Lipoproteins in *Drosophila melanogaster*—Assembly, Function, and Influence on Tissue Lipid Composition

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**Abstract**

Interorgan lipid transport occurs via lipoproteins, and altered lipoprotein levels correlate with metabolic disease. However, precisely how lipoproteins affect tissue lipid composition has not been comprehensively analyzed. Here, we identify the major lipoproteins of *Drosophila melanogaster* and use genetics and mass spectrometry to study their assembly, interorgan trafficking, and influence on tissue lipids. The apoB-family lipoprotein Lipophorin (Lpp) is the major hemolymph lipid carrier. It is produced as a phospholipid-rich particle by the fat body, and its secretion requires Microsomal Triglyceride Transfer Protein (MTP). Lpp acquires sterols and most diacylglycerol (DAG) at the gut via Lipid Transfer Particle (LTP), another fat body-derived apoB-family lipoprotein. The gut, like the fat body, is a lipogenic organ, incorporating both de novo-synthesized and dietary fatty acids into DAG for export. We identify distinct requirements for LTP and Lpp-dependent lipid mobilization in contributing to the neutral and polar lipid composition of the brain and wing imaginal disc. These studies define major routes of interorgan lipid transport in *Drosophila* and uncover surprising tissue-specific differences in lipoprotein lipid utilization.

**Introduction**

Lipoproteins allow the transport of lipids between different organs. In humans, perturbed lipoprotein levels correlate with metabolic disease, but to which extent they contribute to tissue pathology is unclear. Animals synthesize a huge variety of lipids that form cellular membranes, function as signaling molecules, and constitute the major storage and transport form of energy. The lipid composition of different cell types and tissues is important for biological function. To what extent do lipoproteins influence these cellular properties?

Mammals have two types of apolipoproteins that scaffold particles with different functions [1]. Several proteins of the exchangeable apolipoprotein family, including apoA-I, scaffold high-density lipoproteins (HDL), which mediate reverse cholesterol transport. ApoB scaffolds very low-density lipoproteins (VLDL) and chylomicrons, which are secreted by the liver and gut, and deliver fat and sterols to peripheral tissues. Mammalian apoB acquires lipid in producing cells by a process requiring MTP [2,3]. In humans, MTP deficiency blocks secretion of apoB-containing lipoproteins, resulting in abetalipoproteinemia [4]. This causes fatty liver, intestinal lipid malabsorption, and defects in peripheral tissue function including ataxia, retinal degeneration and anemia [5]. On the other hand, elevated levels of apoB-containing lipoproteins are a hallmark of metabolic syndrome, a pathological condition comprising wide-ranging dysfunctions in different tissues. These include obesity, diabetes, heart disease and increased risk of dementia [6,7]. Mammalian tissue culture cells preferentially derive fatty acids and cholesterol from lipoproteins, but can switch to endogenous synthesis if lipoproteins are not provided [8,9]. However, it is not clear to what extent autonomous synthesis suffices for different tissues to maintain a normal lipidome *in vivo*. Furthermore, while the influence of dyslipidemia on the plasma lipidome has been well studied, less attention has been paid to organism-wide changes in tissue lipid composition. Advances in lipid mass spectrometry are only beginning to make such studies possible [10].

To investigate how lipoproteins influence tissue lipid composition requires a system where lipoproteins can be manipulated in a time and tissue-dependent manner. *Drosophila* genetics could provide a tool to easily control lipoprotein levels in an organism whose metabolism shares many similarities with that of mammals [11,12]. In *Drosophila*, the molecular mechanisms controlling storage and mobilization of neutral lipid in cellular lipid droplets resemble mammalian pathways [13,14]. This similarity even extends to the progression of metabolic diseases caused by dysfunctions in lipid metabolism [15]. The major lipoproteins of *Drosophila* and other insects, the lipophorins (Lpp), are similar to mammalian apoB-containing lipoproteins; their scaffolding apolipoproteins, the apolipophorins (apoLpp), are members of the apoB family, which is conserved throughout the animal kingdom [16]. Moreover, *Drosophila* lipoprotein receptors resemble those of mammals [17]. The low-density lipoprotein (LDL) receptor homologues LpR1 and LpR2 promote Lpp internalization [18],...
Author Summary

Lipoproteins transport both dietary and endogenous synthesized lipids between different organs. Lipoprotein dysfunction is associated with many medical disorders, including cardiovascular disease, but the mechanisms underlying pathogenesis are unclear. Simple animal models would be valuable, therefore, to understand basic functions of lipoprotein and their influence on tissue lipids. We develop the fruit fly Drosophila melanogaster as a genetically tractable model to study lipoprotein metabolism. We characterize the major Drosophila lipoproteins, the mechanisms by which they acquire lipid cargo from different organs. By genetically blocking specific interorgan lipid transport routes, we uncover surprising tissue-specific differences in lipoprotein lipid utilization. Our studies define basic features of Drosophila lipoprotein metabolism and suggest novel mechanisms for how lipoproteins might affect animal tissue function in general.

but also appear to increase cellular neutral lipid storage by non-endocytic mechanisms [19]. Similarly, the role of heparan sulfate proteoglycans as endocytic lipoprotein receptors is conserved endocytic mechanisms [19]. Similarly, the role of heparan sulfate proteoglycans as endocytic lipoprotein receptors is conserved. Locusta migratoria in vivo providing the fat body causes accumulation of neutral lipid in the gut, and the brain phospholipid composition can be maintained independently of lipoproteins. Our studies define basic features of Drosophila lipoprotein metabolism and suggest novel mechanisms for how lipoproteins might affect animal tissue function in general.

