In vivo and in vitro loading of lipid by artificially lipid-depleted lipophorins: evidence for the role of lipophorin as a reusable lipid shuttle

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Abstract Lipid transport in the hemolymph of *Manduca sexta* is facilitated by a high density lipophorin in the resting adult insect (HDLp-A, d ~ 1.109 g/ml) and by a low density lipophorin during flight (LDLp, d ~ 1.060 g/ml). Lipophorin presumably shuttles different lipids between sites of uptake or storage, and sites of utilization. In order to shuttle lipid, a lipid-depleted lipophorin should be able to reload with lipid. To test this hypothesis, we used HDLp-A particles that were artificially depleted of either phospholipid (d ~ 1.118 g/ml) or diacylglycerol (d ~ 1.187 g/ml) and subsequently radiolabeled in their protein moiety. Upon injection into adult moths, both particles shifted their density to that of native HDLp-A, indicating lipid loading. Also, upon subsequent injection of adipokinetic hormone, both particles shifted to a lower density (d ~ 1.060 g/ml) indicating diacylglycerol loading and conversion to LDLp. Both phospholipid and diacylglycerol loading were also studied using an in vitro system. The lipid-depleted particles were incubated with fat body that had been radiolabeled in either the phospholipid or the triacylglycerol fraction. Transfer of radiolabeled phospholipid and diacylglycerol from fat body to lipophorin was observed. During diacylglycerol loading, apoLp-III associated with lipophorin, whereas phospholipid loading occurred in the absence of apoLp-III. The results show the ability of lipid-depleted lipophorins to reload with lipid and therefore reaffirm the role of lipophorin as a reusable lipid shuttle.—**van Heusden, M. C., D. J. van der Horst, J. K. Kawooya, and J. H. Law.** In vivo and in vitro loading of lipid by artificially lipid-depleted lipophorins: evidence for the role of lipophorin as a reusable lipid shuttle. *J. Lipid Res.* 1991. **32:** 1789–1794.

Supplementary key words *Manduca sexta* • diacylglycerol • phospholipid • fat body • apolipophorin III

High density lipophorin (HDLp), the multifunctional lipoprotein of insect hemolymph, is composed of two apolipoproteins (apolipophorin I, apoLp-I, $M_r \approx 250 \times 10^3$ and apolipophorin II, apoLp-II, $M_r \approx 80 \times 10^3$) and approximately 50% lipid, mainly diacylglycerol (DAG) and phospholipid (PL) (1–4). In the tobacco hornworm, *Manduca sexta,* HDLp-A of the adult moth contains in addition two molecules of a third apolipoprotein, apolipophorin III (apoLp-III, $M_r \approx 17 \times 10^3$) (5). In insect species that rely on the supply of lipid as fuel for sustained flight, such as *M. sexta* and the migratory locust, *Locusta migratoria,* flight activity necessitates increased transport of lipid from fat body to flight muscles. An adipokinetic hormone (AKH), released from the corpora cardiaca, induces DAG mobilization from fat body (4). By loading DAG from fat body and associating with additional molecules of apoLp-III, HDLp-A is converted to a low density lipophorin, LDLp (5–9).

Insect lipophorin has been suggested to function as a reusable shuttle in the transport of lipids through the hemolymph (10, 11). Supporting this hypothesis is the longer turnover time of the protein components of HDLp-A compared to its lipids (12). Similar to HDLp-A, the flight-specific LDLp is recycled during the transport of DAG to the active flight muscle. LDLp does not enter the muscle cell during hydrolysis of DAG by the lipoprotein lipase (13) but its components (HDLp-A and apoLp-III) are released in the hemolymph and reload DAG at the fat body (14).

