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review

Dynamics of insect lipophorin metabolism

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Introduction and overview

During the past thirty years an enormous effort has been conducted in the field of mammalian lipoprotein biochemistry. Tremendous advances in this area have resulted in major increases in our understanding of lipoprotein structure, function, and metabolism (1, 2). In addition, a number of important enzymes and proteins that play pivotal roles in lipoprotein metabolism have been characterized. During the course of this work investigators have relied extensively upon suitable model systems including human, nonhuman primate, rabbit, and rat. Furthermore, considerable knowledge has been gained with respect to lipoprotein metabolism in other vertebrates including fish and birds (3, 4). In all these systems, in spite of considerable species variation, a number of basic unifying concepts have emerged. One such concept is a model of lipoprotein structure in which a core of hydrophobic lipid is surrounded by a monolayer of amphiphilic lipid and apolipoprotein(s) (5, 6). Packaging nonpolar materials in this way permits solubilization and efficient transport through aqueous plasma with metabolic fine tuning accomplished by specific apoproteins as well as enzymes and transfer proteins.

The transport of hydrophobic material is, however, not limited to vertebrates. All organisms that have a circulatory system possess some mechanism to transport dietary fat or mobilize stored fat. Study of the oftentimes unique lipid transport biochemistry in insects has revealed interesting and novel concepts as detailed in reviews by Chino (7) Beenakkers, Van der Horst, and Van Marrewijk (8), and Shapiro, Law, and Wells (9). The two best characterized insects with respect to lipid transport biochemistry are the locust, *Locusta migratoria* and the tobacco hornworm, *Manduca sexta.* These animals are of relatively large size, possess 0.5 to 3 ml hemolymph (i.e., blood) per animal and can be reared in mass in the laboratory. For example, *M. sexta* fifth instar larval caterpillars weigh up to 14 g and yield >3 ml plasma per animal. This fact coupled to the relatively high concentration of plasma lipoprotein (5-10 mg/ml) permits isolation of large quantities of biological material. From an agricultural standpoint the study of insect lipoproteins is of vital importance. Insect pests impact greatly upon society through destruction of crops and transmission of disease. It is known that hydrophobic pesticides or their metabolites are sequestered and transported by plasma lipoproteins, suggesting an important role in xenobiotic detoxification (10). Moreover, an understanding of insect lipid transport systems may provide insight into the evolution and function of more complex mammalian systems, and materials with unique catalytic and/or biochemical properties may be discovered that prove to be useful biochemical research tools and, therefore, beneficial to man. Recent progress in the field of insect lipoprotein metabolism has been accelerated by increased interest from mammalian lipoprotein biochemists. In an effort to build upon this momentum, the present review is intended to update the reader with respect to current knowledge about lipoprotein metabolism in insects, indicate possible directions for future research, and attempt to point out similarities and distinctions with mammalian lipoproteins.

Visual inspection reveals numerous distinctions between mammals and insects. Obvious differences that have important implications with respect to lipid transport include the occurrence of complete or incomplete metamorphosis and the presence of an open circulatory system. Another important point regards insect diversity. It is oftentimes difficult to generalize observations made in one insect species to another because insects are incredibly diverse animals that, during the course of evolution, have evolved sophisticated ways to adapt to their environment. The ubiquitous insect plasma lipoprotein, lipophorin, provides a vivid example of a biochemical system that has evolved to meet the specific and varied physiological lipid transport demands of the animal throughout its life cycle.

Lipophorin: a multifunctional lipid transport vehicle

The term lipophorin, coined from the Greek words lipos (lipid) and phoros (bearing) was introduced by Chino et al. (11) as a convenient generic term for the

Abbreviations: HDLp, high density lipophorin; HDL, high density lipoprotein; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; apoLp, apolipophorin; VLDL, very low density lipoprotein; AKH, adipokinetic hormone; DAG, diacylglycerol; LDLp, low density lipophorin.

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major lipoprotein in insect plasma. Recently, additional nomenclature has been introduced to designate specific lipophorin subspecies that differ in lipid and/or apoprotein content (12). Lipophorin has been identified in all insect species that have been examined to date and is present in all life stages. Indeed, with the exception of Crustaceà, lipophorins are ubiquitous among arthropods (13, 14). As the only hemolymph component possessing a buoyant density <1.21 g/ml, lipophorin is readily isolated by density gradient ultracentrifugation (15). Although other purification methods have been used (16, 17) most laboratories now use centrifugation to isolate lipophorin and use buoyant density as the physical basis for identification and classification of lipophorin subspecies (12). The versatility of this particle with respect to its lipid-binding capacity may be unparalleled in nature. Stable lipophorin forms that range in density between 1.03 and 1.24 g/ml have been isolated and characterized from *M. sexta* (18-20). These density differences are also reflected in the size of the particles (20). For comparison, the normal density range for human high density lipoprotein (HDL) is 1.063-1.21 g/ml with broad overlap between individual subclasses that coexist in plasma (2). While there is a continuum of lipophorin species with respect to particle density, species isolated from carefully staged animals or after hormone administration fall within a relatively narrow density range. Thus, the characteristics of lipophorin subspecies present in plasma at any given time can be considered to reflect the physiological state of the organism with respect to lipid metabolism.

In the case of high density lipophorin (HDLp), several investigators have reported a molecular mass in the range of 600,000 daltons (16, 21-23). These sphere-like particles are comprised of approximately 50-60% protein in the form of two nonexchangeable, integral apolipoproteins (see below) and 40-50% lipid. **Table 1** presents the properties of HDLp from several insect species that represent four phylogenetic orders. For comparison, the lipid composition of human HDL is also shown. Several features of these compositions are noteworthy. First, in contrast to vertebrate lipoproteins, the major neutral glycerolipid of lipophorin is diacylglycerol (DAG) with much lower levels of monoacylglycerol and triacylglycerol present in all cases. The DAG content of lipophorin varies among species and during development but is generally considered to be the major lipid present. Phospholipid is also an important lipid component of all lipophorin forms. In contrast to mammalian lipoproteins, phosphatidylethanolamine is oftentimes the major phospholipid component in lipophorins (21, 23) which, together with phosphatidylcholine, comprise approximately 90% of the phospholipid mass. The remainder of the phospholipids of lipophorin are largely composed of sphingomyelin, although evidence for the presence of phosphatidylserine, phosphatidylinositol, and cardiolipin has also been reported (25).

TABLE 1. Properties of high density lipophorins and human HDL

Component	*M. sexta*[a]	*L. migratoria*[b]	*P. americana*[b]	*A. mellifera*[c]	Human HDL_2[d]
			Weight %		
Phospholipid	16.7	14.8	21.4	12.8	30
Diacylglycerol	15.7	13.4	7.6	13.3	
Free sterol	1.2	3.2	2.5	6.0	5.4
Triacylglycerol	1.1	0.7	1.0	3.9	4.5
Sterol ester	tr	0.1	0.0	tr	16
Hydrocarbon	2.8	8.7	14.2	2.0	
Protein	62.7	59.0	50.0	59.0	41
Density (g/ml)	1.15	1.12	1.12	1.13	1.12

[a]From Prasad et al. (18).
[b]From Chino and Kitazawa (15).
[c]From Robbs et al. (24); also contains 2.2% free fatty acid.
[d]From Kézdy (6).

