column enhanced LTA of human, pig, and rat plasma.

LIPID TRANSFER ASSAYS

CE-labeled lipoprotein substrates can be obtained from the plasma of animals fed a relatively large dose of [^{14}C]- or [^{3}H]cholesterol. This procedure was used in our laboratory during the early phases of LTA research (100). In a subsequent publication (76), labeled LDL was prepared in vitro by incubating a filter paper disk, impregnated with [^{3}H]cholesterol, with normal rabbit

plasma. The labeled, unesterified cholesterol can be removed by subsequent incubation of the isolated LDL with a large excess of bovine HDL (76) or erythrocytes (7). Alternatively, one may incubate whole plasma or isolated lipoprotein fractions with sonicated phospholipid liposomes containing labeled CE and/or TG (69).

Lipid transfer rates have been measured in different laboratories by somewhat different techniques. In principle, any combination of lipoproteins in which one of the lipoproteins is labeled with the appropriate lipid (donor particle) and the other acts as the acceptor can be used. Which lipoprotein serves as donor or acceptor depends on technical considerations, such as ease of separation of the lipoproteins after incubation or constraints imposed by metabolism. The latter might require that the direction of transfer correspond to the direction of net transfer of CE or TG in vivo. Although in our laboratory we have used radioactive tracers to measure the transfer rates, others have used primarily the measurement of net lipid transfer. We shall briefly consider the merits of both procedures.

In the measurement of lipid transfer by the radioisotope method, equivalent amounts of donor and acceptor lipoproteins or an excess of the acceptor particle may be used. In the latter case, the return of label from acceptor to donor is minimized and can frequently be ignored in short incubations. This simplifies the calculations somewhat (see the section on kinetics). On the other hand, the presence of excess acceptor may reduce the transfer of labeled lipid for at least two reasons: (*a*) the LTP may be preoccupied with the transfer of lipids within the acceptor fraction, or (*b*) an acceptor, such as HDL, may inhibit the transfer reaction or contain a separate inhibitor of lipid transfer (69, 73, 86). Assay procedures for CE, TG, and phospholipid are included in a review by Tollefson & Albers (95).

In the laboratories in which the net change in CE concentration in the HDL or in the lower-density classes is measured, the methodology has to be able to measure very small changes in CE concentrations (22). Equating these measurements with the activity of LTP is probably not warranted, since the latter is only one of the factors involved in this type of net flux. The activity of LCAT must be taken into account or inhibited. In addition, the observed net flux is affected greatly by the backflow of CE, so that, ideally, zero time measurements of flux should be made.

Kinetics

The transfer or exchange of lipids may be expressed simply as the fraction of a labeled lipid in donor particles that is found in the acceptor after a certain length of time. This fraction may not increase linearly with time, however, and may therefore be difficult to translate into a transfer rate for mass. One approach to overcome this difficulty is to arrange the incubations so that little

or no net mass transfer takes place, i.e. at steady-state conditions. The mathematics of such a donor–acceptor system is shown in standard texts of isotope kinetics, e.g. Shipley & Clark (84). We denote the labeled donor fraction as D^* and the acceptor as A^*, with subscripts of 0 or t to reflect zero time and time of incubation, respectively. If the rate constant for lipid transfer from D to A is k_1 and that for transfer in the opposite direction is k_{-1}, then, in a steady state,

$$k_1 \text{ (donor mass)} = k_{-1} \text{ (acceptor mass)},$$

or

$$k_{-1}/k_1 = \text{(donor mass)/(acceptor mass)} = c \text{ (constant)}.$$

Under these conditions,

$$A_t^* = D_0^* \left(1 - e^{-(k_1+k_{-1})t/(1+c)}\right). \quad 1.$$

k_1 is most readily calculated after rearrangement:

$$k_1 t = -\ln[1-A_t^*(1 + c)/D_0^*]/(1 + c). \quad 2.$$

Under conditions in which little or no labeled lipid returns from A to D, Equation 2 simplifies to

$$k_1 t = -\ln(1-A_t^*/D_0^*). \quad 3.$$

Equation 3 was used in publications from our group (76, 77), as was Equation 2 (87), although the published equation in that paper shows a misplaced parenthesis. Barter & Jones (13, 14) used Equation 3 in a somewhat different form. In the same study (14), they tested a molecular model in which the transfer protein interacts with both the donor and acceptor lipoproteins and transfers the CE during this interaction. The authors concluded that the experimental data provided excellent support for this model and that the LTP showed a much greater affinity for HDL than for LDL. In a later study by Barter et al (11), a similar kinetic model was shown to apply to CE exchanges between HDL, LDL, and VLDL.

Ihm et al (58) proposed a somewhat different model for the exchange of CE and phospholipid. The LTP, which normally contains a small amount of lipid (56) and has been designated as a lipid transfer complex, appears to form a ternary collision complex with HDL and LDL, which mediates the lipid transfer process. Binding of LTP to HDL was demonstrated by gel filtration

(77). These studies showed that this binding, which is promoted by negative charges, is readily disrupted by Ca or Mn ions. The interaction of LTP with LDL and VLDL forms a much more labile complex (65), which could be demonstrated only by the use of Sepharose-bound lipoproteins. The binding of LTP to all lipoproteins appears to be a saturable process. Kinetic studies demonstrated that LTA changed in proportion to the extent of binding of LTP to HDL.

LTP exhibits specificity for CE with different fatty acid compositions (66). Whether reconstituted HDL or sonicated phospholipid liposomes with added CE were the donor particles, the transfers were in the following order: cholesteryl oleate > cholesteryl linoleate > cholesteryl arachidonate > cholesteryl palmitate. The transfer of the palmitate ester was 65% of that of the oleate ester. The transfer of CE from HDL to apolipoprotein −B–containing acceptors also depends on the presence of apolipoproteins(a). The apo(a) can be removed from the B protein by reducing the disulfide linkage. In the presence of apo(a), the transfer of CE from HDL to lipoprotein(a) was only about one half that to lipoprotein(a) from which the apo(a) had been removed, which, in turn, was very similar to the transfer to normal LDL (44).