In the accompanying report (15), structural features of lipophorin were deduced by artificially unloading DAG or PL from the particle. The predicted structure was consistent with the postulated function of lipophorin as a lipid

Abbreviations: HDLp, high density lipophorin; LDLp, low density lipophorin; apoLp-I, apolipophorin I; apoLp-II, apolipophorin II; apoLp-III, apolipophorin III; DAG, diacylglycerol; PL, phospholipid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; PLA_2, phospholipase A_2; TAG-lipase, triacylglycerol lipase; AKH, adipokinetic hormone; LTP, lipid transfer particle; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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shuttle. However, the structural characterization alone based on lipid-unloading does not substantiate the shuttle function of lipophorin. This aspect of lipophorin can be proved by determining whether the lipid-depleted particle has the capacity to reload DAG or PL from fat body, to associate with apoLp-III, and to form LDLp. The purpose of this report was, therefore, to investigate the ability of artificially lipid-depleted lipophorin particles to reload DAG and PL from fat body, using both in vivo and in vitro techniques.

EXPERIMENTAL PROCEDURES

Materials

M. sexta were raised from eggs as previously described (16). Adult 1–2-day-old male animals were used in all experiments. Sulphur-protein labeling reagent (^{35}SLR, t-butoxycarbomyl-l-[^{35}S]methionine-N-hydroxysuccinimidyl-ester) was purchased from Amersham Corporation, Arlington Heights, IL. Sources of other materials were as described by Kawooya et al. (15).

Preparation of lipophorins

Lipophorins were isolated from hemolymph by density gradient ultracentrifugation, subsequently treated with phospholipase A_2 (PLA_2) or triacylglycerol lipase (TAG-lipase) and purified as described by Kawooya et al. (15). In order to maintain the same nomenclature system as that used by Kawooya et al. (15), the PLA_2-treated and TAG-lipase-treated HDLp-A will be designated hereafter Lp-4 and Lp-5, respectively. In the report of Kawooya et al. (15), Lp-4 was separated from BSA by density gradient ultracentrifugation in a potassium bromide gradient. However, evidence that this preparation was still contaminated with traces of PLA_2 activity was obtained from pilot in vitro experiments in which the phospholipids taken up by Lp-4 appeared to be entirely in a lysophospholipid form. Therefore, the Lp-4 was subjected to an additional purification step by gel permeation chromatography. The Lp-4 was applied to an Ultrogel AcA22 column (200 × 0.9 cm) which was eluted with phosphate-buffered saline (PBS, 0.1 M Na-phosphate, 0.15 M NaCl, pH 7.0).

ApoLp-III was prepared as described by van Heusden and Law (17). Lipophorin was labeled with ^{32}P in its phospholipids and isolated from hemolymph as described by Kawooya et al. (15).

In vivo experiments

Modified lipophorin particles (treated with lipases as described by Kawooya et al. (15), densities as indicated in Table 1) were radiolabeled in their protein moiety with 50 μl of ^{35}SLR (sp act 800 Ci/mmol) as described in the manufacturer's specifications. Labeled lipophorin was separated from labeling reagent by passing it twice over a PD10 desalting column. Labeled lipophorin (sp act as indicated in legend of Table 1) was injected into animals. Experimental animals were injected after 1 h with synthetic *M. sexta* AKH (10 pmol/animal). Two h after the lipophorin injection, hemolymph was collected and subjected to density gradient ultracentrifugation as described by Kawooya et al. (15). Gradients were fractionated and apparent (non-equilibrium) densities of radiolabeled lipophorins were determined by refractometry.

In vitro experiments

In vitro incubations were performed essentially as described by van Heusden and Law (17). In the experiments investigating PL transport, fat bodies were labeled by incubating a 1/2 fat body in 500 μl lepidopteran saline (18) that contained 32 μCi ^{32}P for 4 h at room temperature. Fat bodies were rinsed 6 times in saline and transferred to tubes that contained incubation medium. In the experiments investigating DAG transport, fat bodies were labeled with [^{3}H]oleic acid (sp act 10 Ci/mmol) as described by van Heusden and Law (17).

Lipophorin and apoLp-III were equilibrated into saline using a PD10 desalting column, prior to addition to the incubation medium. Final incubations were performed for 3 h at room temperature under constant shaking, with a 1/2 fat body in 200 μl saline that contained native or modified lipophorin (5 mg/ml) with or without apoLp-III (5 mg/ml) as indicated under Results. Incubations were ended by careful removal of the fat bodies and the media were subjected to density gradient ultracentrifugation as described above. The media in which ^{32}P-labeled fat body was incubated were desalted over a PD10 column prior to density gradient ultracentrifugation, in order to remove free ^{32}P or any small ^{32}P-containing compounds released into the incubation media. After ultracentrifugation, gradients were fractionated and apparent densities were determined.