Unlike mammals, insects cannot synthesize cholesterol de novo, which suggests that transport of dietary sterol by lipophorin is an important process. Indeed, after its administration in vivo, nearly all labeled cholesterol in hemolymph is associated with lipophorin (7). Aside from its role as a membrane component, cholesterol serves as a precursor of the molting hormone, ecdysone, a steroid hormone required for proper development and metamorphosis (26). Thus the role of lipophorin in transport of diet-derived cholesterol may be critical for survival. In contrast to mammalian lipoproteins, cholesteryl esters are generally observed only in trace amounts as components of lipophorin.

Long chain normal and methyl branched hydrocarbons are found as surface components of many insect species and are thought to protect against desiccation of these animals, which oftentimes have a large surface to volume ratio (see 27 for review). Cuticular hydrocarbons of the american cockroach, *Periplaneta americana* are identical to those found associated with lipophorin. Katase and Chino (28) have studied the transport of hydrocarbons in this species and obtained evidence to suggest that lipophorin functions in the transport of hydrocarbons from their site of synthesis (in oenocytes) to the cuticle where they are deposited by an as yet unknown mechanism. Other minor lipid components of lipophorin include carotenes, which impart a characteristic yellow color and, in some insects, the sesquiterpene juvenile hormone (29), an important regulator of insect development (26).

Apolipophorin I and apolipophorin II

All naturally occurring lipophorins possess a single molecule of apolipophorin I (apoLp-I) and apolipophorin II (apoLp-II). ApoLp-I is the largest lipophorin apoprotein with an apparent molecular weight of ~240,000 and

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is a required structural component of all lipophorin forms. ApoLp-I is insoluble in aqueous solutions outside of the lipophorin complex, but can be solubilized with detergents and/or chaotropic salts. Under these conditions, apoLp-I has been dissociated from the complex and isolated by gel permeation chromatography (15, 21, 30). Amino acid compositions have been reported for apoLp-I from several species and have been shown to possess types of amino acids similar to those present in mammalian apolipoprotein B (15, 23, 24, 29). Sequence information on apoLp-I has not been reported. Thus at present, we are unable to compare apoLp-I with apolipoproteins from vertebrates directly. However, in a manner similar to mammalian apolipoprotein B-containing particles, circular dichroism spectra of native lipophorin reveals a structure that is rich in β-sheet (30). ApoLp-I from a number of species has been shown to contain covalently bound oligosaccharide moieties. Lectin-binding (31) and periodic acid Schiff staining (32) of apoLp-I after SDS-PAGE gives a strong positive reaction indicating apoLp-I is glycosylated and suggests the presence of mannose-containing oligosaccharides.

Like apoLp-I, apoLp-II is a constitutive, integral apoprotein component of all naturally occurring lipophorin particles (31). ApoLp-II has an apparent molecular weight of $\sim$78,000 and is poorly soluble outside the lipoprotein complex. ApoLp-II has been isolated from delipidated lipophorin after solubilization in detergent and/or guanidine HCl and gel permeation chromatography (15, 21, 30). Lectin-binding studies have revealed the presence of mannose-containing oligosaccharide moieties on apoLp-II from several species (31). The covalent structure of asparagine-linked oligosaccharide chains liberated from intact locust lipophorin by almond glycopeptidase have been determined by NMR (33, 34). The two major oligosaccharides were shown to be a high mannose type structure identical to that of bovine thyroglobulin oligosaccharide together with a structure that is identical to the first with the exception that it contains a single glucose residue linked to the nonreducing terminal Man α1–2 residue. Since this analysis was performed on oligosaccharides prepared from intact lipophorin, and both apoproteins are glycosylated, it is not possible to distinguish the distribution of these oligosaccharide types between apoLp-I and apoLp-II.

Amino acid analysis has been performed on isolated apoLp-II from several species and has revealed a high degree of similarity with the composition of apoLp-I (15, 23, 24, 30). As with apoLp-I, amino acid sequence information on apoLp-II is lacking. This information will be important for determination of possible relationships bewteen apoLp-I and apoLp-II and between apoLp-II and vertebrate apolipoproteins. The possibility that apoLp-I and apoLp-II may share some common structural elements was examined by Shapiro, Keim, and Law (15). These workers obtained rabbit polyclonal antibodies directed against isolated apoLp-I and apoLp-II. Immunoblotting experiments showed that *M. sexta* apoLp-I and apoLp-II are antigenically unrelated. Furthermore, when the cross-reactivity of lipophorin apoproteins from different species was examined, it was found that anti-*M. sexta* apoLp-I serum recognized only apoLp-I from another lepidopteran species, the black swallowtail butterfly, *Papilio polyxenes,* but not apoLp-I from species representing six other orders (31, 35). ApoLp-II, on the other hand, was found to crossreact, albeit weakly, with its counterpart from several other species (31). In a study of the immunological properties of lipophorin apoproteins Schulz et al. (36), using anti-apolipophorin monoclonal antibodies, verified the lack of reactivity between individual lipophorin apoproteins and did not detect cross-reactivity between *L. migratoria* apoLp-II and *M. sexta* apoLp-II.

Apolipophorin antibodies have also been used as tools to probe the structural organization of intact lipophorin. Anti-*M. sexta* apoLp-I serum readily precipitates intact lipophorin in double immunodiffusion assays (15). By contrast, anti-apoLp-II serum, although highly specific on immunoblotting, fails to precipitate intact lipophorin. In a similar manner it has been demonstrated that apoLp-II is less exposed than apoLp-I to monoclonal antibodies directed against these respective antigens (36). Furthermore, apoLp-I of *M. sexta* and honeybee, *Apis mellifera,* lipophorin are more susceptible to trypsin cleavage or iodination than their counterpart, apoLp-II (21, 24, 37). These observations have led to the suggestion that apoLp-II may be shielded from the aqueous environment in the native complex while apoLp-I may be relatively more exposed. Cross-linking studies have shown that apoLp-I and apoLp-II are in close contact in HDLp since cross-links are readily formed with dimethylsuberimidate or dimethyladipimidate (30). The apparent internal location of apoLp-II and the lack of significant quantities of nonpolar lipids are yet to be incorporated into an accepted model of *M. sexta* HDLp structure. In the case of cockroach and locust lipophorin which contain a sizable amount of hydrocarbon, differential scanning calorimetry and ^{13}C-NMR relaxation measurements suggest the presence of hydrocarbon-rich regions within these particles (38). The hydrocarbon component of locust and cockroach lipophorins was further localized to the core based on small angle X-ray scattering studies of these lipophorins (39). Taken together, Katagiri, Sato, and Tanaka (39) propose a three-layer model of lipophorin structure that includes *1*) a central hydrocarbon core, *2*) a middle layer composed of DAG and apoLp-II, and *3*) an outer shell with apoLp-I and phospholipid. It is clear from ^{31}P-NMR spectroscopy and enzymatic studies with phospholipase

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A_2 that phospholipid resides in a monolayer on the outer surface of the particle (40). If one considers that DAG partitions between the surface and interior as a function of the lipid composition of the particle, this lipid may play a key role in overall structure determination (21). A dynamic partitioning between particle surface and core would also permit easier access of surface DAG to transfer proteins etc., which, upon removal from the particle, can be replenished from that present in the interior of the particle.

