Conversion of Human Low Density Lipoprotein into a Very Low Density Lipoprotein-like Particle in Vitro*

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T. K. Amarendra Singh‡, Douglas G. Scraba, and Robert O. Ryan‡‡

From the Department of Biochemistry and °Lipid and Lipoprotein Research Group, University of Alberta, Edmonton, Alberta, Canada T6G 2S2.

The lipid substrate specificity of Manduca sexta lipid transfer particle (LTP) was examined in in vitro lipid transfer assays employing high density lipophorin and human low density lipoprotein (LDL) as donor/acceptor substrates. Unesterified cholesterol was found to exchange spontaneously between these substrate lipoproteins, and the extent of transfer/exchange was not affected by LTP. By contrast, transfer of labeled phosphatidylcholine and cholesterol ester was dependent on LTP in a concentration-dependent manner. Facilitated phosphatidylcholine transfer occurred at a faster rate than facilitated cholesterol ester transfer; this observation suggests that either LTP may have an inherent preference for polar lipids or the accessibility of specific lipids in the donor substrate particle influences their rate of transfer. The capacity of LDL to accept exogenous lipid from lipophorin was investigated by increasing the high density lipophorin:LDL ratio in transfer assays. At a 3:1 (protein) ratio in the presence of LTP, LDL became turbid (and aggregated LDL were observed by electron microscopy) indicating LDL has a finite capacity to accept exogenous lipid while maintaining an overall stable structure. When either isolated human very low density lipoprotein (VLDL) apoproteins or insect apolipophorin III (apoLp-III) were included in transfer experiments, the sample did not become turbid although lipid transfer proceeded to the same extent as in the absence of added apolipoprotein. The reduction in sample turbidity caused by exogenous apolipoprotein occurred in a concentration-dependent manner, suggesting that these proteins associate with the surface of LDL and stabilize the increment of lipid/water interface created by LTP-mediated net lipid transfer. The association of apolipoprotein with the surface of modified LDL was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis, and scanning densitometry revealed that apoLp-III bound to the surface of LDL in a 1:14 apoB:apoLp-III molar ratio. Electron microscopy showed that apoLp-III-stabilized modified LDL particles have a larger diameter (29.2 ± 2.6 nm) than that of control LDL (22.7 ± 1.9 nm), consistent with the observed changes in particle density, lipid, and apolipoprotein content. Thus LTP-catalyzed vectorial lipid transfer can be used to introduce significant modifications into isolated LDL particles and provides a novel mechanism whereby VLDL-LDL interrelationships can be studied.

In human plasma, low density lipoprotein (LDL) represents a stable catabolic product of very low density lipoprotein (VLDL) metabolism. Nascent VLDLs are assembled in the liver and secreted as triacylglycerol-rich particles that are rapidly metabolized by lipoprotein lipase and cholesteryl ester transfer protein to cholesterol ester (CE)-rich particles containing apolipoprotein (apo) B as their sole protein component. In studies of the reversibility of this process Deckelbaum et al. (1) produced “modified LDL” by incubation of lipoprotein poor plasma and VLDL with LDL. These particles possess an increased triacylglycerol content, a decreased CE content, and acquired apolipoproteins A, B, and C. In human plasma, however, VLDL catabolism to LDL is not reversible. This is likely due to the influence of lipoprotein lipase and cholesteryl ester transfer protein on the lipid content of VLDL, which together cause a depletion of triacylglycerol and a relative increase in CE (2). The net effect is a loss of low molecular weight surface apolipoproteins producing an apoB-containing CE-rich particle which has been correlated with the incidence of cardiovascular disease (3).

In certain insect species which are capable of long range flight, a metabolic conversion occurs in which the hemolymph lipoprotein, high density lipophorin (HDLp), is converted to a low density lipophorin (LDLp) (4, 5). This interconversion results from the specific uptake of diacylglycerol (DAG, the transport form of neutral lipid in insects) by HDLp, and is accompanied by reversible association of up to 16 molecules of the low molecular weight apolipoprotein, apoLp-III (6). It has been demonstrated that transfer of cell-derived neutral lipid from membrane to lipophorin is facilitated by a hemolymph lipid transfer particle (LTP) (7). LTP is a high molecular weight, very high density lipoprotein that possesses structural (8–10) and catalytic (11–13) properties which distinguish it from mammalian plasma transfer proteins (2, 14). For example LTP possesses three distinct protein components together with 14% by weight lipid, which combine to yield a highly asymmetric M1, ~ 1,000,000 holoparticle. LTP has been shown to induce a dramatic transformation of human high density lipoprotein into larger, apoprotein A-I-poor, particles (13). In other studies we observed LTP catalyzes a bidirec-

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§§ Lipid and Lipoprotein Research Group, University of Alberta, Edmonton, Alberta, Canada T6G 2S2.

