

Lipid transfer from insect fat body to lipophorin: comparison between a mosquito triacylglycerol-rich lipophorin and a sphinx moth diacylglycerol-rich lipophorin

James E. Pennington, Roberto H. Nussenzweig, and Miranda C. Van Heusden¹

Department of Biochemistry and Center for Insect Science, University of Arizona, Tucson, AZ 85721

Abstract Two insect lipoproteins, triacylglycerol-rich *Aedes aegypti* lipophorin and diacylglycerol-rich *Manduca sexta* lipophorin, were compared in their ability to load neutral lipid from fat body. When fat body of *M. sexta* was incubated in vitro with [³H]oleic acid, all radiolabeled fatty acids were esterified, predominantly to triacylglycerol. In *A. aegypti* fat body, however, half of the label remained as free fatty acids. When *A. aegypti* fat body was radiolabeled with [³H]glycerol, most of the radiolabel was incorporated in triacylglycerol. When either *A. aegypti* or *M. sexta* lipophorin was incubated with *A. aegypti* fat body, labeled with [³H]oleic acid, both lipophorins incorporated mainly radiolabeled free fatty acids, while almost no radiolabeled glycerides were transferred. When the same experiment was performed with *A. aegypti* fat body, radiolabeled with [³H]glycerol, very little transfer of radiolabeled glycerides was detected. In contrast, when either *M. sexta* or *A. aegypti* lipophorin was incubated with *M. sexta* fat body, both lipophorins incorporated neutral lipids, predominantly diacylglycerol. *A. aegypti* lipophorin incorporated half the amount of radiolabeled lipid, compared to *M. sexta* lipophorin. Lipophorins from both species were treated with triacylglycerol lipase of the yeast *Candida cylindracea*. Although this lipase readily delipidated *M. sexta* HDLp, it was not able to remove triacylglycerol from *A. aegypti* HDLp. ■ The data presented suggest that, under the conditions used, lipid transfer from fat body to lipophorin in *A. aegypti* is not as efficient as in *M. sexta*.—Pennington, J. E., R. H. Nussenzweig, and M. C. Van Heusden. Lipid transfer from insect fat body to lipophorin: comparison between a mosquito triacylglycerol-rich lipophorin and a sphinx moth diacylglycerol-rich lipophorin. *J. Lipid Res.* 1996. **37**: 1144–1152.

Supplementary key words high density lipoprotein • fatty acid • triacylglycerol lipase • hemolymph • in vitro • *Aedes aegypti* • *Manduca sexta*

In most insects studied so far, lipophorin is the major lipoprotein in hemolymph responsible for lipid transport (1–5). The typical insect lipophorin is a high density lipoprotein (HDLp, 1.063 g/ml < d < 1.210 g/ml), containing 30–50% lipid. It is composed of two apolipo-

proteins, apolipophorin-I and -II, with molecular mass of approximately 250 and 80 kDa, respectively. In certain insect species a third, smaller apolipoprotein (apolipophorin III, ≈18 kDa) can associate in a reversible manner with lipophorin. In general, the major neutral lipid in insect lipophorin is diacylglycerol (DG). In vertebrates, chylomicrons and very low density lipoproteins (VLDL) are the main transport vehicles for neutral lipids from the site of absorbance (the gut) and synthesis (the liver), respectively. These two lipoproteins contain triacylglycerol (TG) as the predominant neutral lipid (6).

A characteristic feature of insect lipophorin is that it acts as a reusable lipid shuttle. The turnover rate of DG in hemolymph is much higher than that of the protein component of lipophorin (7). Lipophorin can load and unload lipid without synthesis or degradation of the lipoprotein (8). This is in contrast to vertebrate chylomicrons and VLDL, which are synthesized and secreted with their full load of lipid (9). Because DG is less hydrophobic than TG, the presence of DG in insect lipophorin (versus TG in vertebrate lipoproteins) may contribute to the ability of lipophorin to exchange lipid with cells without internalization of the lipoprotein.

Recently a TG-rich lipophorin was isolated from the yellow fever mosquito *Aedes aegypti* (10). This was the first insect lipophorin reported to contain TG. A TG-rich lipophorin has been isolated from four more blood-feeding mosquito species (10; and M. C. Van Heusden, unpublished data) as well as from the horseshoe crab *Limulus polyphemus* (11). Thus, as far as lipid composition is concerned, the latter lipophorins resemble the

Abbreviations: HDLp, high density lipophorin; TG, triacylglycerol; DG, diacylglycerol; MG, monoacylglycerol; FA, free fatty acid; PL, phospholipid; LTP, lipid transfer particle.

¹To whom correspondence should be addressed.

vertebrate lipoproteins more than the typical insect lipophorin. In view of this difference in lipid composition, we investigated whether a TG-rich insect lipophorin is able to function as a lipid shuttle, as does the typical DG-rich insect lipophorin, or whether its metabolism is different due to the presence of TG. We analyzed the ability of lipophorin from the mosquito *A. aegypti* to load lipid from fat body, and compared it to the well-characterized DG-rich lipophorin of the sphinx moth *Manduca sexta*.