The recent reconstitution of stable, biologically active lipoprotein particles composed of apoLp-I and phosphatidylcholine by Kawooya, Wells, and Law (41) represents an important step toward complete reconstitution of lipophorin and increased understanding of potential structural and functional roles of apoLp-I and apoLp-II. The observed morphological and immunological similarities between reconstituted particles and native lipophorin suggests that this methodology may also provide useful models for investigations of the mechanism of lipid transport by lipophorin.

Lipophorin biosynthesis

In mammals, chylomicrons synthesized in the intestine serve to transport dietary glycerolipid (in the form of triacylglycerol) and cholesterol (as cholesteryl ester) to adipose tissue and the liver, respectively. Endogenously synthesized triacylglycerol, however, is assembled into very low density lipoproteins (VLDL) in the liver and secreted into the circulation. In insects, the sole site of lipoprotein biosynthesis appears to be fat body tissue, which serves as a major biosynthetic and storage organ and is also the center of intermediary metabolism (42). Thus, lipophorin synthesized by fat body functions both in transport of dietary and endogenously synthesized fat. In larval *M. sexta,* nascent lipophorin particles are assembled in fat body tissue and secreted into the hemolymph where maturation occurs (43). Lipophorin released into the medium in primary cultures of fat body tissue contains apoLp-I, apoLp-II, and phospholipid (approximately 80 molecules). These nascent particles are thought to mature by accepting additional lipid from midgut tissue. Maturation of lipophorin into circulating HDLp results in a density shift from 1.26 to 1.15 g/ml with no change in apoprotein content. Dietary glycerolipids are hydrolyzed to free fatty acids in the gut, taken up by midgut tissue, and converted to DAG prior to uptake by lipophorin. Upon delivery to their storage site at the fat body they are converted to triacylglycerol for storage (44).

In the southwestern cornborer, *Diatraea grandiosella,* lipophorin biosynthesis also occurs in the fat body (45). However in contrast to *M. sexta,* lipophorin secreted by cultured *D. grandiosella* fat body has a density and lipid composition similar to that of circulating lipophorin isolated from hemolymph (46). It is possible that differences in the properties of lipophorins secreted by fat bodies of these respective insects may be related to the fact that *D. grandiosella* can enter a nonfeeding facultative diapause as a fully grown larvae during which stored lipid serves as the major energy reserve. It is clear though, that in both species mechanisms exist whereby lipophorin biosynthesized in fat body can function in transport of dietary lipid from midgut tissue.

Dietary studies conducted in *M. sexta* revealed that animals reared on a high fat diet contain lipophorin particles with more lipid than those raised on control diet (44, 47). It was also observed that increased fat in the diet led to an increased deposition of fat in the fat body. In feeding larvae, over 65% of fed radiolabeled triolein was transported via lipophorin to the fat body after 4 h. On the other hand, when larvae are reared on an essentially fat-free artificial diet, circulating lipophorin has properties very similar to that of nascent particles secreted by cultured fat body in vitro. When these animals were fed a bolus of triolein, however, within a short time DAG appeared on lipophorin which decreased in density. Thus Tsuchida and Wells (44) suggest that, in contrast to mechanisms operative in mammals, lipophorin biosynthesis is not regulated at the level of dietary fat intake.

Lipophorin basic matrix particle

While on the one hand distinct lipophorin subspecies can be identified and isolated, each possesses specific structural properties retained by all lipophorin forms. Thus a basic lipophorin matrix particle is proposed to exist to which lipid and/or additional apoprotein (see below) may be added. Similarly, lipid-rich lipophorin forms may be depleted of lipid to the level of the basic matrix structure. These events occur multiple times during the lifetime of a given particle such that lipophorin can function as a reusable lipid shuttle. Although the precise structure of the lipophorin basic matrix is unknown, the two constitutive lipophorin apoproteins, apolipophorin I and apolipophorin II, as well as phospholipid are the major components.

The reusable shuttle hypothesis proposed by Chino (see ref. 7) is supported by experiments that have shown that *1*) protein biosynthesis is not required for lipid uptake by lipophorin from prelabeled fat body (48), *2*) particle lipid turnover occurs at a faster rate than apoprotein turnover (49), and *3*) uptake of lipophorin-associated DAG by feeding stage *M. sexta* fat body in vitro is accompanied by an increase in particle density that occurs in the absence of apoprotein uptake (44). Implicit in this hypothesis is the concept that lipophorin is capable of accepting or depositing lipid at different tissues without destruction of its basic matrix structure (19). Thus during larval development, just prior to metamorphosis, significant changes in the density of *M. sexta* lipophorin occur on a precisely timed

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developmental schedule that coincides with cessation of feeding behavior and initiation of the prepupal stage. Using larval HDLp labeled in the apoprotein moiety in vivo by injection of ^{3}H-labeled amino acids (18), it was demonstrated that a precursor–product relationship exists for lipophorin forms that appear during this stage of development. During this period lipophorin biosynthesis does not occur, a finding that is consistent with the reported low levels of lipophorin mRNA in fat body at this time (18, 50). Thus new lipophorin forms that arise during larval/pupal metamorphosis result from remodeling the lipid component of pre-existing lipophorin particles with maintenance of the basic matrix structure.

Adipokinetic hormone-induced lipophorin interconversions

All lipophorin particles contain one copy each of apoLp-I and apoLp-II as integral apoprotein components. In adult animals, however, lipophorin forms exist which possess variable amounts of a third low molecular weight apoprotein, apolipophorin III (apoLp-III; 8, 9). As discussed below, apoLp-III association is thought to confer upon lipophorin the ability to bind a greater amount of DAG than lipophorin forms lacking apoLp-III. At least two physiological processes appear to benefit from the increased lipid-binding capacity of lipophorin: flight activity and oogenesis (51–53). Flight activity is one of the most energy-demanding processes in nature. The ready availability of oxygen which diffuses to tissues through a network of tracheoles means that oxygen debt is not a problem (54). It has been suggested that the limiting factor controlling wingbeat frequency in insects is the availability of energy-yielding substrates. Among the insect species that engage in long term migratory flight, oxidation of long chain fatty acid provides the major energy source. Inasmuch as flight muscle is a highly specialized tissue directed toward energy utilization and muscle contraction, it generally does not serve as a fat storage reservoir. Large quantities of fat, stored as triacylglycerol in fat body tissue, are made available to flight muscle via the hemolymph as lipophorin-associated DAG. Under resting conditions lipophorin exists as adult high density lipophorin (HDLp-A) which in *M. sexta* has an M_r = 7.65 × 10^5 and a density = 1.07 g/ml (55).