The abbreviations used are: LDL, low density lipoprotein; VLDL, very low density lipoprotein; DAG, diacylglycerol; apo, apolipoprotein; apoLp, apolipophorin; HDLp, high density lipophorin; CE, cholesteryl ester; PC, phosphatidylcholine; LDLp, low density lipophorin; LTP, lipid transfer particle; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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tional vectorial transfer of DAG from lipophorin to human LDL (12). This reaction results in an altered distribution of DAG between lipophorin and LDL but is not accompanied by apolipoprotein exchange or transfer. Lipophorin particles are depleted of neutral glycerolipid, while LDL particles are enriched. In the present study we have extended this work in an effort to determine the capacity of LDL particles to accept lipophorin-derived lipid as well as the lipid specificity of LTP-mediated transfers between this donor/acceptor pair. The results provide evidence that the lipid content of LDL can be significantly increased and reveal a potentially useful method whereby VLDL-like particles can be produced from LDL in vitro.

MATERIALS AND METHODS

Insects—Manduca sexta were obtained from a continuing laboratory colony reared on a wheat germ-based diet as described by Prasad et al. (15).

Isolation of LTP, LDL, ApoLP-III, and Lipophorins—LTP was isolated from the hemolymph of M. sexta as previously described (9). LDL was isolated from fresh human plasma by sequential density gradient ultracentrifugation between the density limits 1.006 g/ml and 1.063 g/ml and stored at 4 °C under an argon atmosphere. Adult high density lipophorin (HDL-A) was isolated from M. sexta hemolymph by the method of Ryan et al. (16). High density lipophorin-wanderer 1 (HDL-W1) was prepared from prepupal hemolymph as described by Prasad et al. (15). ApoLP-III was prepared from LDLp according to the procedure of Wells et al. (17). Non-B VLDL apoproteins were isolated from the density <1,006 g/ml fraction of human plasma by lipid extraction of lyophilized LDL and resolubilization of the apolipoproteins using the protocol designed for apoLP-III (17).

Preparation of Labeled Lipophorins—[3H]Cholesterol-labeled HDLP-A and [3H]cholesterol ester-labeled HDLP-A were prepared in vitro. Two μCi of [1,2-3H]cholesterol (Amersham Corp., 43.6 Ci/mmol) and 5 μCi of [1,2,3-3H]cholesterol oleate (Amersham, 49.6 Ci/mmol) were separately incubated with HDLP-A (5 mg of protein) in the presence of 10 μg of LTP at 33 °C for 60 min. After incubation the samples were subjected to density gradient ultracentrifugation and the isolated HDLP-A dialyzed against phosphate-buffered saline. The specific activities of [3H]cholesterol-HDLp-A and [3H]cholesterol ester-HDLp-A were 378,000 cpm/mg protein and 356,000 cpm/mg protein, respectively. To obtain [3H]PC HDLp-A, 100 pCi of [3H]PC hydrochloride (Amersham Corp., 378,000 cpm/mg protein) was incubated with HDLP-A (5 mg of protein) for 60 min at 33 °C. After incubation, the samples were subjected to density gradient ultracentrifugation and protein and radioactivity in each of the isolated lipoprotein fractions were determined by refractometry. In some experiments labeled lipoproteins were separated by gel permeation chromatography on a 1.5 X 175-cm column of Sephacryl S200 in phosphate-buffered saline at 8 ml/h to allow for collection of 2-ml fractions.

Electron Microscopy—Electron microscopy was performed in a Philips EM420 as previously described (9). Samples (20 μg of protein/ml) were negatively stained with 2% sodium phosphotungstate (pH 7.0). Samples were photographed at a magnification (calibrated) of 74,400. The average diameters of the lipoprotein particles were then measured on 3X enlarged photographic units.