Other procedures

Protein determination, SDS-PAGE, lipid extraction, lipid analysis by thin-layer chromatography, and radioactivity measurements were performed as described by Kawooya et al. (15).

RESULTS

In vivo studies on the biological activity of lipid-depleted lipophorins

Three lipid-depleted particles were tested for their ability to load lipid in vivo: PLA_2-treated HDLp-A or Lp-4, TAG-lipase-treated HDLp-A or Lp-5, and TAG-lipase-treated LDLp. The latter particle was obtained by limited treatment of LDLp with TAG-lipase such that only part of its DAG was removed. The resulting particle had an

TABLE 1. In vivo lipid loading by lipid-depleted lipophorin particles

	HDLp-A	Lp-4	Lp-5	LDLp	LDLp, Treated with TAG-Lipase
Purified lipophorin	1.109	1.118	1.187	1.060	1.110
After injection into animal		1.112	1.110		1.110
After injection into animal, followed by AKH-injection		1.060	1.060		1.060

PLA_2-treated HDLp-A or Lp-4 (0.43 mg Lp-4 in 10 μl/animal, sp act 12,340 cpm/μg), TAG-lipase-treated HDLp-A or Lp-5 (0.09 mg Lp-5 in 40 μl/animal, sp act 12,740 cpm/μg), and TAG-lipase-treated LDLp (0.10 mg lipophorin in 40 μl/animal, sp act 14,210 cpm/μg) were injected into adult moths. Hemolymph was collected, lipophorin particles were purified and densities of radiolabeled lipophorins were measured as described under Experimental Procedures. Values represent mean densities (g/ml) of three experiments, with a variation of 0.005 g/ml between experiments.

apoLp-III content and a density (1.11 g/ml) similar to that of native HDLp-A. Thus this particle resembled the physiological lipophorin after hydrolysis of DAG from LDLp by the flight muscle lipoprotein lipase. **Table 1** shows the apparent densities of the particles before and after injection into animals. Upon injection, all three particles loaded lipid until they reached the density of native HDLp-A. In the presence of AKH all three particles loaded DAG and were converted to LDLp (Table 1).

In vitro studies on lipid loading by lipid-depleted lipophorin

In order to study in more detail the actual process of DAG- and PL-loading by the artificially altered lipophorins, the latter were incubated in vitro with fat body tissue.

PLA_2-treated HDLp or Lp-4

Uptake of PL by Lp-4 was investigated by ^{32}P-labeling of fat body PL. Incubation of fat body with [^{32}P]orthophosphate resulted in the incorporation of radiolabel in phosphatidylcholine (PC, 65%) and phosphatidylethanolamine (PE, 30%), but not in lysophosphatidylcholine (LPC) or lysophosphatidylethanolamine (LPE) (**Fig. 1a**). In the control experiment, ^{32}P-labeled fat body was incubated in saline (in the absence of lipophorin) (**Fig. 2a**). Absence of radiolabel in the medium after density gradient ultracentrifugation shows that no radiolabeled lipophorin was synthesized and secreted from the fat body. Subsequently, ^{32}P-labeled fat body was incubated with Lp-4, after which lipophorin was isolated by density gradient ultracentrifugation. Radiolabel appeared in the lipophorin (Fig. 2b) indicating that Lp-4 had loaded PL from fat body. Since no radiolabel could be detected in the medium after incubation of ^{32}P-labeled fat body in saline, the radiolabel present in lipophorin upon incubation with ^{32}P-labeled fat body is entirely due to uptake of radiolabeled PL from fat body by lipophorin already present in the medium. Uptake of PL was confirmed by lipid analysis of the labeled lipophorin; after TLC separation of extracted lipids, followed by radioscanning, radiolabel was