In *M. sexta, L. migratoria,* and other species (56, 57) that use lipid as a fuel to power sustained flight, commencement of flight activity elicits secretion of peptidergic adipokinetic hormone(s) (AKH) that results in mobilization of stored fat (58, 59). AKHs comprise a family of peptides that possess lipid or carbohydrate mobilizing and/or cardioacceleratory activities that have been described in a growing number of species. All AKHs possess blocked N-termini and range in size from 8 to 10 amino acid residues (**Fig. 1**). Interestingly, AKH shares sequence identity with the N-terminal segment of human glucagon (60). Indeed, in a manner similar to the action of glucagon on adipose tissue (61), it has been postulated that AKH binds to a fat body cell membrane receptor and activates, via a cyclic AMP-dependent process, a lipase which in turn serves to catalyze breakdown of stored triacylglycerol (8). The biochemical mechanism of lipase activation or the ultimate pathway to DAG formation remains to be elucidated. It is noteworthy that, in contrast to the action of AKH on insect fat body, glucagon binding to mammalian adipose tissue receptors activates a cAMP-dependent phosphorylation of hormone-sensitive lipase which ultimately results in conversion of stored triacylglycerol to free fatty acids which are transported to tissue sites of utilization complexed with serum albumin (61). While insect plasma lacks albumin as such, the reason why DAG in insects and free fatty acid in mammals are the respective transport forms of stored fat remains an open question.

Triacylglycerol that is converted to DAG at the fat body is taken up by pre-existing circulating HDLp-A particles. Concomitant with DAG uptake is the association of several molecules of apoLp-III with the particle to produce low density lipophorin (LDLp), which in *M. sexta* has an M_r = 1.56 × 10^6 and a density of 1.03 g/ml (55). ApoLp-III, which is present in hemolymph in a nonlipoprotein-associated form at a concentration of ~17 mg/ml (62), associates reversibly with the particle as a function of its lipid content. The net result of this process is an elevated hemolymph lipid level which results from an increased amount of lipid per particle rather than a greater number of particles in circulation. The capacity to adapt to changing lipid transport demands without necessitating new lipoprotein biosynthesis undoubtedly decreases the time required to significantly alter hemolymph lipid levels in response to hormonal stimuli. It is important to note that, due to the presence of an open circulatory system in which the hemolymph bathes the tissues present in

L. migratoria AKH-1	pGlu-	Leu-	Asn-	Phe-	Thr-	Pro-	Asn-	Trp-	Gly-	ThrNH2
P. americana CC-2	pGlu-	Leu-	Thr-	Phe-	Thr-	Pro-	Asn-	TrpNH2		
M. sexta AKH	pGlu-	Leu-	Thr-	Phe-	Thr-	Ser-	Ser-	Trp-	GlyNH2	
Human glucagon	...-Gln-	Gly-	Thr-	Phe-	Thr-	Ser-	Asp-	Tyr-	Ser- ...	

Fig. 1. Primary sequences of related insect peptide hormones and the partial sequence (N-terminal residues 3–11) of human glucagon.

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the hemocoel, it is not possible to increase lipid substrate supply to specific tissues by dilation or constriction of blood vessels. This potential physiological disadvantage is circumvented by elevating the plasma lipid concentration and therefore the availability of lipid substrates to flight muscle or other tissues. Since neither lipophorin particles nor their apoprotein components are taken up by tissues (see below), the basic matrix structure as well as apoLp-III can function repeatedly forming a cycle of lipid transport, the net effect of which is the transfer of fat from storage organ to tissue sites of utilization. This reusable shuttle concept of lipid transport by lipophorin (**Fig. 2**) implies the existence of an efficient mechanism whereby transfer of lipid between cell and lipoprotein occurs in the absence of lipoprotein uptake and degradation. As discussed below, the mechanism of this process, which may involve a lipoprotein lipase and facilitated lipid transfer, is an area of active investigation.

Properties of apoLp-III

While apoLp-I and apoLp-II are associated with all lipophorin forms, apoLp-III associates with only certain lipophorin subspecies. In resting *M. sexta* adults, for instance, two molecules of apoLp-III are present per lipophorin particle, yielding an HDLp-A particle apoprotein molar ratio of 1:1:2 for apoLp-I, apoLp-II, and apoLp-III, respectively (62). The apoLp-III associated with HDLp-A, however, represents only a small percentage of that present in adult hemolymph. Indeed, in other species (i.e., *L. migratoria*) HDLp-A in resting adults lacks bound apoLp-III (63–65). ApoLp-III has been purified from isolated HDLp-A, LDLp, and lipophorin-deficient hemolymph (62, 64–66). ApoLp-III associated with *M. sexta* HDLp-A is tightly associated and can be liberated from the particle only by treatment with detergents or chaotropic salts. The purified apoproteins from these sources are indistinguishable with respect to amino acid composition and immunological properties (62, 65). The amino acid sequence of apoLp-III, as determined by protein and cDNA sequencing, from *M. sexta* and *L. migratoria* have been reported (67, 68). *M. sexta* apoLp-III consists of 166 amino acids and is translated with an amino terminal extension of 23 amino acids, not observed in the mature hemolymph protein. Sequence analysis reveals that the protein is composed of tandemly repeating tetradecapeptide units which evidence considerable length variability.