MATERIALS AND METHODS

Insects

Manduca sexta eggs were obtained from USDA, Fargo (ND), and insects were raised as described previously (12). *Aedes aegypti* (NIH-Rockefeller strain) were raised as described by Ford and Van Heusden (10).

Isolation of lipophorin

Hemolymph was collected from adult *M. sexta* as described previously (8). High density lipophorin (HDLp) was isolated from hemolymph as described by Shapiro, Keim, and Law (13). HDLp from *A. aegypti* was purified from total insect homogenate of 4th instars as described by Ford and Van Heusden (10), with the exception that a higher concentration of protease inhibitors was used in the homogenization buffer (4 mM diisopropyl fluorophosphate (DFP) and 4 mM phenylmethylsulfonyl fluoride (PMSF)). HDLp was also isolated from hemolymph of adult female mosquitoes, where indicated under Discussion. Mosquito hemolymph was collected by making a small incision in the cuticle at the penultimate abdominal segment. Subsequently hemolymph was flushed out by injecting, between the head and the thorax, approximately 10 μ l of ice-cold *Aedes* saline (A-saline: 25 mM HEPES, 140 mM NaCl, 4 mM KCl, 1.7 mM CaCl₂, 0.6 mM MgCl₂, 1.8 mM NaHCO₃, pH 7.0; according to Hagedorn et al., 14) containing 20 mM glutathione, 10 mM EDTA, and the protease inhibitors DFP (1 mM), PMSF (1 mM), leupeptin (0.5 μ g/ml), antipain (0.5 μ g/ml), chymostatin (0.5 μ g/ml), and pepstatin (0.1 μ g/ml). The drop of hemolymph with A-saline was collected from the tip of the abdomen into a microfuge tube and kept on ice. Hemocytes were removed by centrifugation at 4000 g for 5 min.

Radiolabeling of fat body

To radiolabel the lipids in *A. aegypti* fat body, fat bodies from adult female mosquitoes (3–7 days after adult emergence) were dissected and incubated in A-saline as described by Hagedorn et al. (14). Ten fat bodies

were incubated in 100 μ l saline containing either 10 μ Ci [9,10-³H]oleic acid (specific activity 9.2 Ci/mmol, DuPont-New England Nuclear) or 15 μ Ci [1(3)-³H]glycerol (specific activity 2.8 Ci/mmol, Amersham Life Science), for 2 h at 28°C and 80% relative humidity (the mosquito rearing conditions). Subsequently the fat bodies were rinsed two times in A-saline and incubated for 1 h in A-saline, followed by lipid analysis or further incubations.

Fat body from adult *M. sexta* was labeled according to the method described by Van Heusden and Law (8), using lepidopteran saline (L-saline: 5 mM KH₂PO₄, 110 mM KCl, 4 mM NaCl, 15 mM MgCl₂, 4 mM CaCl₂, pH 6.5; according to Jungreis, Jatlow, and Wyatt, 15). Fat bodies from 1-day-old moths were dissected, rinsed in L-saline containing 5 mM glutathione and divided in half. A half fat body was incubated in 400 μ l L-saline containing 5 μ Ci [³H]oleic acid, in a 24-well tissue culture plate. Labeling proceeded for 2 h at room temperature on an orbital shaker at 120 rpm. Fat bodies were rinsed two times in L-saline and incubated for 1 h in L-saline prior to lipid analysis or further incubations.

Incubation of fat body with lipophorin

Ten fat bodies of *A. aegypti* were incubated in 100 μ l of A-saline, with or without lipophorin, at 28°C and 80% relative humidity. Four incubations of ten fat bodies each were performed for each experiment. One-half fat body of *M. sexta* was incubated in 400 μ l of L-saline, with or without lipophorin, at room temperature on an orbital shaker at 120 rpm. Four incubations, with one-half fat body each, were performed for each experiment (unless otherwise stated under Results). Lipophorin, which was dialyzed into the respective saline, was added to the medium to a final concentration of 1 mg/ml or 4 mg/ml, as indicated under Results. Incubations lasted for 3 h, after which the media were subjected to KBr density gradient ultracentrifugation as follows: 8 g KBr was added to the incubation media and the final volume was adjusted to 20 ml with phosphate-buffered saline (PBS, 0.1 M Na-phosphate, 0.15 M NaCl, pH 7.0). These samples were overlaid with 20 ml of 0.9% NaCl (containing 0.02% NaN₃). Gradients were formed by centrifugation for 16 h at 50,000 rpm and 4°C in a vertical VTi50 rotor (Beckmann). Radioactivity in lipophorin was quantified by liquid scintillation counting. Density of lipophorin was determined by refractometry.