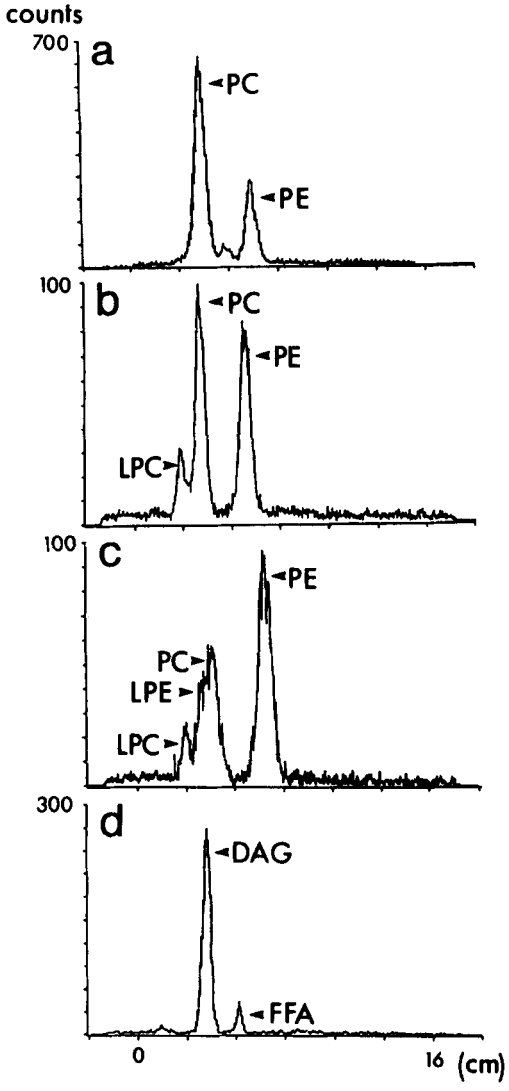

Fig. 1. Localization of radiolabeled lipids in fat body and lipophorin. Thin-layer chromatographic analysis of radiolabeled lipids extracted from (a) fat body after labeling with ^{32}P; (b) Lp-4 after incubation with [^{32}P]-labeled fat body; (c) native HDLp-A after incubation with [^{32}P]-labeled fat body; and (d) Lp-5 after incubation with [^{3}H]-labeled fat body. Incubations were performed as described under Experimental Procedures. Extracted lipids were separated by thin-layer chromatography and scanned with a TLC linear analyzer.

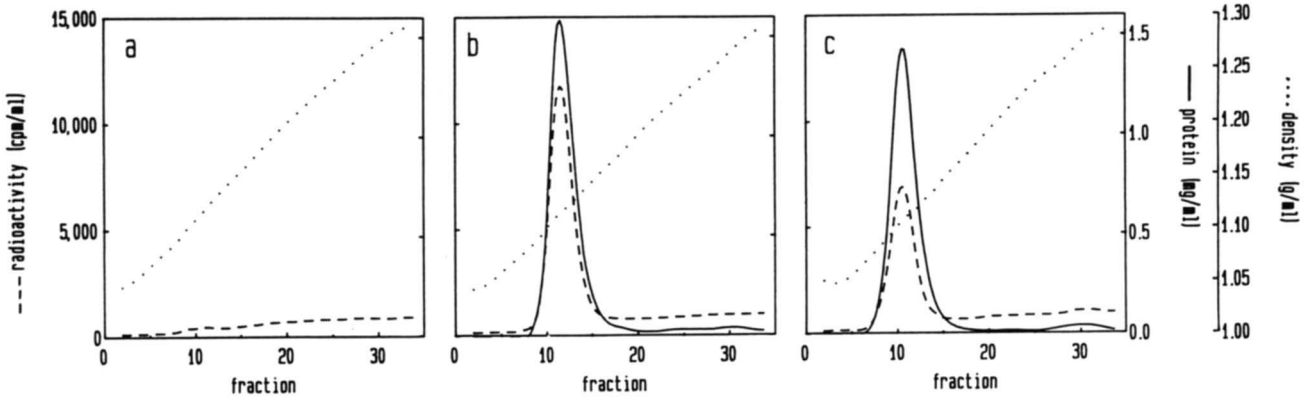

Fig. 2. In vitro phospholipid uptake by lipophorin. [^{32}P]-labeled fat body was incubated (a) in saline; (b) with Lp-4 and (c) with native HDLp-A as described under Experimental Procedures. After incubation, the media were subjected to density gradient ultracentrifugation; gradients were fractionated and protein, radioactivity, and density were determined in each fraction.