Comparison with In Vivo Measures

One purpose of measuring the in vitro transfer rates of CE is to ascertain the rate of CE flux in vivo. The latter is composed of the secretion rate of CE-containing lipoproteins into plasma, the synthesis of CE from unesterified cholesterol by the LCAT reaction, the mass transfer of CE from the higher-density to the lower-density fractions, and the removal of CE-containing lipoproteins from plasma. The in vitro measures of CE transfer can be subdivided into (*a*) transfer of labeled CE between two lipoproteins added to lipoprotein-deficient plasma; (*b*) transfer of labeled CE, usually in HDL, to other lipoproteins in whole plasma; and (*c*) net mass transfer of CE from HDL to the lower-density lipoproteins in whole plasma. All three types of procedures have their limitations, both technically and theoretically.

The transfer of labeled CE between two standard lipoproteins in a $d > 1.21$ or similar lipoprotein-deficient plasma is useful primarily as a measure of LTA in that fraction. This transfer would be affected by the amount of LTP in plasma and the presence of transfer inhibitors. As such, it is a measure of the potential for CE transfer in the plasma from which the $d > 1.21$ was prepared. If this value is very low, one can conclude that the LTA of that plasma is probably the rate-limiting step in the mass transfer reaction. Under such conditions, as with rat plasma, it should not be surprising to find relatively high HDL CE concentrations, if LCAT is active.

The transfer of labeled CE in whole plasma is also dependent on the LTA, as measured in the previous section, but, in addition, it depends on the

relative amounts of donor and acceptor particles and the suitability of the lower-density lipoproteins to act as CE acceptors.

The measurement of net mass transfer in vitro from HDL to the lower-density lipoproteins has been proposed as a measure of CE mass transfer in vivo. For the best estimate of the in vivo transfer rate, the assay conditions must be arranged so that the in vitro process approximates that in vivo. Thus, the change in CE mass of the HDL fractions is generally measured during very short periods. In addition, the formation of additional CE from unesterified cholesterol may be compensated for by inhibiting LCAT or by measuring the newly produced CE separately. Apart from the fact that the assay is technically difficult, whether the in vitro measure of CE mass transfer can adequately mimic the effect of nascent VLDL on the transfer process in vivo is not at all clear, nor can the effect of lipoprotein clearance in vivo on the transfer reaction be estimated.

Apparently, then, the various in vitro assays measure different aspects of the complex interactions of CE production and interchanges that take place in vivo.

REGULATORY ROLE OF LTP

Lipoprotein Composition

Net protein-mediated transfer of CE from LDL and HDL has been observed repeatedly (22, 25, 53, 63, 71). The relative rates of incorporation of CE into VLDL and LDL have been measured by in vitro studies of normal human plasma (9, 10). Esterification of cholesterol in HDL, coupled to transfer of esterified cholesterol to LDL and VLDL, represents the major route whereby CE accumulates in plasma. In this transfer, the TG-rich lipoproteins are the preferential acceptors of CE (63). Even within the VLDL class, subfractions appear to act as preferential acceptors for CE. When nascent VLDL from a perfused rat liver was compared with circulating rat VLDL, the former accepted CE from human HDL more efficiently (74). Among human VLDL subfractions, the larger, TG-rich particles were better acceptors of CE mass, but even the smallest VLDL was better than LDL (31). Viewed from the perspective of exchange reactions, it should not be inferred from these results that LDL CE is not readily exchangeable with HDL CE. Indeed, the author draws attention to the different interpretation of a mass transfer and that of the corresponding labeled species. In an earlier study (71) of CE and TG exchange between different native or reconstituted lipoproteins, a competition of TG and CE for exchange by LTP was observed. Hence, when HDL and VLDL are incubated in the presence of LTP, approximately equimolar amounts of CE and TG move in opposite directions (heteroexchange), and, therefore, net shifts in CE and TG masses are observed (31, 71).

The relative transfer of CE and TG either from a set of "synthetic" HDL particles or from LDL and VLDL fractions with different CE/TG ratios to a lipoprotein acceptor appeared to be independent of the acceptor particle composition (71). However, the relative transfer depended greatly on the lipid composition of the donor particle. In fact, the relative amounts of CE and TG transferred corresponded nearly exactly to the ratio of these lipids in the donor particle, whereas the total amount of apolar lipid remained constant and independent of the relative amounts of these lipids in the acceptor lipoproteins. In the same study, we observed that the transfer of CE in one direction was coupled to the reciprocal transfer of CE (homoexchange) or of TG (heteroexchange). Under conditions in which the only lipid available for "backtransfer" was TG, the forward movement of CE could be reduced greatly by using an inhibitor (*p*-chloromercuriphenyl sulfonate) that specifically inhibited the transfer of TG. A somewhat analogous finding was observed (30) when the transfer of HDL CE to VLDL from normal and hyperlipidemic patients was measured. The transfer of CE to VLDL was correlated with the amounts of TG and phospholipid relative to CE in the acceptor particle.

An additional potent regulator of LTA is the unesterified cholesterol fraction of the lipoprotein fractions (67). An increase in the level of unesterified cholesterol in the VLDL and LDL fractions decreased the unidirectional transfer of their CE by as much as 50% without affecting the transfer of TG. An increase in the level of unesterified cholesterol in the HDL fraction had little or no effect on unidirectional CE transfer to the lower-density acceptors but nearly tripled its TG transfer. These effects on unidirectional transfer resulted in marked changes in net transfer of the apolar lipids. A marked increase in the level of unesterified cholesterol of HDL and VLDL tripled the net transfer of CE from HDL to VLDL and similarly increased the equimolar reciprocal transfer of TG. This finding contrasts with the conclusion of Fielding et al (37) that increases in the level of unesterified cholesterol in lipoproteins, as observed in diabetics, are inversely related to the net transfer of CE from HDL to the lower-density fractions.