Lipid analysis

Total lipids were extracted either from the incubation media containing lipophorin or from fat bodies after homogenization in chloroform, following the method described by Bligh and Dyer (16). Neutral lipids were separated by thin-layer chromatography on silica gel

TABLE 1. Lipid composition of fat body

Fat Body	Lipid			
	PL	DG	FA	TG
<i>A. aegypti</i>				
μg/10 fat bodies	19 ± 5	11 ± 5	10 ± 4	707 ± 194
%	2.6	1.4	1.4	94.6
<i>M. sexta</i>				
μg/0.5 fat body	533 ± 45	100 ± 25	477 ± 129	10609 ± 3199
%	4.5	0.9	4.1	90.5

Values represent the mean ± SD of 8 determinations for *A. aegypti* and of 4 determinations for *M. sexta*.

plates (Si250, Baker) using a solvent system consisting of hexane–ethyl ether–acetic acid 60:40:1 (v/v/v) modified from Mangold (17). Mass and specific activity of individual lipid classes were determined by scraping lipids from the thin-layer plates (after visualizing with iodine vapor) and extracting the silica with chloroform. In the resulting sample, radioactivity was measured by liquid scintillation counting. Glycerides and fatty acid (FA) were quantified using the vanillin assay (18) with diolein as a standard. Phospholipid (PL) was quantified by phosphorus analysis according to Bartlett (19).

Lipase treatment of lipophorin

HDLp of both *A. aegypti* and *M. sexta* were treated with triacylglycerol lipase (TG-lipase) under identical conditions. HDLp was delipidated with TG-lipase from the yeast *Candida cylindracea* as described by Kawooya et al. (20). In short, 9.5 mg HDLp was incubated with 225 units of TG-lipase (Fluka, specific activity 36 U/mg) in the presence of 200 mg fatty acid-free bovine serum albumin (Boehringer Mannheim) in a total volume of 2 ml. Reactions proceeded for 3 h at 34°C and were stopped by the addition of EDTA to a final concentration of 10 mM. The reaction media were subjected to KBr density gradient ultracentrifugation as described above (16 h at 50,000 rpm and 4°C). Gradients were fractionated and lipophorins were identified by absor-

bance at 450 nm. Density of lipophorins was determined by refractometry.

RESULTS

Radiolabeling of fat body

The lipid composition of fat body did not differ significantly between *A. aegypti* and *M. sexta* (Table 1). Upon incubation of *M. sexta* fat body with [³H]oleic acid, radiolabel was incorporated mainly in TG (Table 2). In *A. aegypti* fat body, however, the majority of the [³H]oleic acid was divided equally among FA and TG. The same result was obtained when *A. aegypti* fat body was radiolabeled with [³H]palmitic acid (result not shown). Radiolabeling followed by a subsequent incubation with glucose (40 mM), glycerol (40 mM) or trehalose (40 mM) did not chase the radiolabeled FA into TG.

When *A. aegypti* fat body was radiolabeled with [³H]glycerol (Table 3), most of the radiolabel was incorporated in TG. When *A. aegypti* fat body was incubated in saline for 3 h prior to radiolabeling with [³H]glycerol, the extent of incorporation of radiolabel was similar to that in fat bodies radiolabeled immediately after dissection. This result indicates that, as far as lipid synthesis is concerned, the fat bodies were viable for at least 5 h.

Transfer of lipid from fat body to HDLp

To investigate whether neutral lipid is transferred from fat body to HDLp, radiolabeled fat bodies were incubated in vitro in medium containing HDLp from either *A. aegypti* or *M. sexta*. The data in Fig. 1 show the time course of transfer of radiolabeled lipid from fat body to HDLp. For both species an equilibrium was reached within 3 h of incubation, the time point chosen for further incubations.

The data in Fig. 2A show that when *A. aegypti* fat body was radiolabeled with [³H]oleic acid, radiolabeled lipid

TABLE 2. Radiolabeling of fat body

Fat Body	Lipid				
	PL	MG	DG	FA	TG
	% radioactivity				
<i>A. aegypti</i> ^a , incubated in:					
A-saline	8.3 ± 1.2	0.3 ± 0.1	2.1 ± 0.5	49.2 ± 5.5	40.1 ± 5.8
A-saline + 40 mM glucose	7.9	0.3	3.9	52.9	34.9
A-saline + 40 mM glycerol	7.3	0.3	3.4	43.5	45.5
A-saline + 40 mM trehalose	6.3	0.2	3.0	44.8	45.7
<i>M. sexta</i> ^a , incubated in:					
L-saline	10.9 ± 4.2	0.2 ± 0.1	7.8 ± 2.1	7.8 ± 5.3	73.3 ± 9.2

Fat bodies were homogenized and lipids were extracted, fractionated, and quantified as described in Materials and Methods. Values represent single measurements or the mean ± SD of 4 incubations of 10 fat bodies each for *A. aegypti* or of 10 incubations of one-half fat body each for *M. sexta*.

^aFat bodies were radiolabeled by incubation for 2 h with [³H]oleic acid, rinsed in saline, and incubated 1 h with indicated additions.