Analytical Procedures—Protein concentrations were determined according to Smith et al. (19). SDS-PAGE (20) was performed in 4-15% acrylamide gradient slab gels containing a 2.5% stacking gel. After electrophoresis at a constant 30 mA the proteins were visualized by staining with Coomassie Brilliant Blue. Lipid analyses were performed using enzyme based commercial kits for choline-containing phospholipids, unesterified cholesterol, total cholesterol, and neutral glycerolipid (Boehringer Mannheim). ApoLP-III was quantitated by staining with Coomassie Brilliant Blue. Lipid analyses were performed using enzyme based commercial kits for choline-containing phospholipids, unesterified cholesterol, total cholesterol, and neutral glycerolipid (Boehringer Mannheim).

RESULTS

Lipid Specificity of LTP-mediated Transfer/Exchange—The relative ability of LTP to facilitate transfer of different radiolabeled lipids from HDLP-A to LDL was studied in in vitro transfer assays. Lipid-labeled HDLP-A was incubated with unlabeled LDL in the presence and absence of LTP, after which the substrate lipoproteins were resolubilized by density gradient ultracentrifugation and the distribution of radioactivity and protein determined. Under the conditions employed LDL serves as lipid acceptor and apoprotein exchange, or transfer among the lipoprotein substrates does not occur (12, 21). When [3H]cholesterol-labeled HDLP-A was employed as substrate no differences were observed between control and LTP-containing assays (Table 1). In all cases up to 50% of labeled cholesterol was recovered in LDL indicating that unesterified cholesterol can spontaneously transfer between these substrate lipoproteins. By contrast, when HDLP-A containing [3H]CE was incubated for 60 min in the absence of LTP, less than 5% of the labeled lipids were recovered in LDL. LTP, however, induced a concentration-dependent increase in the amount of radioactive CE recovered in LDL.

It has been shown previously that after incubation with LDL and LTP, lipophorin retains its complement of phospholipid despite a net loss of neutral lipid (12, 21). To determine whether this results from a lack of facilitated phospholipid transfer or arises from facilitated phospholipid exchange, transfer assays were conducted using [3H]PC-labeled HDLP-

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<th>Table 1</th>
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**Effect of LTP on lipid transfer from HDLP-A to LDL**

<table>
<thead>
<tr>
<th>Labeled substrate</th>
<th>LTP specific activity*</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>LTP (3 μg)</td>
</tr>
<tr>
<td>HDLP-A (0.25 mg of protein)</td>
<td></td>
</tr>
<tr>
<td>[3H]PC-HDLp-A</td>
<td>25.0 ± 2.0</td>
</tr>
<tr>
<td>[3H]CE-HDLp-A</td>
<td>2.8 ± 0.8</td>
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<tr>
<td>[3H]PC-HDLp-A</td>
<td>3.7 ± 0.9</td>
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* HDLP-A (0.25 mg of protein) prelabeled separately with [3H] cholesterol, [3H]PC, or [3H]CE (80,000 cpm/mg protein) was incubated with 0.5 mg of LDL protein in the presence and absence of LTP for 60 min at 33 °C. After incubation, LDL was resolubilized by density gradient ultracentrifugation and the distribution of radioactivity and protein determined. Under the conditions employed LDL serves as lipid acceptor and apoprotein exchange, or transfer among the lipoprotein substrates does not occur (12, 21). When [3H]cholesterol-labeled HDLP-A was employed as substrate no differences were observed between control and LTP-containing assays (Table 1). In all cases up to 50% of labeled cholesterol was recovered in LDL indicating that unesterified cholesterol can spontaneously transfer between these substrate lipoproteins. By contrast, when HDLP-A containing [3H]CE was incubated for 60 min in the absence of LTP, less than 5% of the labeled lipids were recovered in LDL. LTP, however, induced a concentration-dependent increase in the amount of radioactive CE recovered in LDL.

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In other experiments using unlabeled lipoproteins the HDLP-LDL protein ratio was increased to 1:1, 2:1, 3:1, and 10:1. Incubations were conducted at 33 °C for 60 min in the presence or absence of LTP (20 μg). Where stated a specified amount of apoLP-III or non-B VLDL apolipoproteins were added. After incubation the density of the samples was adjusted with KBr (6.68 g of KBr/20 ml final volume), transferred to 39-ml Quick-Seal tubes, overlaid with 0.9% saline, and centrifuged in a VTI 50 rotor at 50,000 rpm for 4 h at 4 °C. After centrifugation the tube contents were fractionated and protein and/or lipid analyses conducted. The relative densities of density gradient fractions were determined by refractometry. In some experiments lipoproteins were separated by gel permeation chromatography on a 1.5 X 175-cm column of Sephacryl S200 in phosphate-buffered saline at 8 ml/h to allow for collection of 2-ml fractions.