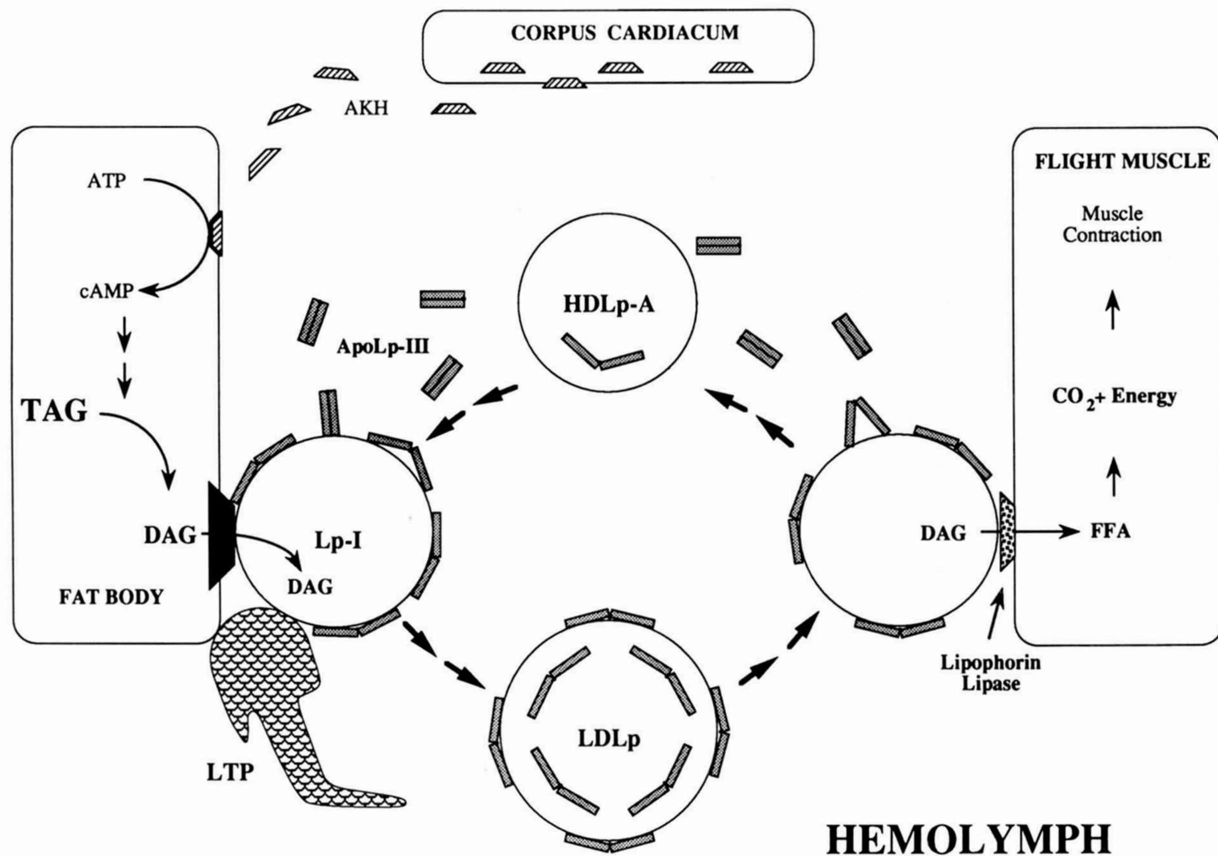

Fig. 2. Model of the effect of adipokinetic hormone on fat mobilization and transport by lipophorin in insects which utilize lipid as the fuel for sustained flight. Abbreviations are AKH, adipokinetic hormone; TAG, triacylglycerol; DAG, diacylglycerol; ApoLp-III, apolipophorin III; HDLp-A, high density lipophorin-adult; LDLp, low density lipophorin; LP-I, lipophorin loading intermediate; FFA, free fatty acid; LTP, lipid transfer particle.

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A hydropathy plot of *M. sexta* apoLp-III shows a regularly alternating pattern of hydrophobicity and hydrophilicity throughout the entire sequence and secondary structure predictions suggest a high percentage (63%) of the sequence may exist in α-helical conformation. Comparative analysis of *M. sexta* apoLp-III with that of mammalian apolipoproteins revealed little sequence identity but suggests the existence of significant functional homology (67).

L. migratoria apoLp-III is a protein of 161 amino acids (68) that has 29% overall sequence identity with *M. sexta* apoLp-III and contains two putative NH_2-linked glycosylation sites, which is consistent with the observed presence of covalently bound oligosaccharide moieties. It is composed of 12 repeating peptides that evidence greater length variability than the corresponding repeats in *M. sexta* apoLp-III. While the overall sequence identity between *M. sexta* and *L. migratoria* apoLp-III is relatively low, the two apoproteins possess a high degree of functional equivalence (see below). *L. migratoria* apoLp-III has been crystallized (69) and recently its three-dimensional structure has been determined by X-ray analysis to a resolution of 3.0 Å (H. M. Holden, personal communication). The protein contains five long anti-parallel α-helices which are connected by short loops. The molecule is organized such that hydrophobic residues are oriented toward the interior while hydrophilic residues face outwards. The structural model is consistent with the hypothesis that apoLp-III unfolds to expose its hydrophobic interior when presented to a lipid surface. Importantly, this work represents the first apolipoprotein structure to be determined.

N-terminal sequence analysis of apoLp-IIIs isolated from two grasshopper species (*Barytettix psolus* and *Melanoplus differentialis*) reveals >50% sequence identity with *L. migratoria* apoLp-III, reflecting the closer phylogenetic relationship of these species compared to *M. sexta* (70). As more sequences become available, comparative analysis with respect to molecular evolution should provide insight into possible relationships between mammalian and insect apolipoproteins as well as the limits of sequence diversity among functionally equivalent proteins.

In *L. migratoria,* Goldsworthy, Miles, and Wheeler (71) have reported the existence of two glycosylated apoLp-III species with molecular weights of 16,000 and 20,000, respectively, both of which are competent to associate with lipophorin during AKH-induced DAG mobilization. On the other hand, others have found evidence for three (65) or two (72) distinct isoforms of apoLp-III in *L. migratoria.* These apoLp-IIIs were indistinguishable by SDS-PAGE but were separable by ion exchange chromatography. Amino acid analyses revealed no difference in composition between the different apoLp-III isoforms (65). At present no acceptable explanation has been put forward to explain these conflicting results although it has been suggested that apoLp-III isoforms may represent molecules with a variable level of oligosaccharide phosphorylation (65). An interesting aspect of apoLp-III is the species diversity that exists with respect to covalently bound oligosaccharide moieties. While *M. sexta* apoLp-III lacks carbohydrate (62), that from the grasshopper, *Gastrimargus africanus* (56), contained 5.3% sugar by weight composed of mannose (3.4%) and glucosamine (1.9%). *G. africanus* apoLp-III also showed a strong positive reaction when stained with fluorescein isothiocyanate-conjugated concanavalin A (FITC-Con A). *L. migratoria* apoLp-III has been reported to contain 11% (65) or 12.5% (64) carbohydrate whose components were fucose, mannose, and glucosamine (65). These studies, however, relied wholly or in part on colorimetric assays of sugar components. In a recent study we examined the carbohydrate composition of three orthopteran species by gas-liquid chromatography and found that apoLp-III from these species possess approximately 5% carbohydrate with a fucose:mannose:N-acetyl glucosamine ratio of ~2:2:1 (70). The structure of these apoLp-III carbohydrate moieties has yet to be determined. Interestingly, although the compositions of these apoLp-IIIs are very similar, *B. psolus* and *M. differentialis* apoLp-IIIs react positively with FITC-Con A while *L. migratoria* does not. Explanation of this apparent anomaly must await oligosaccharide structure determination.

LDLp formation and properties

The mechanism of LDLp formation from HDLp-A and apoLp-III has been the focus of considerable research interest. Characterization of the DAG-loaded LDLp particle has revealed that specific incorporation of DAG as well as association of several molecules of apoLp-III occurs. In response to injected AKH, the DAG content of *M. sexta* lipophorin increases from 25 to 46% of the particle mass (19) and 16 molecules of apoLp-III associate with the particle (55). In *L. migratoria,* reports of 10, 14, and 28 apoLp-III per LDLp particle have appeared (72–74). It is known that apoLp-III association with HDLp-A does not occur in the absence of fat body- supplied DAG and, likewise, LDLp formation requires the presence of apoLp-III. In in vivo studies of LDLp formation, Wells et al. (55) found evidence of a transient loading intermediate that had a size and density between that of HDLp-A and LDLp. In further studies using density gradient ultracentrifugation, it was found that the content of apoLp-III increased more rapidly than the content of DAG in the region of the gradient corresponding to the loading intermediate, suggesting preferential uptake of apoLp-III compared to DAG. A hypothesis emerging from this observation and its known surface properties is that apoLp-III may bind to the lipoprotein surface in two different ways. The relative small increase in particle surface area

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caused by the addition of DAG compared to the number of apoLp-III molecules bound suggests that initially apoLp-III binds with its minor axis parallel to the surface of the lipoprotein, creating the "loading intermediate." Then as more DAG is added, further expanding the surface area of the lipoprotein, the area occupied by each molecule of apoLp-III increases dramatically, suggesting that the molecule unfolds on the surface.