LTA and Density Shifts of LDL

Severe hypertriglyceridemia both in humans and in animals is frequently associated with low levels of LDL in plasma (64). In instances in which the LDL apo B concentration is abnormally low, this association can be most readily explained by a slowed degradation of the VLDL complex to LDL by a decreased activity of lipoprotein lipase. In other instances the primary mechanism may involve the heteroexchange of CE and TG between LDL and VLDL (25, 40, 64).

A striking instance of this phenomenon was demonstrated in our study of

cholesterol-fed diabetic rabbits, in which the plasma TG concentrations may reach extremely high values (64). In a typical set of experiments in which alloxan-diabetic rabbits were fed 0.5 g of cholesterol and 2.5 or 7.5 g of fat added to commercial rabbit chow, the plasma TG level varied between 100 and 16,000 mg/dl. Nondiabetic rabbits fed the same diets maintained plasma LDL plus HDL cholesterol levels of about 350 mg/dl, whereas in one group of diabetic animals with plasma TG levels around 6,000 mg/dl, the LDL plus HDL cholesterol level averaged 21 mg/dl. When LDL from a hypertriglyceridemic rabbit was labeled with ^{125}I and then injected into either a hypertriglyceridemic or a normotriglyceridemic rabbit, the rate of disappearance of the LDL protein from the circulation was the same in the two recipient animals. However, when labeled LDL from a normotriglyceridemic rabbit was injected into the same two recipients, the disappearance of that LDL from the circulation of the hypertriglyceridemic rabbit was greatly accelerated, and, in 4 h, large quantities of iodine-labeled LDL protein were present in the intermediate-density lipoprotein (IDL) fraction. This and other experiments in vitro clearly demonstrated that normal LDL, in the presence of excess VLDL, becomes enriched in TG at the expense of CE to the extent that such LDL is recovered in IDL or even in the VLDL class. The amount of LDL involved in a density shift to a lower-density class was directly related to the plasma TG concentrations.

This phenomenon would be expected to occur only in animals or humans with greatly elevated plasma TG fractions and with sufficient LTA to promote the heteroexchange reaction. The observation mentioned above, that individuals with severe hypertriglyceridemia have lower than normal plasma LDL concentrations, may have led some to believe that such individuals are protected against the development of atherosclerotic lesions. However, to the extent that the abnormally low LDL concentrations are the result of LDL density shifts, the risk for cardiovascular disease may be underestimated.

LTA and CE Metabolism

Two experimental approaches have been taken to determine whether, in vivo, LTA is an important regulator of lipoprotein CE metabolism. After the discovery of neutral-lipid exchange and/or transfer among lipoproteins in rabbit plasma in vitro, Barter and co-workers reexamined the intravascular metabolism of lipoprotein CE and TG in the rabbit. At 30 min after injection of isotopically labeled LDL, more than half of the esterified [^3H]cholesterol was recovered in the HDL fraction of the recipient rabbits (15, 48), but no transfer of [^{125}I]apoprotein was observed (48). In a similar experiment with rabbits, there were bidirectional transfers of [^3H]TG between HDL and LDL and, to a lesser extent, transfer of [^3H]TG to VLDL (16). In pigs, which are essentially deficient in plasma LTA, no such equilibration of [^3H]CE among

lipoproteins was observed (49). Hence, indirect evidence was provided that, in vivo, LTP activity was necessary for the exchange of CE and TG among plasma lipoproteins.

Experiments have also been performed in vivo to determine whether LTP activity drives a net transfer of CE from HDL to the potentially atherogenic, lower-density lipoproteins. In these experiments, LTP activity was administered intravenously to rats that are inherently deficient in LTP activity; the transfer protein–induced changes in lipoprotein composition and concentrations were then determined (45, 47, 79). In rats, intravenous injections of partially purified human LTP did not affect total cholesterol levels in plasma, but they had profound effects on the distribution of CE among plasma lipoproteins. In normolipidemic rats, the administration of plasma LTP activity to a level similar to that in rabbits was associated with up to a 45% reduction in the concentration of HDL-esterified cholesterol and concomitant increases in VLDL- and LDL-esterified cholesterol levels (45, 79). Similarly, Ha & Barter (47) reported decreased HDL and increased VLDL cholesterol levels 24 h after LTP injections in rats, which were made hypertriglyceridemic by dietary sucrose feeding. The LTP-mediated transfer of CE out of the HDL fraction resulted in a complete disappearance of the large, apo E–rich HDL subfraction that is normally present in rat plasma (47, 50). A reciprocal net transfer of TG from VLDL to HDL was not evident from increased HDL TG levels in the LTP-treated rats, possibly because of high levels of hepatic lipase in rats (45). These studies of LTP administration in a species that is inherently deficient in LTP activity demonstrate that, in vivo, LTP activity mediates the net transfer of CE from HDL to VLDL and LDL and that the preponderance of HDL cholesterol in the rat is attributed, at least in part, to the deficiency of LTP activity in this species. In the reverse experiment, it was reported that HDL-esterified cholesterol levels were increased in rabbits after inhibition of endogenous LTP activity by means of a monospecific antibody (98). In contrast, HDL cholesterol levels were unchanged in two rabbits after near-complete inhibition of LTA in a similar experiment (2). However, the results of the latter experiment are difficult to interpret because the blood-sampling protocol was associated with a 50% reduction in hematocrit, erratic changes in plasma TG levels, and marked reductions in the plasma HDL cholesterol (30%) and LTA (50%) levels for control rabbits that received injections of nonimmune immunoglobulin G (IgG) (2).