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A. The results (Table I) show that $[^3]$HPC underwent LTP-mediated transfer from HDLp-A to LDL and was transferred at a faster rate than CE. The mass of choline-containing phospholipid associated with LDL and HDLp-A before and after lipid transfer, however, was largely unchanged. This corresponded to a decrease in the specific activity of HDLp-A-associated choline-containing phospholipids from 200 cpm/µg in control incubations to 78 cpm/µg in LTP-containing incubations (60-min incubation). Taken together with the observed increase in LDL choline-containing phospholipid specific activity from 3 to 16 cpm/µg, the results suggest LTP mediates exchange of substrate particle phospholipid rather than net transfer.

Capacity of LDL to Serve as a Lipid Acceptor—The experiments described above employed an HDLp-A:LDL protein ratio of 1:2; and in such mixtures LDL was capable of accepting all of the lipophorin-associated neutral lipid while retaining a stable particle structure. The capacity of LDL to accept exogenous neutral lipid from lipophorin was measured by increasing the lipophorin:LDL ratio. The lipophorin subspecies HDLp-W1 was chosen for these studies because it has a relatively high DAG content, yet lacks apoLp-III. This apolipoprotein, unlike apoLp-I, apoLp-II, and apoB, is water-soluble and may itself exchange/transfer between the substrate lipoproteins as lipid transfer proceeds. Increasing the ratio of HDLp-W1 to LDL has the effect of increasing the total pool of HDLp-W1 lipid available for transfer to LDL. Based on the compositions of LDL (22) and HDLp-W1 (15), a 3:1 HDLp-W1:LDL protein ratio corresponds to an HDLp-W1:LDL core lipid ratio of 1:2. Thus, if LDL accepted all of the DAG from lipophorin, its core lipid content would increase by 50%.

No observable changes in LDL particle density or stability occurred in incubations without LTP. In the presence of LTP, however, as the protein ratio of HDLp-W1:LDL was increased there was a corresponding increase in the amount of glycerolipid transferred from HDLp-W1 to LDL (Fig. 1). At HDLp-W1:LDL protein ratios of 1:1 or 2:1 LTP-induced lipid transfer resulted in a decrease in LDL density with apparent retention of a stable LDL particle structure. At a 3:1 HDLp-W1:LDL protein ratio, however, sample turbidity developed with time, suggesting that LTP-mediated net transfer of lipid into LDL may have decreased its stability. This interpretation was supported by analysis of the density distribution of protein and CE from control and LTP containing incubations (3:1 HDLp-W1:LDL protein ratio; Fig. 2) which revealed that the density decrease in LDL induced by facilitated redistribution of glycerolipid from lipophorin to LDL occurred in the absence of significant change in the relative distribution of cholesteryl ester and protein. SDS-PAGE analysis confirmed that apolipoprotein exchange or transfer between the substrate lipoproteins did not occur. Examination of the modified LDL by electron microscopy provided evidence of particle aggregation (Fig. 6C), suggesting that LDL particles have a finite capacity to accept exogenous HDLp-W1-derived neutral lipid and above a specific threshold become destabilized.

Apolipoprotein Stabilization of Modified LDL—The hypothesis that the development of turbidity in lipid-enriched LDL is due to creation of lipid/water interface which results in particle aggregation was examined by adding isolated apolipoproteins to transfer assays. In preliminary experiments, when either apoLp-III or non-B VLDL apoproteins were added to incubations of HDLp-W1, LDL, and LTP, the amount of turbidity produced was observed to be reduced. To characterize this phenomenon we determined the effect of apoLp-III concentration on the turbidity of LDL produced by incubation with HDLp-W1 and LTP. The results (Fig. 3) show that the turbidity of LDL (estimated by absorbance at 350 nm) decreased from a value of 0.45 (no apoLp-III) to approximately 0.10 to 0.50 µg of apoLp-III/mg of LDL protein. Addition of apoLp-III to incubations after development of turbidity failed to cause a decrease in absorbance at 350 nm suggesting that the alterations in LDL structure caused by LTP-mediated increases in particle lipid content are irreversible.