present in both PE and PC and to a minor extent in LPE and LPC (Fig. 1b). On a one-dimensional thin-layer chromatogram (after staining) PC and LPE could be detected as distinct spots despite their close migration. However, when scanned for radioactivity with the TLC linear isotope analyzer, the activity peaks in PC and LPE overlapped to some extent. Therefore, phospholipid classes could not be quantified accurately.

In an attempt to understand further the process of phospholipid transfer, in vitro incubations were performed in which ^{32}P-labeled fat body was incubated with native HDLp-A. The results presented in Fig. 2c show that even native HDLp-A incorporated labeled PL from fat body, to a similar extent as Lp-4. TLC analysis of lipids extracted from the native HDLp-A after incubation with fat body showed that radiolabel was present mainly in PE and PC and to some extent in LPE and LPC (Fig. 1c). The distribution of radiolabel was very similar to that in the PL of Lp-4 after incubation with ^{32}P-labeled fat body (Fig. 1b).

TAG-lipase-treated HDLp or Lp-5

Lp-5 (depleted in DAG) had a density of 1.187 g/ml (versus 1.109 g/ml for intact HDLp-A) and was devoid of apoLp-III (15). Lp-5 and apoLp-III were incubated in vitro with fat body that was labeled with [^{3}H]oleic acid. After incubation, Lp-5 contained radiolabel and had shifted to a density similar to that of HDLp-A (1.11 g/ml). Since secretion of radiolabeled nascent HDLp from ^{3}H-labeled fat body is not detectable under the conditions used (as described by van Heusden and Law, 17), the results presented here show that Lp-5 had loaded radiolabeled lipid from ^{3}H-labeled fat body. Thin-layer chromatographic analysis of lipids, extracted from lipophorin after incubation with ^{3}H-labeled fat body, showed that radiolabel was incorporated in DAG (Fig. 1d). Moreover, SDS-PAGE analysis of the lipophorin (**Fig. 3**) showed that Lp-5 had reassociated with apoLp-III during DAG-loading.

DISCUSSION

Whereas different lipoprotein populations of varying density and with specific functions have been described in mammalian species (19), insects have one major multifunctional lipoprotein particle, the high density lipophorin or HDLp (1–4, 11). The lipid transport mechanism of

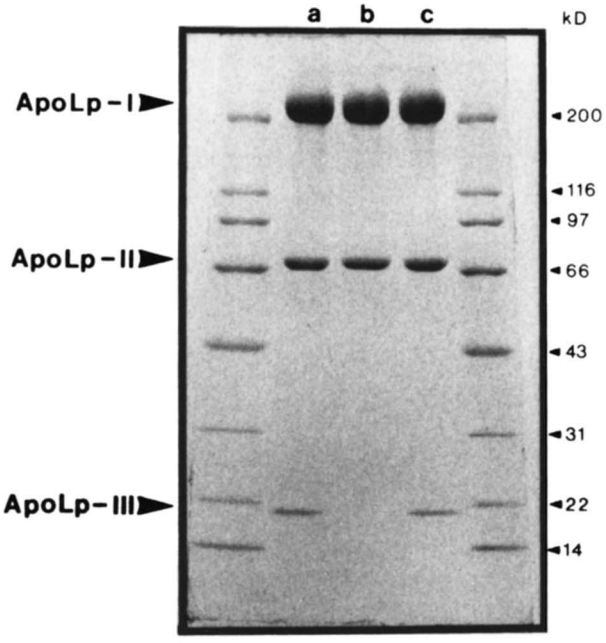

Fig. 3. Apolipoprotein composition of lipophorin. Apolipoprotein composition, as determined by SDS-PAGE analysis (4–15% slab gel), of (a) native HDLp-A, (b) Lp-5, and (c) Lp-5 after incubation with fat body and apoLp-III (as described under Results). Apolipoproteins and molecular weight markers are indicated.