LTA and Hyperalphalipoproteinemia

Experimental elevation of LTP activity in rats (45, 47, 79) or reduction in rabbits (98) indicates that LTP activity is a regulatory component of intravascular cholesterol metabolism and can affect the distribution of CE

among the plasma lipoproteins. Therefore, several investigators have attempted to determine whether the abnormally high levels of the HDL cholesterol associated with hyperalphalipoproteinemia in humans can be explained mechanistically by a deficiency of LTP or LTA. Relatively few hyperalphalipoproteinemic patients have been studied thus far, and the results of the investigations are equivocal. At present there appears to be considerable heterogeneity among these patients with respect to plasma LTA.

Three independent investigations of a total of five hyperalphalipoproteinemic patients indicate that the high levels of HDL cholesterol in these particular cases may be caused by a deficiency in the transfer of CE from HDL to lower-density lipoproteins. By using LTP-monospecific antibodies in Western immunoblot and radioimmunoassay procedures, Brown et al (18) reported a complete deficiency of plasma LTP for two familial hyperalphalipoproteinemic siblings; plasma LTP levels for normolipoproteinemic controls were 2.0 ± 0.5 μg/ml. In studies of LTA levels in three different hyperalphalipoproteinemic patients, the CE transfer activity of $d > 1.21$ plasma ranged from completely absent (61) to less than 50% that of a normolipidemic control (62). The HDL cholesterol levels of these three patients were 174–372 mg/dl, and the HDL particles were reported to have an abnormally high CE: apolipoprotein A-I ratio (61). Their plasma also exhibited minimal transfer of isotopically labeled CE among the endogenous lipoproteins in vitro; however, owing to technical factors, these data are difficult to interpret. Koizumi et al (61) reported little transfer in whole plasma of [^{3}H]CE from HDL to lower-density lipoproteins for two hyperalphalipoproteinemic patients but the high endogenous HDL:LDL ratio in these plasma samples would, in and of itself, produce a lower percentage of transfer of labeled HDL CE to the relatively small pool of acceptor particles (VLDL and LDL). Measuring CE transfer in the opposite direction, Yokoyama et al (99) reported little transfer of [^{3}H]CE from LDL to the disproportionately large HDL pool in the $d > 1.006$ plasma of a hyperalphalipoproteinemic patient. The authors concluded that the HDL of this patient were poor acceptors of CE, but they were unable to replicate the observation when plasma from the same individual was tested on a second occasion (99).

The aforementioned case studies of plasma LTP and CE transfer activity in five remarkably hyperalphalipoproteineimic patients suggest that this rare dyslipoproteinemia, which appears to be associated with longevity and a paucity of coronary artery disease, might be associated causally with a deficiency of plasma LTA. However, from these limited observations, it is not possible to decipher the extent to which this deficiency of LTA might be attributable to a relative or absolute deficiency of LTP, the presence of LTP inhibitors, or possible alterations in plasma lipoproteins that might affect their interaction with the transfer protein.

In sharp contrast to the results of the case studies of LTA in hyperalphalipoproteinemia, Groener et al (42) reported that CE transfer was not markedly low for a kindred of 21 familial hyperalphalipoproteinemic individuals compared with their unaffected spouses. The kindred were classified as hyper- or normoalphalipoproteinemic according to HDL cholesterol concentrations (greater or less than the 90th percentile, respectively). The HDL cholesterol concentrations (mean ± standard deviation) for the respective groups were 95 ± 38 mg/dl (n = 9) and 56 ± 8 mg/dl (n = 12), whereas that of the spouses was 38 ± 11 mg/dl (n = 6). Transfer activity was determined by two different assays. In an estimation of the activity of "lipoprotein-deficient-like" plasma, VLDL and LDL were precipitated from whole plasma by means of polyethylene glycol, and the capacity of the supernatant to transfer isotopically labeled CE from LDL to HDL was determined in a standardized system of exogenous substrates. Although such a procedure could have resulted in the inclusion of variable amounts of endogenous HDL in the assay, the authors state that the volumes of supernatants that were used for the different types of subjects were selected such that the endogenous HDL did not confound the results. By this assay, CE transfer activity for the hyperalphalipoproteinemic patients was only 23% lower ($p < 0.01$) than that of the unaffected spouses. Furthermore, the authors emphasize that the transfer activity for the proband with the highest HDL level was similar to the mean value for the spouses. In the second transfer assay, the CE transfer activity of whole plasma was determined as the transfer of [^{3}H]CE from HDL to lower-density lipoproteins during a 3-h incubation of whole plasma in the presence of 1 mM sodium iodoacetate (LCAT inhibitor). The calculation of CE transfer for this assay assumed no backflow of labeled CE and no mass transfer. The assumption of no mass transfer might have been inappropriate in this case, because plasma TG levels were significantly lower for the hyperalphalipoproteinemic patients than for the other two groups of subjects. When whole-plasma transfer activity was calculated as the percentage of labeled CE transferred out of the HDL fraction, as Koizumi et al did previously (61), transfer for these patients appeared to be markedly lower than that for the other two groups. However, as Groener et al (42) note, this apparent deficiency most likely results from the relatively large donor pool (HDL) present in their plasma. More appropriately, when whole-plasma transfer activity was calculated as the percentage of label transferred multiplied by the HDL pool size, the activity for the hyperalphalipoproteinemic group was only 22% lower than that for the unaffected spouses. Furthermore, the transfer activity for the normoalphalipoproteinemic kindred, whose HDL cholesterol levels were significantly higher than that of the spouses, was actually higher than that of the spouses. These data clearly indicate that this particular family of hyperalphalipoproteinemics is not characterized by a gross deficiency of CE transfer activity or

a major disturbance in the CE transfer process. This study also illustrates the importance of carefully choosing the appropriate method for calculating lipid transfer in a whole-plasma assay system (42).