**Fig. 1.** Effect of HDLp-W1:LDL ratio on LTP-mediated glycerolipid transfer. LDL (1.0 mg of protein) was incubated with HDLp-W1 (1.6 mg of protein, closed squares; 2.0 mg of protein, open circles; or 3.0 mg of protein, closed circles) in the presence of 20 µg of LTP for 60 min at 33 °C. A control incubation (1:1 HDLp-W1:LDL protein ratio) was conducted in the absence of LTP (open squares). After incubation the samples were subjected to density gradient ultracentrifugation as described under “Materials and Methods” and fractionated (1.5 ml), and the density and glycerolipid (DAG + TG) content (enzyme-based colorimetric assay) of each fraction was measured.

**Fig. 2.** Effect of LTP on the density distribution of protein and cholesteryl ester between lipophorin and LDL. LDL (1 mg of protein) and HDLp-W1 (3 mg of protein) were incubated in the absence (open circles) and presence (closed circles) of LTP (20 µg) for 60 min at 33 °C. After incubation the samples were subjected to density gradient ultracentrifugation, as described under “Materials and Methods” and fractionated, and the density, protein, and cholesteryl ester content of each fraction determined.
ApoLp-I11 or apoVLDL serve as inhibitors of LTP—which contained a HDLp-W1:LDL protein ratio of 3:1 in the enriched LDL. We repeated the experiments with concentrations ranging from 0 to 1.0 mg. After incubation LDL was reisolated and its protein concentration and absorbance at 350 nm determined. LDL from the control incubation (no LTP) had an absorbance of 0.05 at 350 nm. Although the apoLp-III content of the modified LDL increased, since the protein assay method does not detect apoLp-III appreciably (25) its contribution to the LDL protein determinations was ignored.

A trivial explanation for the observed effect of exogenous apolipoprotein on the LTP-induced creation of turbid LDL is that apoLp-III or apoVLDL serve as inhibitors of LTP-mediated lipid transfer. In order to test this possibility the extent of LTP-mediated glycerolipid transfer in incubations which contained a HDLp-W1:LDL protein ratio of 3:1 in the presence and absence of added apoLp-III was determined. The results (data not shown) revealed that apoLp-III did not affect the extent of LTP-mediated glycerolipid transfer, suggesting it has stabilized the lipid-enriched LDL.

Capacity of Apolipoprotein-stabilized LDL to Accept Lipid—Experiments were conducted to determine the capacity of LDL to accept exogenous lipid in the presence of water soluble apolipoprotein. The ratio of HDLp-W1:LDL was increased to 10:1 so that lipid availability from the donor particle would not be limiting. We repeated the experiments described above and found that, whereas turbidity invariably developed in LTP-containing incubations lacking apoLp-III, in the presence of apoLp-III the sample did not become turbid. Apoprotein-stabilized modified LDLS produced in these incubations were isolated by either density gradient ultracentrifugation or Sepharose 4B gel permeation chromatography (Fig. 4). Control and modified LDL samples were then analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 5). In control incubations no apoLp-III was recovered in the LDL-containing fractions (lane 3), whereas when LTP was present apoLp-III associated with LDL (lane 4). Scanning densitometry of the stained gel and comparison with a standard curve constructed from known amounts of apoLp-III electrophoresed in parallel indicated a molar ratio of 1 apoB:14 apoLp-III in modified LDL. No evidence of apoLp-I or apoLp-II was found in the LDL-containing fractions, verifying that facilitated net lipid transfer was not accompanied by apolipoprotein transfer from lipophorin to LDL. The development of turbidity in modified LDL samples after reisolation by density gradient ultracentrifugation prompted us to compare the apoprotein composition of modified LDL isolated by density gradient ultracentrifugation versus gel filtration. The results indicated a reduction in the amount of apoLp-III relative to apoB in modified LDL isolated by density gradient ultracentrifugation versus gel filtration, consistent with the possibility that some apoLp-III dissociated from the surface of modified LDL during density gradient ultracentrifugation.