TABLE 3. Radiolabeling of *A. aegypti* fat body with [³H]glycerol

Incubation	Lipid				
	PL	MG	DG	FA	TG
2 h Radiolabeling with [³ H]glycerol + 3 h incubation in A-saline					
dpm × 10 ³	35 ± 9	0.3 ± 0.1	26 ± 12	0.7 ± 0.2	352 ± 59
% Radioactivity	8.5	<0.1	6.2	0.2	85.1
3 h Incubation in A-saline + 2 h radiolabeling with [³ H]glycerol					
dpm × 10 ³	47 ± 15	0.8 ± 0.3	39 ± 16	1.7 ± 0.2	389 ± 95
% Radioactivity	9.9	0.2	8.2	0.4	81.5

A. aegypti fat bodies were radiolabeled by incubation (for 2 h) in A-saline containing [³H]glycerol either preceded or followed by 3 h incubation in A-saline. After a total of 5 h of incubation, fat bodies were rinsed and homogenized; lipids were extracted, fractionated, and incorporation of radioactivity was quantified as described in Materials and Methods. Values represent the mean ± SD of 4 incubations.

was transferred from fat body to HDLp of both *A. aegypti* and *M. sexta*. *M. sexta* HDLp incorporated approximately twice as much radiolabeled lipid (41×10^3 dpm, total radioactivity in the peak) compared to *A. aegypti* HDLp (18×10^3 dpm). In the absence of HDLp in the medium, no radioactivity was released from the fat body (Fig. 2A), indicating that there was no detectable synthesis and secretion of radiolabeled HDLp by the fat body and that

radioactivity recovered in HDLp was due to loading of lipid from fat body by HDLp already present in the medium. Analysis of radioactivity in the individual lipid classes showed that most of the radioactivity in both lipophorins was in FA, whereas very little radiolabeled TG or DG was incorporated (Table 4).

When *A. aegypti* fat body was radiolabeled with [³H]glycerol (so that most of the radioactivity was localized in glycerides) and subsequently incubated with HDLp from either species, very little radioactivity could be detected in HDLp: 2.3×10^3 dpm and 1.6×10^3 dpm for *M. sexta* and *A. aegypti*, respectively (Fig. 2B).

When *M. sexta* fat body (radiolabeled with [³H]oleic acid) was incubated with *M. sexta* HDLp, transfer of radiolabeled lipid to HDLp occurred (Fig. 2C) and radioactivity in HDLp was localized mainly in DG (Table 4). When the same fat body was incubated with *A. aegypti* HDLp, radiolabeled lipid was transferred to HDLp. However, the amount of radiolabeled lipid transferred was approximately half (35×10^3 dpm) compared to that transferred to *M. sexta* HDLp (67×10^3 dpm). Also, in *A. aegypti* HDLp the radiolabel was localized mainly in DG (Table 4).

We investigated the possibility that the lack of TG transfer between fat body and HDLp in *A. aegypti* could be due to the physiological condition of the fat body. It could be argued that in sugar-fed adult females the overall need for lipid and thus the mobilization and transport of TG are low. It may be that lipid transport, and thus lipid transfer from fat body to HDLp, is more prominent, for example, after ingestion of a blood meal when egg development (requiring lipid accumulation) is initiated. A three-fold increase in HDLp concentration in female *A. aegypti* 48 h after ingestion of a blood meal has been described (21), indicating an increase in lipid transport. We repeated the experiment shown in Fig. 2B with fat bodies dissected from females 24 h after ingestion of a blood meal. However, the transfer of radiolabeled lipid to HDLp was identical to that obtained with fat bodies from females prior to blood feeding (results not shown).

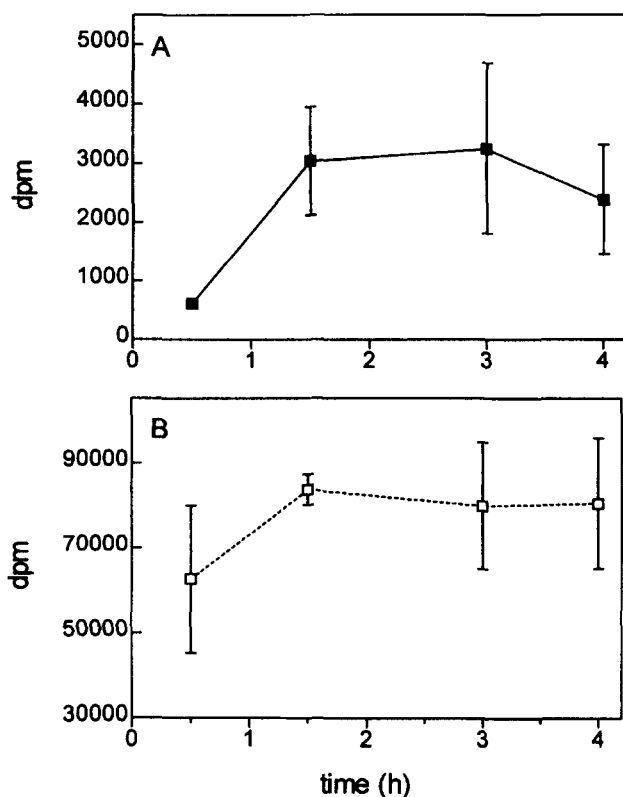


Fig. 1. Time course of transfer of radiolabeled lipid from fat body to HDLp. Radiolabeled fat body was incubated with HDLp as described under Materials and Methods. Incubation media were subjected to KBr density gradient ultracentrifugation and gradients were analyzed for radioactivity. Values represent the mean ± SD for 4 determinations. A: *A. aegypti* fat body with *A. aegypti* HDLp (—); B: *M. sexta* fat body with *M. sexta* HDLp (---).