From the studies reported to date of LTA in hyperalphalipoproteinemic people, it appears that transfer activity is at least marginally lower than normal for some hyperalphalipoproteinemic patients. However, in most cases, the mechanism(s) for the reduced level of LTA is not known, and the generalization that hyperalphalipoproteinemia is caused by a deficiency of LTA cannot be made. An abnormally low CE transfer process could certainly account for the CE enrichment and large size of HDL particles in hyperalphalipoproteinemic patients, but whether such an alteration might also affect the rates of secretion or removal of apolipoprotein A-I–containing particles from plasma remains to be demonstrated.

LTA and HDL Metabolism

The LTP-mediated heteroexchange of lipoprotein core lipids provides a mechanism for the low levels of HDL cholesterol in hypertriglyceridemic humans. The inverse relationship between the concentrations of plasma TG and HDL cholesterol has been well established, and decreased levels of HDL cholesterol in humans appear to be associated predominantly with decreases in the HDL_2 subfraction; HDL_2 are larger and more CE rich than HDL_3. Hypertriglyceridemic humans show a deficiency of HDL_2 particles, and the mean particle size of HDL_3 reportedly decreases as the level of plasma TG increases (23). To identify the mechanism for these abnormal HDL, Hopkins et al (54, 55) demonstrated that when HDL_3 was incubated with TG-rich emulsions or lipoproteins in the presence of LTP, it was converted to larger, TG-rich particles. However, subsequent hydrolysis of the HDL-TG by hepatic or lipoprotein lipase resulted in the formation of very small HDL particles, which were similar in size and composition to those in the plasma of hypertriglyceridemic humans. Similarly, Deckelbaum et al (26a) demonstrated retroconversion of HDL_2 to HDL_3 upon incubation of HDL_2, VLDL, and LTA ($d>1.21$) and subsequent hydrolysis of HDL TG; the resultant HLD_3-like particles were depleted of CE, and the VLDL were reciprocally enriched. These in vitro studies provide evidence suggesting that LTA is likely to deplete HDL of CE in hypertriglyceridemic humans and, in doing so, to decrease the particle size of HDL. Whether the decreased particle size of HDL is associated causally with an accelerated clearance of HDL protein has not been determined.

Conversely, a lack of LTA appears to be responsible for the grossly enlarged, CE-rich HDL_2 particles in the plasma of abetalipoproteinemic humans, who are deficient in apo B–containing particles. Deckelbaum et al (26) demonstrated that abetalipoproteinemic HDL could be almost completely

normalized with respect to size and composition when plasma was supplemented with VLDL followed by lipoprotein lipase.

These in vitro studies of HDL, LTA, and acceptor lipoproteins demonstrate the likely role of core lipid exchange in intravascular mebalolism of CE and illustrate the importance of TG-rich acceptor particles in the exchange process. It is suggested that although hypertriglyceridemia is not often recognized as a significant risk factor in coronary atherosclerosis, an expanded plasma TG pool creates a "sink effect" by which LTA can deplete HDL of CE and reciprocally enrich the potentially atherogenic, lower-density lipoproteins with this lipid component.

Lipid Transfer from Extravascular Structures

Stein et al (90) reported that the efflux of CE and cholesteryl ether from bovine aortic smooth muscle cells, previously exposed to these labeled lipids, was increased 3–10-fold when bovine plasma albumin was replaced by lipoprotein-free human serum. No increase in efflux was observed when rat serum, which does not contain LTA, was used in the incubation medium. The increased efflux of CE by LTA, was probably CE localized in the extracellular matrix. When cells were made permeable with saponin, the LTA-induced efflux was increased, probably because intracellular CE then became available for efflux (89).

The LTP-induced uptake of CE from HDL by HepG2 cells has also been reported (41). A somewhat smaller effect on uptake of CE was observed with rabbit aortic smooth muscle cells and with human skin fibroblasts. The uptake of CE by porcine aortic endothelial cells and J774 macrophages was not stimulated by LTA (41). Morton (68) confirmed the inability of LTA to stimulate the uptake of lipoprotein CE by J774 cells but demonstrated an accelerated efflux of labeled CE as well as a net efflux of CE. Suprisingly, however, the transfer activity–induced efflux of CE was not dependent upon acceptor particles in the media.

REGULATION OF LTP ACTIVITY

Regulation of LTA: Rabbit d > *1.21*

The specific mechanisms for the regulation of plasma LTP activity have yet to be determined. However, studies with rabbits indicate that in this species, there appears to be an inseparable relationship between the level of plasma cholesterol and the LTP activity of lipoprotein-deficient plasma. Son & Zilversmit (87) first reported that hypercholesterolemia was associated with marked increases (two- to threefold) in the CE and TG transfer activities in Watanabe heritable hyperlipidemic (WHHL) rabbits and in New Zealand White rabbits that were made hypercholesterolemic by means of a high-cholesterol diet (0.5%, wt/wt) or a cholesterol-free, casein-sucrose diet. More

recently we reported (80) that a low-cholesterol diet and a cholesterol-free, high-coconut-oil diet were associated with moderate and comparable increases (~50%) in plasma cholesterol and CE and TG transfer activities. For a variety of hyperlipidemic diets, LTA increased to new steady-state levels within 2 weeks, with little additional change for up to 3 months, even when plasma cholesterol levels continued to rise (80). The increased plasma LTA level probably reflects increased LTP mass, as Quinet et al (82) have recently reported similar magnitudes of change for plasma LTP mass and hepatic LTP mRNA in cholesterol-fed rabbits. Collectively, these studies indicate that moderate and gross hypercholesterolemias in the rabbit are associated with increases in the CE and TG transfer activities of lipoprotein-deficient plasma. The increased LTA level may be related to the development of atherosclerosis in these rabbit models (87).