Examination of lipoprotein morphology by electron microscopy (Fig. 6) showed that LDL from control incubations were of the expected size (22.7 ± 1.9 nm diameter; n = 50) while turbid LDL produced by LTP-mediated net lipid transfer without apoLp-III (panel C) showed aggregated particles. ApoLp-III-stabilized modified LDL (10:1 HDLp-W1:LDL protein ratio) (panel B), on the other hand, were spherical entities of increased diameter (29.2 ± 2.6 nm; n = 50). This change in particle diameter corresponds to a 2.1-fold increase in particle volume and is consistent with the observed association of apoLp-III as well as lipid analyses which showed that modified LDL can accept up to 2 mg of exogenous DAG/mg of LDL protein, an amount approximately equal to its original core lipid (CE plus triacylglycerol) mass. At this HDLp-W1:LDL ratio some CE transfer from LDL to lipophorin occurred suggesting that, in the presence of an in-
DISCUSSION

The results of transfer experiments employing HDLp-A particles containing radiolabeled lipids illustrate the capability of LTP to facilitate redistribution of different lipoprotein-associated lipids. Previous studies have demonstrated that LTP can catalyze vectorial movement of DAG from lipophorin to LDL, thereby producing lipoprotein products with dramatically altered lipid compositions and lipid/protein ratios (12, 23, 24). This property of LTP is entirely consistent with its postulated physiological function in transformation of preexisting HDLp-A into a low density lipophorin, in response to adipokinetic hormone (4, 5). The lipophorin-LDL substrate pair is useful for studies of LTP activity because distinctive differences in their structural properties and compositions permit rapid quantitative separation of the particles following incubation. Previous studies have suggested that LTP is relatively nonspecific with respect to lipid substrate (11, 25). Recently, however, evidence of an apparent specificity for DAG versus long chain aliphatic hydrocarbons has been obtained (24). In the present study we have compared the rates of LTP-mediated transfer of labeled PC and CE from HDLp-A to LDL. The results indicate that, although each of the labeled lipids serve as substrate, they were transferred at different relative rates of (PC > CE). Thus LTP exhibits an apparent preference for more polar lipids. Whether this preference represents an intrinsic lipid specificity of LTP or is a reflection of the availability/location of lipid substrates in the donor particle remains an open question.

Earlier we observed that LTP did not facilitate transfer of [3H]CE from LDL to HDLp when incubated at a 1:1 protein ratio (12). By contrast, when [3H]CE-HDLp-A was employed as substrate, an LTP concentration-dependent transfer of [3H]CE from HDLp-A to LDL occurred. This result provides support for the concept that the direction of LTP-mediated lipid transfer may be significantly influenced by the properties of the donor/acceptor substrate lipoproteins. It is likely that under the assay conditions employed LDL provides a more stable hydrophobic environment for neutral core lipids since LTP-mediated lipid redistribution proceeds, the neutral lipid core of HDLp particles is significantly depleted yielding a very high density lipophorin product (12, 21). It is noteworthy that when the lipid pool size of HDLp was increased relative to LDL in transfer assays, such that LDL did not have the capacity to accept all available HDLp neutral lipid, facilitated transfer of CE mass from LDL to HDLp was observed. When taken together with previous results which suggest facilitated vectorial DAG transfer in this system occurs via a bidirectional process (12), it is plausible that LTP-mediated lipid redistribution facilitates the establishment of a new equilibrium distribution of lipid mass that is dictated by the properties of the respective donor/acceptor particles.

In spite of significant depletion of its neutral lipid content, lipophorin particles maintain the bulk of their phospholipid mass (21). When [3H]PC-labeled HDLp-A was employed in transfer assays with unlabeled LDL, significant facilitated PC transfer was observed. However, there was not a corresponding decline in choline-containing phospholipid mass associated with HDLp-A. Based on changes in the specific activity of choline-containing phospholipids associated with lipophorin and LDL we conclude that LTP-mediated redistribution of phospholipid from lipophorin to LDL represents a bidirectional exchange reaction. Whereas neutral “core” lipids prefer to be associated with LDL, apparently there is not a preferred environment for choline-containing phospholipid provided by either lipophorin or LDL. Thus, transfer of labeled PC from lipophorin is accompanied by exchange of LDL-associated phospholipid to lipophorin, maintaining the original balance of phospholipid mass while the two pools equilibrate. Unesterified cholesterol, on the other hand, was found to transfer spontaneously from lipophorin to LDL in the absence of LTP. A similar result was observed with lipophorin-associated free fatty acid in a previous study (24), suggesting that these lipids redistribute evenly via the aqueous phase or upon contact between lipoproteins in solution.