the insect lipophorin is very efficient: supposedly the particle recycles by repeatedly loading, transporting, and delivering a range of lipids between various tissues, without cellular uptake and degradation of its basic matrix structure (10, 11). A continuous exchange and net transfer of DAG and PL between lipophorin particles occurs in hemolymph and is mediated by a lipid transfer particle (LTP) in both *M. sexta* (20, 21) and *L. migratoria* (22). Moreover, a continuous exchange of DAG between lipophorin and fat body in vitro has been described (17). Since lipophorin seems to load and unload certain lipids quite easily without major structural changes in the particle, the exchangeable lipids have to be readily accessible to the environment. Also lipophorin should be stable both in a loaded and unloaded state. In the accompanying paper (15) it was shown that PL and part of the DAG are readily accessible to lipases and are therefore located at the lipophorin–water interface. Furthermore, it was shown that a DAG-depleted lipophorin is stable and morphologically similar to native lipophorin. According to the shuttle hypothesis, a lipid-depleted lipophorin should be able to reload lipid from fat body and function in a manner similar to native lipophorin. To investigate this, lipase-treated particles were injected into the animal and their capacity to reload PL and DAG from fat body was investigated. As judged by changes in densities, DAG-depleted HDLp-A appeared to adjust rapidly its density to that of native HDLp-A. Subsequently, the particle was converted to LDLp upon AKH injection. Although loading of PL by PL-depleted HDLp-A cannot be shown experimentally in vivo, the particle did convert to LDLp under the influence of AKH. The results show that both PL- and DAG-depleted lipophorins are biologically active.

Further analysis of PL and DAG loading by the modified lipophorin particles was performed by in vitro experiments in which lipophorins were incubated with isolated fat body, radiolabeled in its lipids. Lp-4 was shown to be able to load PL from the fat body. The results shown in Fig. 1 demonstrate that radioactivity in the lipophorin was present in both PC and PE in equal amounts. However, fat body contained more radiolabeled PC than PE. Although differential rate of ^{32}P-incorporation into fat body PL (resulting in different specific activities) cannot be excluded, the results reported here might indicate an enhanced uptake of PE into lipophorin, compared to PC. ^{32}P-labeled fat body did not contain radiolabeled lysophospholipids. However, both Lp-4 and native HDLp-A contained radiolabeled LPC and LPE upon incubation with labeled fat body (Fig. 1b,c). This indicates that some PL loaded by lipophorin is converted to lysophospholipid in the lipophorin particle, or during its transfer from fat body to lipophorin. Whether lipophorin contains phospholipase activity itself, or the activity is present at the fat body cell surface, is not known. Uptake of radiolabeled PL by native HDLp-A upon incubation with ^{32}P-labeled fat body (even though HDLp-A is "normally loaded" with PL) suggests that a continuous exchange of PL occurs between HDLp-A and fat body. This would be similar to the exchange of DAG between fat body and HDLp-A or LDLp (17). The exchange of PL between HDLp-A and the fat body cell could be partly explained by diffusion due to possible differences in PL composition between the plasma membrane and HDLp-A. Also, it will be of interest to investigate whether LTP is involved in this PL-transfer, as it is in DAG-transfer (17). The in vitro experiments show that Lp-4 loads PL from fat body, up to a content of native HDLp-A. This indicates that, when injected into animals, Lp-4 probably rapidly adjusts its density to that of native HDLp-A by loading PL, and explains the normal LDLp formation upon subsequent injection of AKH.