Conversely, the plasma LTA level decreases with reductions in plasma cholesterol levels. In pregnant New Zealand White and WHHL rabbits, the marked progressive hypocholesterolemia during gestation was associated with parallel decreases in CE and TG transfer activities; at term, plasma cholesterol and LTA levels were decreased by about 80% for both strains of rabbits (78). Similarly, the postpartum increases in the plasma cholesterol level were paralleled by an increased LTA level. We have also reported (80) concomitant decreases in plasma cholesterol and LTA levels in rabbits fed a 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) inhibitor (Lovastatin ®) for 7–14 days; similar relative decreases for the two variables were exhibited in rabbits fed chow or chow plus coconut oil. These longitudinal studies provide further evidence that, in rabbits, plasma cholesterol and LTA levels change in unison, whether the increase or decrease in cholesterol levels is affected as a result of genetic aberration, endocrine status, or dietary or pharmacologic manipulation.

What is the mechanism for the close association between plasma cholesterol and LTA levels? We will examine the experimental evidence that appears to discredit one hypothesis and then consider alternatives. In rabbits the liver appears to be the principal source of LTP (72, 82); plasma LTP, LTA, and hepatic cholesteryl ester transfer protein mRNA levels are increased in association with hypercholesterolemia in this species. Hypercholesterolemias in rabbits are also associated with enhanced hepatic secretion of lipoprotein cholesterol; this has been determined for the three models of diet-induced hypercholesterolemia that have been used in our studies of LTA (81). Therefore, we hypothesized that the close association between plasma cholesterol and LTA levels in rabbits was caused by a concerted hepatic secretion of lipoprotein cholesterol and LTA (81). To test this hypothesis, we fed rabbits coconut oil (14%, wt/wt) and/or Lovastatin to alter plasma cholesterol and LTA levels, and we measured the rate of secretion of lipoprotein cholesterol into plasma in rabbits that had fasted for 18 h (81). Coconut oil feeding

increased the plasma cholesterol level by 68%, the LTA level by 42%, and hepatic cholesterol (unesterified) secretion by 69%. Lovastatin® (75 mg/day) for 14 days lowered the plasma cholesterol level 40–50% and the LTA level 40–60% in chow-fed and coconut oil–fed rabbits but did so without affecting the rates of secretion of cholesterol into plasma. Nonetheless, additional evidence appears to discredit the hypothesis. In pregnant rabbits, plasma cholesterol and LTA levels are also markedly lowered, despite a normal to slightly increased rate of secretion of cholesterol into plasma (101). Thus, the close association between plasma cholesterol and LTA levels appears not to be caused solely by a concerted hepatic secretion of cholesterol and LTA. Alternatively, since LTA is found in plasma only in association with lipoproteins (65, 94), we have proposed (78) that plasma LTA is regulated not only by a balance between its secretion and removal from plasma, but also by the availability of lipoprotein particles to which LTP can bind. It has also been proposed that cholesterol enrichment of extrahepatic cells, such as peripheral macrophages, may accelerate the secretion of LTA by these cells (81), and some in vitro evidence seems to support this idea (35).

Regulation of LTP Activity: Dyslipoproteinemias

LIPOPROTEIN-DEFICIENT PLASMA Increased LTA levels may exacerbate the atherogenicity of hypercholesterolemias in humans by enhancing the transfer of CE from HDL to atherogenic lipoproteins. Several investigators have studied plasma CE transfer activity in a variety of dyslipoproteinemias. However, most probably owing to differences in methodologies, the results of these studies appear equivocal. Most controversial are studies to estimate the rates of CE transfer in whole plasma in vitro. Least controversial are the studies in which the CE transfer capacity of lipoprotein-deficient plasma in the absence of endogenous lipoproteins was determined. Consistent with the close association between the levels of plasma cholesterol and LTA in the rabbit, the LTA level in lipoprotein-deficient plasma in humans appears to change in parallel with plasma cholesterol.

The LTA level of lipoprotein-deficient plasma is increased in some hyperlipidemias in humans. Groener et al (46) first reported that the mean CE transfer activity of delipidated plasma in a group of hyperlipidemic humans was approximately double compared to normolipidemic controls. The hyperlipidemic subjects were not characterized with respect to specific hyperlipoproteinemias, but the group mean values of plasma cholesterol and triglycerides in these individuals were approximately 3–10 times higher, respectively, than those in normolipidemic controls. Plasma CE transfer activity was highly correlated with plasma total and VLDL plus LDL cholesterol levels but was not correlated with plasma TG levels. Groener et al (43) also observed an association between the transfer activity of delipidated plasma and VLDL plus LDL cholesterol levels in a longitudinal study of five hyperlipidemic subjects.

Subjects were fed hypocaloric, high- or low-fat diets for 1 to 2 weeks, and marked changes in VLDL plus LDL cholesterol levels were paralleled by similar changes in CE transfer activity. Transfer activity was unaltered in subjects in whom VLDL plus LDL cholesterol levels were not affected by the diets. Hence, it has been demonstrated that the transfer activity of lipoprotein-deficient plasma can be altered by diet and by hyperlipidemia in humans.