Having shown that lipophorin becomes depleted of neutral lipid by LTP-mediated transfer to LDL, we used this system to evaluate the capacity of LDL particles to accept exogenous lipophorin-associated neutral lipid. At lipophorin:LDL protein ratios up to 2:1, LDL has the capacity to accept available lipophorin lipids. At a 2:1 HDLp-W1:LDL protein ratio LTP facilitated redistribution of up to 80% of the glycerolipid pool into LDL. This corresponds to an approximate increase in lipid mass of 640 mg/mg LDL protein and, based on the lipid composition of LDL, represents an 18% increase in neutral lipid content. When the amount of lipophorin was increased to a 3:1 HDLp-W1:LDL protein ratio, sample turbidity developed in LTP-containing incubations due to LDL particle aggregation and fusion. These results suggest that somewhere between 640 and 1000 mg of exogenous lipophorin-derived lipid can be introduced into LDL particles via LTP-mediated lipid transfer with maintenance of an overall stable LDL particle structure, but that above a threshold of neutral lipid enrichment, LDL become destabilized and aggregate. The capacity of LDL to accept exogenous lipid and the stability of the lipid-enriched particle was increased by the addition of apolipoproteins. In the presence of non-B VLDL apoproteins or apoLp-III, even at a lipophorin:LDL ratio of 10:1, aggregation did not occur, and stable modified LDL of significantly increased diameter and glycolipid content were created. The exogenous apolipoprotein associated with LDL particles presumably increased their capacity to accept lipophorin-derived neutral lipid while maintaining a stable particle structure.
The apoB:apoLp-III molar apolipoprotein stoichiometry of modified LDL was 1:14.

In recent years the details of an extremely efficient lipid transport system associated with the physiology of insect flight have been elucidated. A shuttle mechanism of lipid transport exists in which lipoprotein lipid is delivered to tissues in the absence of lipoprotein internalization. Thus circulating lipophorin cycles between lipid-rich and lipid-poor states that are dictated by the activity of LTP. ApoLp-III functions in this cycle by reversibly binding to the surface of LDLp particles where it is thought to stabilize the increased lipid content of the particle (4, 5). Results presented in this paper provide evidence that apoLp-III also associates with the surface of human LDL as the neutral lipid content of the particle increases via LTP-mediated redistribution of lipophorin-associated lipid. They further suggest apoLp-III is serving to stabilize the increased lipid content of LDL in a manner analogous to that postulated for LDLp (6, 26, 27). Similar results were obtained when non-B VLDL apoproteins were substituted for apoLp-III providing evidence for a similar function for these apolipoproteins. This functional homology between insect apoLp-III and human water-soluble apolipoproteins is corroborated by x-ray crystallographic data which showed that the three-dimensional structures of apoLp-III (27) and a 22,000-Da fragment of human apoE (28) are remarkably similar.

The apparent nonspecificity of LTP-mediated lipid transfer from lipophorin to LDL suggests that if lipophorins containing the appropriate mix of neutral lipid can be prepared it should be possible to use lipophorin and LTP as tools to introduce a wide variety of lipids into LDL and perhaps other lipoproteins as well. For example it may be possible to engineer lipophorins that contain triacylglycerol or CE, rather than DAG, thereby providing a methodology whereby further defined alterations of the lipid content and composition of LDL particles can be made. Such modified particles may be useful in studies aimed at determining the role of LDL particle lipid in apoB epitope exposure or in the binding, uptake and degradation of LDL particles via receptor mediated endocytosis.

Several approaches have been used to modify LDL particles for such purposes since it is known that LDL isolated from individuals with hypertriglyceridemia (29), diabetes (30), and other dyslipoproteinemias (31, 32) possess altered lipid compositions and size when compared with normal LDL. Phospholipase A*-induced alterations in the lipid composition of LDL do not affect the overall structural integrity of the LDL particle (33), but modified particles exhibit altered immuno-reactivity and altered binding to cultured skin fibroblasts (34). Cholesterol esterase has been employed to alter LDL lipid composition (35), and this modification affects binding to receptors. Other have employed transferrin proteins to alter the lipid moiety of LDL (1, 36, 37). To date, however, attempts to modify LDL through the use of transfer proteins have been confined to the human plasma cholesteryl ester transfer protein, which facilitates a 1:1 exchange of triacylglycerol and CE between lipoprotein substrates (2, 14). We have shown that LTP-mediated net lipid transfer from donor lipophorin particles can be used to significantly alter the lipid content of LDL particles in vitro and this methodology may provide a means for the introduction of a variety of specific types and amounts of lipids into LDL.

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REFERENCES