Lp-5 was incubated with [^{3}H]oleic acid-labeled fat body. ApoLp-III was added to the incubation medium, since native HDLp-A contains two molecules of apoLp-III, and apoLp-III is stripped from HDLp-A during treatment with TAG-lipase. Lp-5 was able to load DAG up to the density of native HDLp-A (Table 1). Most interestingly, the particle had associated with apoLp-III as shown in Fig. 3. These results indicate that the apoLp-III is necessary for the stabilization of the extra DAG on lipophorin. It has been shown that some LTP is present at the fat body cell surface and promotes DAG transfer from fat body to lipophorin as part of a continuous DAG exchange process between HDLp-A and fat body. However, this fraction of LTP is apparently not sufficient for net transfer of large quantities of DAG; additional LTP has to be added to the medium in order to achieve extensive mobilization of DAG from fat body to lipophorin, resulting in LDLp-formation (17). Our results show that when a DAG-depleted particle is incubated with fat body, some net DAG-transfer and association with apoLp-III occurs, apparently in the absence of added LTP. It is likely that the LTP present at the fat body cell surface is involved in this limited process of DAG-loading. Both in vivo and in vitro results show that DAG-depleted lipophorin loads DAG up to a density similar to that of native HDLp-A. This limited uptake of lipid could be due to the reaching of an equilibrium state between the accessible pool of DAG in lipophorin and in the fat body. However, it could also be that other factors, such as AKH and/or LTP are essential for further DAG-loading of lipophorin.

Lipid loading of HDLp-A at the fat body involves receptor-mediated binding of lipophorin to the fat body cell surface (23, 24). Our results show that the removal of PL does not abolish this interaction. Therefore PL (with the possible exception of sphingomyelin, which is not removed by PLA_2, [15]) is not directly involved in receptor-binding and its removal does not eliminate the exposure of the receptor-binding domain(s) of lipophorin. Apparently, removal of DAG also does not abolish the interaction of lipophorin with its receptor. Kawooya et al. (15)

suggested that when DAG is enzymatically removed from the particle surface, it could be replaced by DAG which moves from the core of the particle to the surface. Therefore a DAG-depleted particle could still have some DAG located at the particle–water interface and an involvement of DAG in the interaction of lipophorin with its fat body receptor cannot be excluded.

Conclusions

Although the experiments reported here are qualitative rather than quantitative, the results do show that a PL-depleted lipophorin is able to reload PL from fat body. In addition, the results suggest that a continuous exchange of PL occurs between fat body and HDLp-A. A lipophorin particle that is stripped of a major part of its DAG is able to reload DAG from fat body up to its original density. During DAG-loading the particle associates with apoLp-III and under the influence of AKH it converts to LDLp. These data support the hypothesis that lipophorin functions as a reusable shuttle for the transport of DAG and PL. The possible shuttle function of lipophorin for lipids other than PL and DAG remains unknown.