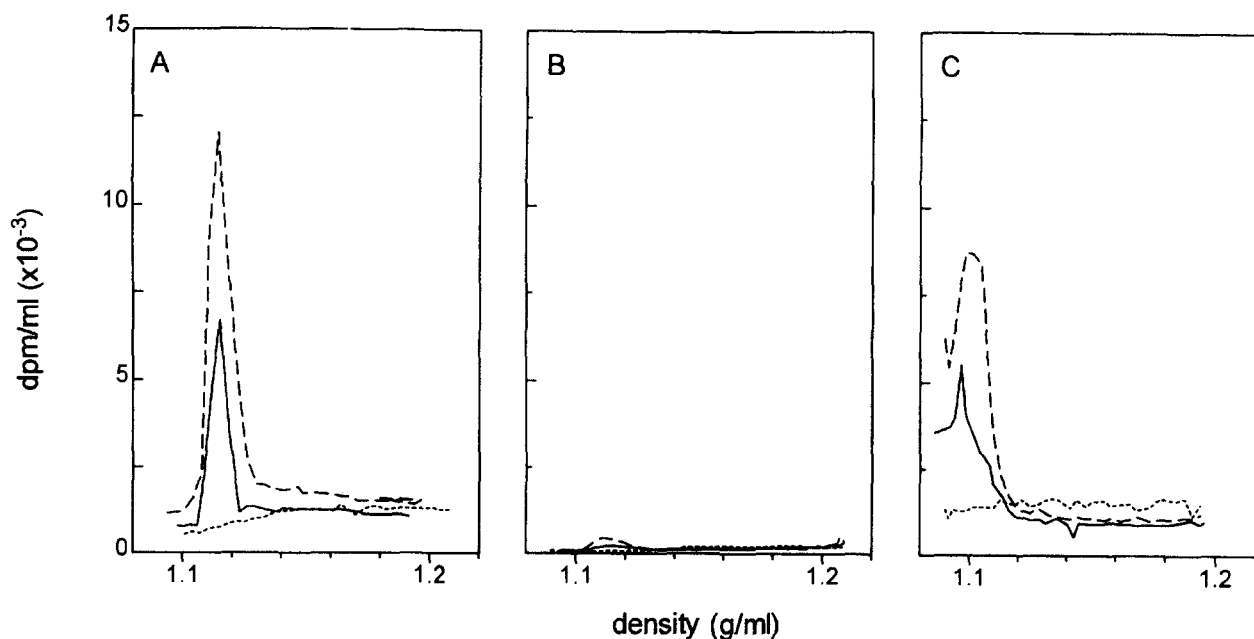


Fig. 2. Transfer of radiolabeled lipid from fat body to HDLp. A: Ten *A. aegypti* fat bodies were radiolabeled with [^3H]oleic acid and incubated in 100 μl A-saline without (control) or with 100 μg HDLp. B: Ten *A. aegypti* fat bodies were radiolabeled with [^3H]glycerol and incubated in 100 μl A-saline without (control) or with 400 μg HDLp. C: One-half fat body of *M. sexta* was radiolabeled with [^3H]oleic acid and incubated in 400 μl L-saline without (control) or with 400 μg HDLp. Incubation media were subjected to KBr density gradient ultracentrifugation. Gradients were fractionated and analyzed for density and radioactivity. For each experiment 4 incubations were performed, of which one is shown in this figure; (..) control, (- -) *M. sexta* HDLp, (-) *A. aegypti* HDLp.

Density of lipophorin

In both fat body experiments (*A. aegypti* fat body in Fig. 2A and *M. sexta* fat body in Fig. 2C) the density of HDLp of the two species was identical at the end of the incubation, even though the starting particles have different densities (Table 5). Because apolipoprotein-I and -II are integral components of HDLp that do not exchange, a change in density of HDLp implies a change in lipid composition. When incubated with *A. aegypti* fat body, *M. sexta* HDLp increased in density to that of *A. aegypti* HDLp, indicating that there was a net transfer of lipid from *M. sexta* HDLp to *A. aegypti* fat body. How-

ever, when incubated with *M. sexta* fat body, *A. aegypti* HDLp decreased in density to that of *M. sexta* HDLp, indicating that *A. aegypti* HDLp loaded lipid from *M. sexta* fat body (Table 5).