The CE transfer activity of lipoprotein-deficient plasma also appears to be increased in association with hyperlipidemia in some insulin-dependent diabetics (29) and in patients with dysbetalipoproteinemias (93). For the diabetics ($n = 25$), transfer activity ($d > 1.21$) was correlated with the levels of cholesterol and apolipoprotein B in LDL and VLDL, the level of glycosylated hemoglobin, and micro- and macrovascular complications (29). For patients with dysbetalipoproteinemia ($n = 4$), who are characterized by the apolipoprotein E-2/E-2 phenotype and an accumulation of CE-rich remnants, the transfer activity of $d > 1.21$ plasma was twice that for normolipidemic controls. The authors demonstrated that the difference in transfer activity between the two groups was not caused by the presence or absence of LTP "activators" or inhibitors (93).

In contrast to increased LTA levels in hyperlipidemic humans, a decreased LTA level ($d > 1.21$) has been found in markedly hypocholesterolemic, abetalipoproteinemic patients. Pappu & Illingworth (75) reported that the CE and TG transfer activities of $d > 1.21$ plasma were 50 and 60% lower, respectively, for two abetalipoproteinemic humans than for 12 normolipoproteinemic controls. Interestingly, addition of the $d > 1.21$ plasma from the abetalipoproteinemics to that of controls resulted in a dose-dependent reduction in both CE and TG transfer activities. Hence, this rare genetic disorder, which is characterized by an absence of acceptor lipoproteins, may be associated with an inhibitor of LTP activity that appears to be trypsin sensitive (75). Inhibitors of LTP activity are discussed further below. Frohlich et al (39) concluded that CE transfer activity ($d > 1.21$) was 30% lower than normal for a group of LCAT-deficient kindred who exhibited hypoalphalipoproteinemia, but plasma total cholesterol levels for 8 of the 10 kindred were similar to those of normolipidemic controls. Essentially normal levels of $d > 1.21$ LTA have also been reported for hypoalphalipoproteinemic but normocholesterolemic patients afflicted with Tangier disease (29, 52). Collectively, these data indicate that the LTA of lipoprotein-deficient plasma is abnormally low in hypocholesterolemic humans but not necessarily in hypoalphalipoproteinemics who are otherwise normocholesterolemic. As in rabbits, the LTA capacity of lipoprotein-deficient plasma in humans is closely related to plasma cholesterol levels.

WHOLE PLASMA Whether the changes in the LTA capacity of lipoprotein-deficient plasma in humans reflect changes in the rate of transfer of CE from

HDL to potentially atherogenic lipoproteins in vivo is not known. In contrast to other studies (88, 93), Fielding et al (38) reported that the whole plasma from 5 dysbetalipoproteinemics (apolipoprotein E-2/E-2 phenotype), compared with 10 normolipoproteinemic controls, exhibited a negligible rate or even a reversal in the transfer of CE between HDL and VLDL plus LDL. As regards other hyperlipidemias, net CE mass transfer from HDL to VLDL plus LDL also appeared to be lower than that in controls for 6 hyperbetalipoproteinemic subjects, who did not display the apolipoprotein E-2/E-2 phenotype. Hypertriglyceridemic subjects with documented cardiovascular disease also appeared to exhibit low CE transfer, but those without cardiovascular disease appeared to transfer CE from HDL to apolipoprotein B–containing lipoproteins at a rate similar to that for controls. In contrast to the other study (93), the incubations of whole plasma were much shorter (60 min) and the changes in HDL CE levels were reported to be linear with time. For the three types of hyperlipidemias that were associated with decreased CE mass transfer, normal rates of transfer were observed when endogenous acceptor VLDL plus LDL were replaced with VLDL plus LDL from control subjects. The authors concluded that CE transfer from HDL to other lipoproteins is abnormally low in plasma from humans with dysbetalipoproteinemia, hyperbetalipoproteinemia, and a specific hypertriglyceridemia, as a result of the presence of VLDL plus LDL that are abnormally poor acceptors of LCAT-derived CE (38). In a subsequent study, Fielding et al (37) also observed lower rates of CE transfer in non-insulin-dependent human diabetics and concluded that the transfer defect was associated with an increased unesterified cholesterol content in the acceptor lipoproteins (VLDL and LDL).

Measuring the rate of transfer of [^{3}H]CE from HDL to lower-density lipoproteins in plasma obtained from dysbetalipoproteinemics, Tall et al (93) and Sparks et al (88) concluded that these patients had 30–100% higher LTA levels than normolipidemic controls did. It should be noted that the labeled HDL in these experiments was obtained from normolipidemic donors. Whether [^{3}H]CE equilibrates with and therefore truly represents that of the endogenous HDL of dysbetalipoproteinemics was not reported. Tall et al (93) also reported greater net mass transfer of CE from HDL to $d < 1.063$ lipoproteins for the dysbetalipoproteinemics. However, the plasma was incubated for 6 h, and HDL cholesterol was measured only at two time points; most likely an initial rate might reflect that in vivo more closely.

All of the CE mass transfer assays discussed above were performed in the presence of an LCAT inhibitor. The reasons the results of the assays are so conflicting are unclear, but the discrepancies may be related to the different incubation times used to determine the transfer rate. An initial rate would probably be more representative of the rate in vivo, but even an early rate in vitro does not include the potential influence of newly secreted lipoprotein

particles present in vivo. Further research is needed to determine whether changes in the LTA of $d > 1.21$ plasma are paralleled by similar changes in the rate of CE transfer in vivo.