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REFERENCES

1. Beenakkers, A. M. T., D. J. van der Horst, and W. J. A. van Marrewijk. 1985. Insect lipids and lipoproteins, and their role in physiological processes. *Prog. Lipid Res.* **24:** 19–67.
2. Chino, H. 1985. Lipid transport: biochemistry of hemolymph lipophorin. *In* Comprehensive Insect Physiology, Biochemistry and Pharmacology. G. A. Kerkut and L. I. Gilbert, editors. Vol. 10. Pergamon Press, Oxford. 115–135.
3. Shapiro, J. P., J. H. Law, and M. A. Wells. 1988. Lipid transport in insects. *Annu. Rev. Entomol.* **33:** 297–318.
4. Kanost, M. R., J. K. Kawooya, J. H. Law, R. O. Ryan, M. C. van Heusden, and R. Ziegler. 1990. Insect haemolymph proteins. *Adv. Insect Physiol.* **22:** 299–396.
5. Wells, M. A., R. O. Ryan, J. K. Kawooya, and J. H. Law. 1987. The role of apolipophorin-III in in vivo lipoprotein interconversions in adult *Manduca sexta*. *J. Biol. Chem.* **262:** 4172–4176.
6. Mwangi, R. W., and G. J. Goldsworthy. 1977. Diglyceride-transporting lipoproteins in *Locusta migratoria*. *J. Comp. Physiol.* **114:** 177–190.
7. van der Horst, D. J., J. M. van Doorn, and A. M. T. Beenakkers. 1979. Effects of the adipokinetic hormone on the release and turnover of haemolymph diglycerides and on the formation of the diglyceride-transporting lipoprotein system during locust flight. *Insect Biochem.* **9:** 627–635.
8. van der Horst, D. J., J. M. van Doorn, and A. M. T. Beenakkers. 1984. Hormone-induced rearrangement of locust haemolymph lipoproteins: the involvement of glycoprotein C_2. *Insect Biochem.* **14:** 495–504.
9. Shapiro, J. P., and J. H. Law. 1983. Locust adipokinetic hormone stimulates lipid mobilization in *Manduca sexta*. *Biochem. Biophys. Res. Commun.* **115:** 924–931.
10. Chino, H., and K. Kitazawa. 1981. Diacylglycerol-carrying lipoprotein of hemolymph of the locust and some insects. *J. Lipid Res.* **22:** 1042–1052.
11. Chino, H., R. G. H. Downer, G. R. Wyatt, and L. I. Gilbert. 1981. Lipophorins, a major class of lipoproteins of insect haemolymph. *Insect Biochem.* **11:** 491.
12. Downer, R. G. H., and H. Chino. 1985. Turnover of protein and diacylglycerol compounds of lipophorin in insect hemolymph. *Insect Biochem.* **15:** 627–630.
13. van Antwerpen, R., W. A. M. Linnemans, D. J. van der Horst, and A. M. T. Beenakkers. 1988. Immunocytochemical localization of lipophorins in the flight muscles of the migratory locust (*Locusta migratoria*) at rest and during flight. *Cell Tissue Res.* **252:** 661–668.
14. van Heusden, M. C., D. J. van der Horst, J. Voshol, and A. M. T. Beenakkers. 1987. The recycling of protein components of the flight-specific lipophorin in *Locusta migratoria*. *Insect Biochem.* **17:** 771–776.
15. Kawooya, J. K., D. J. van der Horst, M. C. van Heusden, B. L. J. Brigot, R. van Antwerpen, and J. H. Law. 1991. Lipophorin structure analyzed by in vitro treatment with lipases. *J. Lipid Res.* **32:** 1781–1788.
16. Prasad, S. V., R. O. Ryan, J. H. Law, and M. A. Wells. 1986. Changes in lipoprotein composition during larval-pupal metamorphosis of an insect, *Manduca sexta*. *J. Biol. Chem.* **261:** 558–562.
17. van Heusden, M. C., and J. H. Law. 1989. An insect lipid transfer particle promotes lipid loading from fat body to lipoprotein. *J. Biol. Chem.* **264:** 17287–17292.
18. Jungreis, A. M., P. Jatlow, and G. R. Wyatt. 1973. Inorganic ion composition of haemolymph of the cecropia silkmoth: changes with diet and ontogeny. *J. Insect Physiol.* **19:** 225–233.
19. Nestel, P. J. 1987. The regulation of lipoprotein metabolism. *In* New Comprehensive Biochemistry. A. M. Gotto, Jr., editor. Vol. 14: Plasma lipoproteins. Elsevier, Amsterdam. 153–182.
20. Ryan, R. O., K. R. Senthilathipan, M. A. Wells, and J. H. Law. 1988. Facilitated diacylglycerol exchange between insect hemolymph lipophorins. *J. Biol. Chem.* **263:** 14140–14145.
21. Ryan, R. O., A. Howe, and D. G. Scraba. 1990. Studies of the morphology and structure of the plasma lipid transfer particle from the tobacco hornworm, *Manduca sexta*. *J. Lipid Res.* **31:** 871–879.
22. Hirayama, Y., and H. Chino. 1990. Lipid transfer particle in locust hemolymph: purification and characterization. *J. Lipid Res.* **31:** 793–799.
23. van Antwerpen, R., H. J. A. Wynne, D. J. van der Horst, and A. M. T. Beenakkers. 1989. Binding of lipophorin to the fat body of the migratory locust. *Insect Biochem.* **19:** 809–814.
24. Tsuchida, K., and M. A. Wells. 1990. Isolation and characterization of a lipoprotein receptor from the fat body of an insect, *Manduca sexta*. *J. Biol. Chem.* **265:** 5761–5767.