Consistent with this interpretation are the results of physical studies by Kawooya et al. (75) on *M. sexta* apoLp-III. Hydrodynamic, gel permeation, and viscometry experiments suggest that apoLp-III assumes a solution conformation as a prolate ellipsoid with an axial ratio of 3 and circular dichroism spectra reveal that apoLp-III contains about ~50% α-helix. Monolayer studies revealed that at low surface pressures (<1 dyne/cm) apoLp-III forms a stable monolayer with a limiting molecular area of 3795 $Å^2$. Upon compression of the monolayer to 22.1 dynes/cm, rather than collapsing, the monolayer undergoes a phase transition and can be compressed to a limiting molecular area of about 480 $Å^2$. Since a globular protein of molecular weight 18,000 would occupy a molecular area of 2000 $Å^2$, it is hypothesized that apoLp-III may unfold at the air/water interface at low surface pressures in a manner analogous to the conformation it assumes when bound to LDLp. Moreover, the second stable orientation, observed at high surface pressures, has been suggested to correspond to the orientation of apoLp-III during the initial stages of LDLp formation in vivo when the "loading intermediate" is detected.

Although apoLp-III binding to DAG- or phosphatidylcholine-coated polystyrene beads occurs spontaneously (75), it is not known whether apoLp-III association with HDLp during formation of LDLp is dictated by the surface properties of the apolipoprotein or is induced by some additional factor. Such a factor would likely be provided by fat body tissue, however, since in vitro loading studies have revealed LDLp formation from purified HDLp, AKH, apoLp-III, and fat body (76, 77). The requirement for fat body is absolute in this instance and the extent of loading is dependent upon the amount of apoLp-III present. Furthermore, the conversion is Ca^{2+}-dependent (77). Interestingly, when *L. migratoria* apoLp-III is limiting in in vitro incubations, two populations corresponding in density to LDLp and HDLp are observed rather than a single partially transformed intermediate density species. This observation suggests that there is inherent stability in the LDLp species formed which is preferred over partially loaded species. Recent evidence has appeared that suggests that transfer of DAG from fat body to lipophorin during LDLp formation in response to AKH requires a hemolymph lipid transfer particle (see below) that appears to facilitate vectorial transfer of DAG mass from membrane to lipoprotein (78).

The interaction of lipophorin with fat body membranes has been studied in *L. migratoria*. Ligand binding studies have been conducted to investigate the interaction of *L. migratoria* HDLp with fat body tissue (79). Saturable binding of labeled HDLp was observed that was displacable by a 20-fold excess of unlabeled HDLp. Scatchard analysis revealed a dissociation constant, $K_d = 3.1 \times 10^{-7}$ with a maximum binding capacity of 9.8 ng/μg tissue protein. The DAG-rich LDLp had an approximate 20-fold lower affinity for the binding site versus HDLp, perhaps suggesting that HDLp displacement of newly formed LDLp is partially responsible for the observed directional flux of DAG out of the fat body in response to AKH. Important questions remaining to be addressed, with regard to this fat body lipophorin binding site, include whether lipoprotein internalization or docking is involved and whether this putative receptor is the site of hormone-induced lipophorin transformations. Answers to these questions may be forthcoming, however, as a lipophorin receptor has recently been isolated from *M. sexta* larval fat body membranes which shares properties with human LDL receptor as well as the chicken oocyte VLDL receptor (80). The larval fat body receptor has an M_r = 120,000, an absolute requirement for Ca^{2+} and is inhibited by Suramin. It has been postulated that the relative binding affinities of different lipophorin subspecies to this receptor may function in controlling the directional flux of lipid from midgut to fat body during this life stage.

In an effort to understand factors that control LDLp formation, novel experimental approaches have been employed. Wheeler and Goldsworthy (81) and Van der Horst et al. (82) examined the adipokinetic response of immature locusts, which do not possess wings. It was observed that larval *L. migratoria* respond to AKH injection with a hemolymph lipid increase of only 2 mg/ml in contrast to the 10- to 20-fold increase observed in adult locusts. These animals contain much less apoLp-III (one-fifth the level found in adults) but the poor response could not be attributed solely to the lower concentration of apoLp-III, since injection of exogenous, purified apoLp-III or apoLp-III plus HDLp-A, failed to increase hemolymph lipid levels. By contrast, in vitro lipid loading experiments showed that larval fat body is not fully competent to induce lipophorin conversions even when incubated with adult hemolymph proteins. Moreover, the hemolymph components of larval animals are restrictive for LDLp formation since, when incubated with adult fat body and AKH in vitro, only a partial adipokinetic response was observed. The results suggest that the ability to form LDLp from high density lipophorins is a function of the ability of fat body to respond to lipid mobilization stimuli as well as the presence of specific hemolymph protein components.

A major action of AKH is considered to be its role in

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flight-related lipid mobilization. Thus a potentially useful model for study of this process is the grasshopper, *B. psolus,* which, during the course of evolution, has secondarily lost the ability to fly. Corpora cardiaca extracts from this species contain large quantities of an AKH-like peptide that is functional when injected into the locust *Schistocerca americana* but fails to elicit lipid mobilization in *B. psolus* (83). In further experiments it was shown that *B. psolus* lipophorin has the capacity to form LDLp when injected into *S. americana* followed by AKH. Furthermore *B. psolus* was shown to possess a functional apoLp-III that had a similar amino acid composition and 50% sequence identity with the N-terminal 30 amino acids of *L. migratoria* apoLp-III (70). The presence of functional hemolymph components as well as abundant AKH but the lack of an adipokinetic response in *B. psolus* may therefore reflect an inability of fat body tissue to respond to AKH which is a prerequisite for mobilizing DAG. If this is true, however, important questions remain that pertain to the physiological role of *B. psolus* AKH and apoLp-III. On the one hand, the observed reduced lipid mobilizing response is consistent with the evolutionary loss of energy demanding flight. Alternatively, it is possible that AKH may function in regulating other aspects of metabolism including protein synthesis, carbohydrate metabolism or myoactivity (58).

Using a novel approach to address the question of the competence of immature animals to respond fully to AKH, Van der Horst et al. (84) employed precocene to induce adult forms from fifth instar animals and azadirachtin to induce over-aged nymphs. In precocene-treated animals it was observed that the response to AKH differed markedly from untreated controls and closely resembled the response of adults. In azadirachtin-treated animals the conversion of HDLp to LDLp was less complete and produced lipophorin species intermediate in size between HDLp and LDLp. This latter result was explained by the presence of less apoLp-III than in normal adults.

Interactions of lipophorin with flight muscle

The delivery of LDLp-associated DAG to flight muscle assumes an interaction between lipoprotein and cell. In mammals, circulating triacylglycerol-rich lipoproteins are a substrate for lipoprotein lipase on the capillary endothelium. Extracellular lipolysis yields free fatty acids which enter adipose tissue cells and are converted to triacylglycerol. In the case of VLDL, triacylglycerol removal results in surface apoprotein and phospholipid migration (i.e., to HDL) ultimately resulting in conversion to LDL, which is cleared from the circulation via receptor-mediated endocytosis and degraded (85).

L. migratoria flight muscle possesses a lipoprotein lipase activity that has a substrate specificity that favors LDLp versus HDLp-A (86–88). The enzyme activity is not heparin-releasable (89) and, to date, purification has not been reported. Subcellular fractionation studies, however, suggest that it is localized on the plasma membrane (87). If flight muscle lipase resides on the extracellular surface of the plasma membrane, it is plausible that DAG hydrolysis to free fatty acid occurs extracellularly in a manner analogous to mammalian lipoprotein lipase. After entering the flight muscle cell, free fatty acid needs to reach the mitochondria for subsequent β-oxidation. Since a high flux of fatty acid is required to sustain flight, it is likely that an intracellular receptor protein plays a role in this transport. Recently, Haunerland and Chisholm (90) discovered an abundant fatty acid-binding protein in flight muscle of the desert locust, *Schistocerca gregaria,* that is reminiscent of mammalian fatty acid binding proteins (91). It is conceivable that this protein may play an important role in fatty acid trafficking within the flight muscle cell.