Tissue Sources

To identify sites of synthesis and secretion of LTP, low levels of LTP-like activity were first detected in the concentrated perfusates of rabbit livers (4, 27). Similar to plasma LTA, the transfer activity in the liver perfusates mediated the net transfer of CE from HDL to VLDL and LDL (27), and the partially purified LTP transferred CE, TG, and phospholipids (4). By using human cell lines, Albers and co-workers subsequently demonstrated that monocyte-derived macrophages and HepG2 and Hep3B cells synthesize and secrete a LTP that closely resembles the LTP derived from human plasma (32, 96). Studies of plasma LTA and hypercholesterolemia in the rabbit performed in our laboratory indicate that diet-induced increases in LTA levels are delayed by about 3–5 days in comparison with immediate, diet-induced increases in plasma cholesterol levels. Extending those observations, Quinet et al (82) have also reported increases in plasma LTP mass and hepatic LTP mRNA levels in rabbits that had been fed a high-cholesterol diet for 3 days. As a possible mechanism for this delayed response in LTA, we suggested that accumulation of cholesterol or its esters in hepatocytes and/or hepatic and peripheral macrophages may be a prerequisite for accelerated secretion of LTP by these cells (80). Albers and co-workers have recently provided support for this hypothesis, at least for macrophages. Increased amounts of CE transfer activity were recovered in the media of human monocyte-derived macrophages that were enriched with CE by means of exposure to acetylated LDL, free cholesterol, or phorbol 12-myristate 13-acetate, and secretion of CE transfer activity was correlated with cellular CE mass (35). Albers and associates have also demonstrated secretion of LTP by CaCo-2 cells that were cultured on permeable membranes (33). Transfer activity was detected only in the lower culture compartment, which suggests vectorial secretion from the basolateral cellular domain (33). The CaCo-2 enterocytes secreted transfer activity at a rate about twice that of HepG2 cells, and secretion of CE transfer activity by CaCo-2 cells, but not by HepG2 cells, was increased when culture media were supplemented with fatty acids. From these observations with cultured carcinoma cells, the authors speculated that the intestine may be the principal source of human plasma LTP (33).

In contrast to humans, the intestines of rabbits are not likely to be the principal source of LTP. Preliminary evidence with humans suggests that plasma $d > 1.21$ LTA increases after a single high-fat and cholesterol meal, but in rabbits, $d > 1.21$ LTA is unaffected by one high-fat and cholesterol feeding (80). In addition, LTA was not detected in concentrated $d > 1.21$

subnatant of chylous lymph that was collected from a rabbit during a 24-h duodenal infusion of a TG-rich emulsion (Quig & Zilversmit, unpublished observations). Consistent with this apparent difference between the two species, LTP mRNA in rabbits is found mainly in the liver, occurs at low levels in the adrenals and kidneys, but is below the level of detection in the intestine (72, 82). In humans, LTP mRNA has been detected in the intestine, liver adrenals, and spleen (28). Thus, in contrast to humans, the rabbit liver is likely to be the principal regulatory site for plasma LTP activity (82).

Alimentary Hyperlipidemia

Castro & Fielding (21) observed a near doubling of the net transfer of CE during postprandial lipemia (an increase that indicates an increase in CE synthesis). Tall et al (94) reported that feeding 135 g of TG in the form of heavy cream to human subjects tripled the unidirectional transfer of CE from HDL to lower-density fractions. A number of factors appeared to be responsible: the binding of LTP to phospholipid-enriched and enlarged HDL, the increased amount of TG-rich acceptor lipoproteins, and, to a lesser extent, a possible increase in LTP mass. Feeding a high-fat meal to normal rabbits did not increase LTA levels when assayed in the $d > 1.21$ fraction with standard substrates (80). The increment of LTP in human plasma after a TG-rich meal may not be surprising, because the human intestine is among the several human tissues that contain mRNA for LTP (28). In contrast, no LTP mRNA was detected in the rabbit intestine (72), which might explain why $d > 1.21$ LTA in rabbits did not increase after a high-fat meal (80).

Lipid Transfer Protein Inhibitor

Barter et al reported that little or no LTA was present in rat (6) or pig (8) plasma. To clarify whether this was due to a lack of transfer activity or to the presence of an inhibitor, we observed that the addition of lipoprotein-deficient rat or pig plasma to partially purified rabbit LTP decreased the transfer activity of the LTP (69). More surprisingly, the addition of human lipoprotein-deficient plasma to partially purified human LTP also reduced the transfer activity of both CE and TG. Although the inhibitions of CE and TG transfer were quite similar, the degree of inhibition for both lipids depended greatly on the acceptor and donor lipoproteins in the assay. The inhibitor was most effective on the bidirectional transfer between VLDL and LDL and least effective in suppressing the transfer of TG and CE from VLDL to HDL. When the total cholesterol contents of VLDL and HDL were the same, the transfer of both lipids from VLDL to HDL was diminished only about 6%, whereas that in the opposite direction was decreased by about 30%. Thus, under these conditions, the inhibitor also appears to affect the net transfer of lipid species between lipoproteins.

In a subsequent study (86), we showed that the activity of the inhibitor was independent of the amount of LTP in the assay system but that the inhibitory effect was substantially decreased after the LDL and HDL concentrations in the assay had been increased. This observation, coupled with the finding that the inhibitor did not suppress the transfer of CE between unilamellar phospholipid vesicles, indicates that the inhibitor does not interact directly with the LTP. Instead, the inhibitor appears to exert its effect on one or both of the lipoprotein substrates (65, 69, 86).

An apparently different type of LTP inhibitor has been investigated by Tollefson et al (97). When the inhibitor was removed by phenyl-Sepharose chromatography, not only did the LTA of human lipoprotein-deficient plasma increase, but also, significant transfer activity was observed in plasma from rats and pigs. The inhibitor was shown to inhibit both CE and TG transfer activity, as well as the activity of a second protein that stimulates the transfer of phospholipids (LTP-II) (73). Removal of the inhibitor by immunoaffinity chromatography again enhanced the LTA of human, rat, and pig lipoprotein-deficient plasma, even though the inhibitor was concluded to be present in a subfraction of HDL that constitutes only about 1% of the HDL mass. Faust et al (34) have reported that HepG2 cells in culture secrete an LTP inhibitor protein that is associated with apolipoprotein E–containing lipoprotein particles, but this protein has yet to be characterized.

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