Delipidation of lipophorin by treatment with lipase

Kawooya et al. (20) have described the removal of DG from *M. sexta* HDLp with a TG-lipase from *C. cylindracea*, resulting in a density shift from 1.103 g/ml to 1.187 g/ml. The results in Fig. 3 show that, under identical conditions, TG was not removed from *A. aegypti* HDLp. Lipid analysis of the lipase-treated *A. aegypti* HDLp

TABLE 4. Transfer of radiolabeled lipid from fat body to lipophorin

Lipid	<i>A. aegypti</i> Fat Body Radiolabeled with [^3H]Oleic Acid		<i>M. sexta</i> Fat Body Radiolabeled with [^3H]Oleic Acid		<i>A. aegypti</i> Fat Body Radiolabeled with [^3H]Glycerol
	<i>Aa</i> -HDLp	<i>Ms</i> -HDLp	<i>Aa</i> -HDLp	<i>Ms</i> -HDLp	
	% radioactivity				
PL	2.9 \pm 0.5	2.8 \pm 0.7	3.4 \pm 1.0	1.5 \pm 0.4	85.0
MG	0.8 \pm 0.1	0.6 \pm 0.1	3.9 \pm 0.5	2.4 \pm 0.9	-
DG	1.5 \pm 0.2	1.3 \pm 0.4	79.6 \pm 4.1	84.4 \pm 4.8	14.0
FA	92.2 \pm 1.0	93 \pm 0.9	11.9 \pm 4.6	10.4 \pm 4.4	-
TG	1.7 \pm 0.3	1.6 \pm 0.3	1.1 \pm 0.2	0.5 \pm 0.2	1.0

Fat bodies of *A. aegypti* or *M. sexta* were radiolabeled with [^3H]oleic acid or [^3H]glycerol and incubated with HDLp of *A. aegypti* (*Aa*-HDLp, 4 mg/ml) or of *M. sexta* (*Ms*-HDLp, 1 mg/ml). Values represent the mean \pm SD of 4 incubations for the first 4 rows. For the last row, the media from 4 incubations were pooled. Isolation of HDLp and lipid analysis was performed on the pooled sample.

TABLE 5. Densities of HDLp before and after incubation with fat body

Fat Body	HDLp	Density g/ml
-	<i>A. aegypti</i>	1.117 ± 0.003
<i>A. aegypti</i>	<i>A. aegypti</i>	1.113 ± 0.002
<i>M. sexta</i>	<i>A. aegypti</i>	1.098 ± 0.003
-	<i>M. sexta</i>	1.103 ± 0.003
<i>A. aegypti</i>	<i>M. sexta</i>	1.111 ± 0.002
<i>M. sexta</i>	<i>M. sexta</i>	1.100 ± 0.001

Fat bodies from *A. aegypti* and *M. sexta* were incubated with HDLp from *A. aegypti* and *M. sexta* as described under Materials and Methods. Incubation media were subjected to KBr density gradient ultracentrifugation and densities of lipoproteins were determined. Values represent the mean ± SD of 4 incubations.

revealed that TG was not hydrolyzed (results not shown). It cannot be excluded that the inability of this TG-lipase to hydrolyze TG is due to the substrate specificity of the enzyme. Using a different TG-lipase, from the fungus *Rhizopus arrhizus*, TG could be removed efficiently from *A. aegypti* HDLp (result not shown), producing a particle with a density of 1.153 g/ml. However, compared to the delipidation of *M. sexta* HDLp with *R. arrhizus* TG-lipase, 5 times more enzyme was needed to delipidate *A. aegypti* HDLp, indicating that TG in *A. aegypti* HDLp is removed less efficiently than DG in *M. sexta* HDLp. The delipidated *A. aegypti* particle was physiologically inactive: it did not reload lipid from fat body of either *A. aegypti* or *M. sexta*. Similarly, *M. sexta* HDLp, delipidated with *R. arrhizus* TG-lipase, was not able to reload DG from *M. sexta* fat body (results not shown).

DISCUSSION

M. sexta HDLp, in which DG is the major neutral lipid, continuously exchanges DG with the fat body (8). We investigated whether the TG-rich HDLp of *A. aegypti* is able in a similar way to exchange TG with the fat body. When *A. aegypti* fat body was radiolabeled with [³H]oleic acid and subsequently incubated with *A. aegypti* HDLp, transfer of lipid from fat body to HDLp occurred (Fig. 2A). However, radioactivity was found mainly in FA (Table 4), even though fat body TG was labeled (Table 2). When *M. sexta* HDLp was incubated with *A. aegypti* fat body (labeled with [³H]oleic acid), it also incorporated mainly radiolabeled FA (Table 4). These results do not exclude the possible transfer of TG: because the major form of storage of lipid in the fat body is TG, the specific activity of TG in the fat body is much lower than that of FA. To circumvent this problem, *A. aegypti* fat body was radiolabeled with [³H]glycerol. When the latter fat body was incubated with *A. aegypti* HDLp, there

was little transfer of radiolabeled lipid to HDLp (Fig. 2B) and most of the radiolabel was localized in PL (Table 4).