There is now strong evidence to support the concept that the protein portion of lipophorin remains in the extracellular space and that the basic matrix particle cycles between lipid loaded (LDLp) and unloaded (HDLp) states during which apoLp-III binds reversibly to the particle. It has been demonstrated that after hydrolysis of LDLp-associated DAG by flight muscle lipoprotein lipase, the apoLp-III that dissociates is competent to reassociate with HDLp-A to form new LDLp (72). The question of whether lipophorins enter the flight muscle at rest or during flight has been addressed by Van Antwerpen et al. (92) by indirect immunofluorescence and immunogold labeling in cryosections of dorsolongitudinal flight muscles. Lipophorins were located only in the wider spaces of the extracellular matrix, in the basement membranes of the individual muscle fibers and in the extracellular spaces that surround intrafibrillar tracheoles. Cellular internalization of lipophorins by flight muscle was not observed. Taken together the results suggest that delivery of lipophorin-bound DAG to flight muscle involves DAG hydrolysis by a lipoprotein lipase that results in apoLp-III dissociation but no lipoprotein particle internalization. Indeed, the released apoLp-III and HDLp particles may recombine upon reloading of HDLp with DAG at the fat body thereby forming a cycle of lipid transport, the net effect of which is the net transfer of DAG from its storage site at the fat body to its site of utilization at the flight muscle (see Fig. 2). Thus, on the one hand, the apparent extracellular hydrolysis of LDLp-associated DAG is analogous to triacylglycerol-rich lipoprotein metabolism in mammals; the reuse of the lipophorin basic matrix particle to make new LDLp is a departure from the accepted metabolic cacade of mammalian triacylglycerol-rich lipoproteins.

One hypothesis emerging from studies of *L. migratoria* (64) suggests that the oligosaccharide moiety serves as an

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activator of flight muscle lipophorin lipase, akin to the action of apolipoprotein C-II in mammalian systems (93). An alternative hypothesis, proposed by Wheeler, Boothby, and Goldsworthy (94), is that free apoLp-III serves as an inhibitor of flight muscle lipoprotein lipase and that upon lipid loading and apoLp-III association with lipophorin, lipase inhibition is relieved. The question of the role of apoLp-III carbohydrate as a modulator of flight muscle lipase was tested experimentally by preparing hybrid LDLp particles composed of *L. migratoria* apoLp-I and apoLp-II and *M. sexta* apoLp-III (74). Comparison of the abilitiy of whole dorsolongitudinal flight muscle preparations to hydrolyze hybrid or native LDLp-associated DAG revealed a similar efficiency. Since *M. sexta* apoLp-III lacks covalently bound carbohydrate, it was concluded that the carbohydrate moiety of *L. migratoria* apoLp-III is not required for interaction with flight muscle lipase. Furthermore, it is also suggested that specific epitopes of *L. migratoria* apoLp-III are not required for lipase activation since *M. sexta* apoLp-III lacks immunological cross-reactivity with *L. migratoria* apoLp-III (74).

Lipid transfer particle

As described above, in *M. sexta* a variety of lipophorin forms exist which possess very different densities and lipid compositions. At the same time it is recognized that these forms are interconvertible. In studies directed toward characterization of this process, it was initially observed that a nondialyzable heat-labile factor, present in lipophorin-deficient hemolymph, caused a dramatic alteration of the density distribution of isolated lipoproteins upon incubation in vitro (19). Upon further characterization of the reaction promoted by this factor, it was ascertained that neither substrate lipoprotein particle fusion nor apoLp-I or apoLp-II transfer was responsible for the observed changes in lipoprotein density distribution. In experiments using radiolabeled lipids, direct evidence of facilitated DAG and PL transfer was obtained (19). Thus it was concluded that the observed changes were due to facilitated redistribution of lipophorin-associated lipid. Ultimately a new equilibrium was established in which a lipoprotein species of density between that of the starting lipophorins was produced. It was concluded that facilitated vectorial net transfer of lipid mass from lipid-rich donor lipoprotein to relatively lipid-poor acceptor lipoprotein redistributed the lipid complement of the original substrate particles such that, ultimately, each particle possessed similar amounts of lipid while retaining its original apoLp-I and apoLp-II complement. This is possible because the basic matrix structures of the substrate lipophorins are identical.

The production of a lipophorin of intermediate size and density when two lipophorin species of different density are incubated in the presence of the transfer factor was used as an assay method whereby purification of the material responsible was monitored. A facile, three-step purification scheme from lipophorin-deficient hemolymph using ammonium sulfate precipitation, gel permeation chromatography, and density gradient ultracentrifugation was developed (95). Subsequent introduction of an ion exchange chromatography step increased the purity of the product (96). Two important characteristics of the lipid transfer catalyst were apparent from the purification scheme. First, all transfer activity eluted as a high molecular weight material when subjected to gel permeation chromatography (>500,000) and second, when subjected to a modified vertical rotor density gradient ultracentrifugation (97), all of the transfer activity floated above the other hemolymph proteins to an equilibrium density of 1.23 g/ml suggesting that this material contains lipid. Further characterization by SDS-PAGE provided evidence of three apoprotein components and resulted in the term lipid transfer particle (LTP) (98). The molecular weight of the apoprotein components of LTP as well as its lipid composition are given in **Table 2.** Comparison of the properties of LTP from *M. sexta* and that recently isolated from *L. migratoria* by Hirayama and Chino (99) reveals similarities in apoprotein size and lipid content. Thus it is possible that LTP may be present in a wide variety of insects, supporting the concept that it plays an important role in lipid and lipoprotein metabolism. Native molecular weight estimates of LTP by native pore limiting PAGE (98) as well as sedimentation equilibrium studies (100) suggest a particle mass of ~900,000. Although densitometric scanning of apoproteins stained with Amido Black 10B indicate a mass ratio of 4.5:1.0:0.34 for apoLTP-I, apoLTP-II, and apoLTP-III, respectively, the precise apoprotein stoichiometry of the native complex is not known. LTP contains 5% by weight covalently

TABLE 2. Properties of insect lipid transfer particle

Component	M_r	
	M. sexta[a]	*L. migratoria*[b]
Apoprotein (86% of particle mass)		
ApoLTP-I	~320,000	310,000
ApoLTP-II	85,000	89,000
ApoLTP-III	55,000	68,000
	Weight %	
Lipid (14% of particle mass)[c]		
Phospholipid	58	18
Diacylglycerol	28	44
Free fatty acid	8	13
Triacylglycerol	6	
Hydrocarbon		17
Cholesterol		8

[a]Adapted from ref. 98.
[b]Adapted from ref. 99.
[c]Composition subject to change upon incubation with lipid donor substrates.

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bound sugar (mannose and N-acetyl glucosamine) and each of the three apoproteins is glycosylated (98).

Given the apparent unusual physical properties of *M. sexta* LTP compared to other known lipid transfer proteins, an important question is whether the active principle involved in facilitating lipid transfer is the entire LTP complex or merely one of its apoproteins. In studies aimed at addressing this question, evidence for the involvement of the lipid component was obtained (98). First, when lipophorin containing labeled DAG was incubated with a stoichiometric amount of LTP, radiolabeled DAG was recovered at a density corresponding to that of LTP. Furthermore, upon incubation of [^{3}H]DAG-LTP with excess unlabeled lipophorin, the labeled DAG was transferred back to lipophorin. This result suggests that the lipid complement of LTP is not merely a structural component of the particle but rather is dynamic and participates in the observed lipid transfer reaction. Secondly, when the lipid component of LTP is removed with ethanol–diethyl ether extraction or by detergent treatment, transfer activity is lost. When the morphology of LTP was examined by electron microscopy, evidence for an unusual asymmetric shape was obtained (96; see Fig. 2). LTP has two major structural features: a roughly spherical head region and an elongated cylindrical tail section that possesses a central hinge. The precise location of LTP apoproteins within this complex is not known, but specific perturbation of the morphology of the head region of the particle upon exposure to SDS has led to the suggestion that the lipid moiety of the particle resides in this section (96). Polyclonal antibodies have been raised against purified LTP in rabbits. This antiserum, which is specific for LTP, has been demonstrated to inhibit the activity of LTP in lipid transfer assays and recently has been used in experiments directed toward determination of potential physiological roles of LTP. Van Heusden and Law (78) studied the process of AKH-induced LDLp formation in vitro. It was shown that lipid transfer occurred from fat body containing labeled lipid to either HDLp or LDLp in vitro. This transfer was inhibited by preincubation of the fat body with anti-LTP IgG. Addition of LTP, however, restored the transfer process, demonstrating an ability of LTP to promote lipid transfer between tissue and lipoprotein. In addition, the AKH-induced conversion of HDLp-A to LDLp was demonstrated to require the presence of LTP, which is consistent with the observed activity of LTP when isolated lipoproteins are used as substrate. Interestingly, lipid exchange or transfer in the reverse direction (from lipophorin to fat body) was not influenced by LTP.

From the above discussion it is clear that LTP possesses unique structural and functional properties. Indeed, it appears well suited to mediate the lipid transport processes observed within the framework of the lipophorin shuttle hypothesis. It is likely that further functional roles of LTP in insect lipid metabolism will be discovered (see next section). In addition, however, it may also be that other more general uses of this catalyst may be found. Recent studies have focussed on the ability of LTP to facilitate lipid transfer between other lipoproteins. We have obtained evidence that LTP can utilize a broad spectrum of substrate lipoproteins/particles including an unrelated insect plasma very high density chromolipoprotein (101, 102), human LDL and a human apolipoprotein A-I-stabilized phospholipid/triolein microemulsion (103). The latter two substrates have been used as donor/acceptor species in experiments that illustrate the difference in catalytic activity of LTP versus human plasma cholesteryl ester transfer protein (CETP; 104). While CETP promotes heteroexchange of triacylglycerol and cholesteryl ester between these pools (105, 106), LTP catalyzed net transfer of TG and phospholipid mass from emulsion to LDL, producing a lipid-enriched LDL that retains its original apolipoprotein profile. Other studies have shown that lipophorin can serve as a lipid donor upon incubation with LDL and LTP. Facilitated vectorial transfer of DAG from lipophorin to LDL produces a DAG-enriched LDL of lower density and increased lipid:protein ratio and a lipid-depleted lipophorin whose properties are similar to those of egg very high density lipophorin (VHDLp-E; 20, 107). These reactions serve to illustrate the remarkable capacity of LTP to catalyze significant redistribution of lipid mass among lipoproteins and suggest that donor/acceptor lipoprotein structure plays an important role as determinant of the direction and extent of LTP-catalyzed lipid flux. The potential utility of LTP in the production of "designer lipoproteins" will undoubtedly be an area of interest to many researchers in the lipoprotein field.

Lipophorin in oocytes

A second physiologically relevant alteration of the lipophorin basic matrix structure that appears to require LTP is the transformation of HDLp-A to egg very high density lipophorin, VHDLp-E. In *M. sexta* and silkworm, *Philosamia cynthia,* oocytes a lipophorin species can be isolated that contains greatly reduced amounts of lipid and is virtually devoid of DAG (20, 108). VHDLp-E, however, contains intact apoLp-I and apoLp-II and has been shown to retain its capacity to function as a lipid acceptor. Kawooya, Osir, and Law (20) demonstrated that HDLp-A labeled in the protein moiety is converted to VHDLp-E in vivo and in vitro upon uptake by oocytes via an apparent receptor-mediated endocytosis. Conversion of HDLp-A to VHDLp-E is accompanied by dissociation of its two apoLp-III molecules. The product VHDLp-E is composed of 80% protein and 20% lipid of which 75% is phospholipid. This transformation, which results in a particle density increase from 1.08 g/ml to 1.24 g/ml, involves specific removal of lipids from the particle. DAG removed

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from HDLp-A is ultimately esterified and stored as triacylglycerol in the oocyte. It is possible that the removal of lipid from HDLp-A may be the result of lipolysis or facilitated net lipid transfer. Since sterols, hydrocarbons, and carotenes are also removed from the particle during transformation (20), however, lipase activity alone cannot account for VHDLp-E production. Furthermore, since LTP has exhibited a capacity to induce alterations in the net mass of lipid associated with lipophorin particles, it was hypothesized that LTP plays a role in this transformation. Consistent with this hypothesis are the observations that LTP can catalyze a similar transformation in vitro, that active LTP can be isolated from *M. sexta* oocyte homogenates, and that anti-LTP IgG specifically inhibits transformation of ^{125}I-labeled HDLp-A to VHDLp-E by oocyte homogenates (Liu, H., and R. O. Ryan, unpublished results). These results provide additional evidence that LTP is a normal component of the oocyte and, as such, is involved in mediating redistribution of lipid between pools within this cell. Previously, LTP has been observed only in the plasma compartment and, based on this observation, it will be important to examine other tissues (e.g., fat body) for LTP activity as well.

While the uptake and transformation of HDLp-A to VHDLp-E serves to supply lipids to the developing oocyte, another process involving nonendocytotic delivery of LDLp-associated DAG to the oocyte appears to be quantitatively more important (53). It has been shown, using dual-labeled LDLp, that significant uptake of LDLp-associated DAG in the absence of protein uptake occurs. The mechanism of this delivery process has not been elucidated but it is intriguing to speculate whether LTP may mediate this transfer process as well.

Concluding remarks

Although substantial progress has been made in recent years, it is also clear that many gaps remain with respect to our understanding of invertebrate lipoproteins. With the tools of modern biochemistry and molecular biology, however, purification and characterization of many of the poorly understood structural and catalytic proteins involved in insect lipid transport and metabolism should now be possible. It is vital that more complete characterizations of intact lipophorin, apoLp-I, apoLp-II, LTP, fatty acid-binding proteins, as well as purification and initial characterizations of lipophorin lipase, fat body triacylglycerol lipase, and several others be performed so that the dynamics and control of this remarkable lipid transport system can be more fully understood, evaluated, and compared with distinct or analogous systems which function in vertebrates. ■

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