To confirm that *A. aegypti* HDLp does not exchange TG as efficiently as *M. sexta* HDLp exchanges DG, we calculated how many moles of DG were transferred from fat body to HDLp in *M. sexta* and the amount of transfer of radioactivity that would be expected if a similar number of moles of TG would be transferred in *A. aegypti*. The amount of radiolabeled lipid transferred from fat body to HDLp in *M. sexta* amounted to 6.7×10^4 dpm (Fig. 2C); 84.4% of this radiolabel, or 5.6×10^4 dpm, was localized in DG (Table 4). With a specific activity of 4.7×10^6 dpm/mg DG (Table 6), this means that 12 µg or 19.3 nmol DG was incorporated in HDLp. If *A. aegypti* would transfer TG from fat body to HDLp as efficiently as the transfer of DG in *M. sexta*, then 19.3 nmol or 17.1 µg of TG should be transferred. However, the total amount of TG in 10 fat bodies of *A. aegypti* was 798 nmol, whereas the total amount of DG in one-half

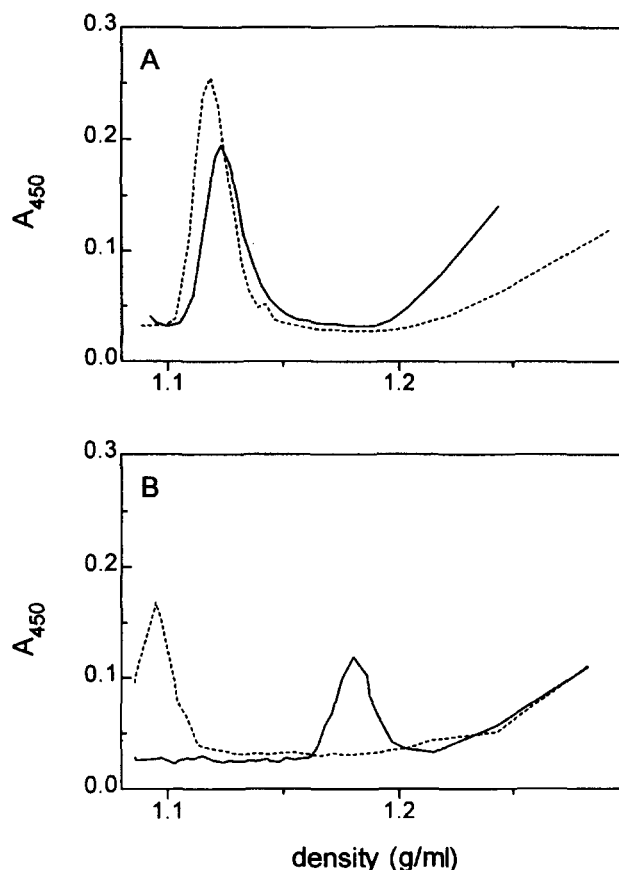


Fig. 3. Delipidation of HDLp with TG-lipase. HDLp from either *A. aegypti* (A) or *M. sexta* (B) was incubated with TG-lipase from *Candida cylindracea* under the conditions described under Materials and Methods. Incubation media were subjected to KBr density gradient ultracentrifugation. Gradients were fractionated and analyzed for lipoprotein (A₄₅₀) and density; (-) with TG-lipase, (- -) control without TG-lipase.

TABLE 6. Specific activity of radiolabeled DG and TG in fat body

Fat Body	Specific Activity	
	DG	TG
	<i>dpm</i> × 10 ³ / <i>mg</i>	
<i>A. aegypti</i>	5,247 ± 440	892 ± 91
<i>M. sexta</i>	4,663 ± 572	84 ± 16

Fat body of *A. aegypti* was radiolabeled with [³H]glycerol and fat body of *M. sexta* was radiolabeled with [³H]oleic acid for 2 h. Fat bodies were rinsed and homogenized; lipids were extracted, fractionated, and their specific activities were determined. Values represent the mean ± SD of 4 incubations.

fat body of *M. sexta* was 161 nmol (based on Table 1). Therefore, the incubation of *A. aegypti* fat body contained 5 times more TG, compared to DG in the incubation of *M. sexta* fat body. Thus, we could expect 5 times more, or 85.5 μg TG, to be transferred out of *A. aegypti* fat body. With a specific activity of 8.9 × 10⁶ dpm/mg for TG (Table 6), this means that 7.6 × 10⁴ dpm could be expected. The value observed, 1.6 × 10³ dpm (Fig. 2B) is approximately 39 times lower than the expected (calculated) value. Moreover, only 1% of the label in *A. aegypti* HDLp was localized in TG (Table 4). Thus we conclude that, under the conditions used, the transfer of TG from *A. aegypti* fat body to HDLp is much less efficient or absent, compared to the transfer of DG in *M. sexta*. In these calculations we assumed that the pool of radiolabeled DG in *M. sexta* fat body is homogeneous. If during the incubation the pool of DG is replenished from TG (which has a lower specific activity than DG, Table 6), the specific activity of DG would decrease. Thus the amount of DG transferred would be underestimated, and therefore the calculated transfer of TG in *A. aegypti* would even be higher than 85.5 μg. We also assumed that the pool of TG in *A. aegypti* fat body is homogeneous. Two reasons for a non-homogeneous distribution of radiolabeled TG in the fat body are possible: First, TG could be replenished from DG (which has a higher specific activity than TG, Table 6) during the incubation period, which would result in an increase of specific activity of TG. Second, the newly radiolabeled TG could remain localized more closely to the surface of the fat body and not partition equally over the entire fat body, which would mean that the specific activity of TG is underestimated. Because in both cases the specific activity of TG would only increase, the amount of expected radiolabeled TG transferred to lipophorin would only be higher. Thus, the two assumptions made do not affect the validity of the conclusion.

Adult *M. sexta* HDLp has a lower density than larval *A. aegypti* HDLp (Table 5). Upon incubation with *A. aegypti* fat body, lipophorins from both species equilibrated at identical density (Fig. 2A and B), which coin-

cides with the density of *A. aegypti* HDLp (Table 5). Because the protein content of lipophorin remains constant, a net transfer of lipid from *M. sexta* HDLp to *A. aegypti* fat body occurred. On the other hand, upon incubation with *M. sexta* fat body (Fig. 2C), both lipophorins equilibrated at the density of *M. sexta* HDLp (Table 5). Therefore, *A. aegypti* HDLp had loaded lipid from *M. sexta* fat body. However, the lipids incorporated by *A. aegypti* HDLp were mainly DG and FA, but not TG. The results show that upon incubation with fat body, the final density of lipophorin (and thus the net transfer of lipid) as well as the type of lipid that is transferred, is dictated by the fat body.

When the two lipophorins are compared in their capacity to exchange FA with *A. aegypti* fat body (Fig. 2A), it is clear that *A. aegypti* HDLp has incorporated half as much radiolabeled FA. Similarly, when comparing their capacity to load DG from *M. sexta* fat body (Fig. 2C), *A. aegypti* HDLp incorporated half as much radiolabeled DG. Although these results indicate that lipid transfer to *A. aegypti* HDLp is less efficient than to *M. sexta* HDLp, it is not possible to conclude, based on the experiments presented here, what causes this difference.

If HDLp functions as a reusable shuttle, it should be able not only to load, but also to unload lipid without destruction of the particle. In support of its shuttle function is the fact that both DG and PL can be removed from HDLp by *in vitro* treatment with lipases (20, 22). The delipidated lipophorin is able to reload DG or PL at the fat body (22, 23). When *A. aegypti* HDLp was treated with TG-lipase, no TG was removed. This result suggests that TG cannot readily be removed from *A. aegypti* HDLp and indicates that there are structural differences between the lipophorins of the two species.

Insects store lipid in the form of TG in their fat body. Although part of the lipid may be synthesized from carbohydrate precursors, part is also synthesized from FA originating in the hemolymph. Our results indicate that, under the conditions used, there is a difference in the utilization of FA and the incorporation into TG in the fat body, between *A. aegypti* and *M. sexta*. When *M. sexta* fat body was incubated with [³H]oleic acid, all FA incorporated into the fat body was converted mainly into TG. In *A. aegypti*, however, half of the [³H]oleic acid that was taken up by the fat body was not esterified and remained as FA (Table 2). This phenomenon of partial incorporation of FA into glycerides is not understood yet. Synthesis of glycerides may depend on the physiological state of the fat body. In *A. aegypti*, lipid reserves in the adult fat body are carried over from the larval stage (24) and therefore synthesis of TG may be very low in adult fat body. The presence of [³H]oleic acid in the fat body also could be related to the relative abundance

(\approx 14%) of FA in *A. aegypti* HDLp (10). However, because the latter determination was performed on a lipophorin preparation isolated from a homogenate of total insects ("homogenate-HDLp"), the abundance of FA could be an artifact introduced by the homogenization step. We collected hemolymph from 500 adult female mosquitoes and isolated HDLp. The lipid composition of this "hemolymph-HDLp" was identical to that of "homogenate-HDLp" (results not shown), indicating that homogenization of total mosquitoes does not affect the lipid composition of HDLp. We did, however, measure a lower content of FA (\approx 7%), along with a lower content of DG (\approx 7%) and a higher content of TG (\approx 41%) compared to the previously reported values (\approx 14%, \approx 14%, and \approx 32% for FA, DG, and TG, respectively; 10). This shift in relative abundance of lipid classes can be explained by the lower concentrations of protease/lipase inhibitors used in the first analysis (2 mM of DFP and 2 mM of PMSF; 10) compared to this study (4 mM of each inhibitor).

In conclusion, the data presented here indicate that, under the conditions used, transfer of neutral lipid from fat body to HDLp is less efficient in *A. aegypti* than in *M. sexta*. The fat body dictates the amount of net lipid transfer, whereas HDLp influences the efficiency of lipid exchange. Therefore, the inefficiency of lipid exchange between fat body and HDLp in *A. aegypti* may be attributed to both fat body and lipophorin.

The inefficient lipid transfer in *A. aegypti* could be due to the presence of TG in *A. aegypti* HDLp. It may be more difficult to transfer TG between a cell membrane and a lipoprotein. If this is the case, then it could be that *A. aegypti* HDLp does not act as a reusable lipid shuttle, but instead is synthesized and secreted from the fat body with its full load of lipid, and is degraded after delivery of the lipid to target tissues. Another explanation for the inefficient lipid transfer in *A. aegypti* could be that a lipid transfer particle (LTP; 25–28) is necessary to promote transfer of TG from fat body to HDLp. In *M. sexta*, LTP is present at the surface of the fat body and mediates DG transfer from fat body to HDLp (8). Perhaps an "LTP-like" molecule is present in *A. aegypti* that occurs only in hemolymph. If it does not associate with the fat body, it would be absent in the experimental conditions used here. We are currently investigating this possibility.

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