Results

Drosophila Possesses Two Homologues of ApoB, but No ApoA

We started our study of Drosophila lipoprotein metabolism with a genome search for potential apolipoproteins. Many proteins involved in interorgan lipid transport harbor vitellogenin-N domains, including apoB, MTP and vitellogenins [16,32,33]. BLAST searches with the vitellogenin-N domain of human apoB yield four fly genes: apolpp [34] and apoLTPI [31], as well as two novel genes, CG15828 and CG31150 (Figure 1A). apolpp and CG15828 seem to have arisen by gene duplication of an ancestral insect apoB homologue (Figure 1B). As will be shown below, the protein encoded by CG15828 assembles a lipoprotein that functions similarly to a lipid transfer particle, LTP, identified in Locusta and Manduca [35,36]. We therefore refer to it as apoLTP. CG31150, which has been recently shown to be mutated in crossing d (cv-d) [37], is most closely related to vitellogenins (Figure 1B).

To ask whether the fly genome encoded exchangeable apolipoproteins like those scaffolding mammalian HDL, we searched for sequences similar to apolipoproteins A-I and E. No Drosophila protein had significant homology. Neither was there a homologue of apoLipophorin III, a structurally related exchangeable apolipoprotein in Locusta and Manduca [38]. Thus, there is no evidence for apolipoproteins of this family in Drosophila.

Drosophila Larvae Have Three Circulating Lipoproteins of Different Densities—Lpp, LTP, and Cv-d

To ask which of the vitellogenin-N domain proteins might scaffold lipoproteins, we fractionated hemolymph from feeding third instar larvae in isopycnic gradients and probed for apoLpp, apoLTPI and Cv-d. These proteins are all present in circulation (Figure S1A), and fractionate at densities consistent with different degrees of lipidation (Figure 1C). ApoLpp is posttranslationally cleaved into apoLI and apoLII, which assemble the lipoprotein Lpp (1.13–1.14 mg/ml). Like apoLpp, apoLTP harbors furin cleavage sequences C-terminal to the vitellogenin-N domain, and is cleaved into two polypeptides, which we denote apoLTPPI and apoLTPII. ApoLTPPI and apoLTPII assemble a higher density (1.23 mg/ml) lipoprotein, LTP. Cv-d is poorly lipidated (1.24 mg/ml), consistent with its similarity to vitellogenins, which contain little lipid [39].

To investigate the relative amounts of Lpp, LTP and Cv-d in circulation, we used silver and Coomassie staining to detect them in hemolymph fractionated by density and size (Figure 1D, Figure S1B). These methods detect two prominent bands corresponding to apoLI and apoLII. ApoLTPPI and Cv-d are detectable, but much less abundant. Each of these proteins is also present in embryos and adults (Figure S1C). We do not detect any other proteins in low-density fractions, suggesting that no other abundant lipoproteins exist in larval hemolymph.

Lipophorin Is the Major Hemolymph Lipid Carrier

To assess the amount of lipid associated with each lipoprotein, we quantified hemolymph lipids in different density fractions by shotgun mass spectrometry. More than 95% of hemolymph lipids co-fractionate with Lpp (Figure 1E, Figure S1D). The fractions containing LTP and Cv-d account for less than 1% and 0.5% of hemolymph lipids, respectively. Thus, Lpp carries the bulk of lipids in circulation.
Figure 1. Drosophila lipoproteins and their lipid content. (A) Fly and human proteins harboring vitellogenin-N domains. Drosophila possesses two apoB homologues, apoLpp and apoLTP, an MTP and a vitellogenin-like protein, Cv-d. Humans possess one apoB gene, which gives rise to two proteins, apoB-100 and apoB-48, and an MTP. Both apoLpp and apoLTP give raise to two polypeptides. The N-terminal and C-terminal parts generated from full-length apoLpp are denoted apoLII and apoLI, respectively. The N-terminal and C-terminal parts generated from full-length Drosophila lipoprotein metabolism.
apoLTP are denoted apoLTPII and apoLTPI, respectively. Numbers in the protein boxes denote the predicted total number of amino acid (aa); numbers below the boxes indicate the first and last amino acid of the predicted vitellogenin-N and von Willebrand factor D domains. (B) Phylogenetic tree of animal vitellogenin-N domain proteins. Alignment and tree were constructed from vitellogenin-N domain sequences with ClustalW and PHYLIP-NEIGHBOR using http://toolkit.tuebingen.mpg.de and default settings. See also [16]. (C) Immunoblot of larval 3rd instar hemolymph lipoproteins fractionated on an isopycnic KBr gradient. ApoLi and apoLII scaffold the lipoprotein Lpp; apoLTPII and apoLTPI scaffold the lipoprotein LTP. Note that minor amounts of uncleaved apoLpp (*) are present in the hemolymph. (D) Silver staining of an isopycnic gradient run and processed in parallel to the gradient shown in (C). Silver-stained bands corresponding to immunoblot bands in (C) are indicated by red arrowheads. Note that the non-lipidated larval serum proteins (LSP) 1 and 2 (blue arrowhead) peak in the last gradient fraction. (E) Larval hemolymph fractionated on an isopycnic KBr density gradient, analyzed by mass spectrometry and immunoblotting. Shown is the total amount of lipid present in each gradient fraction from 1 μl hemolymph of one representative experiment. More than 95% of all hemolymph lipids co-fractionate with Lpp. (F) Lipid composition of the larval hemolymph, quantified by mass spectrometry (n = 8, free sterols n = 4). The left y-axes indicate the amount of each lipid class normalized to total hemolymph protein, the right y-axes the mol% of each lipid class. (G) Chain length distribution of fatty acid residues in DAG and PE in larval hemolymph, quantified by mass spectrometry (n = 8). The amount of each lipid species is normalized to total hemolymph protein. Lpp DAG contains fatty acid residues with an average chain length of 12–14 carbons (medium-chain); Lpp PE contains fatty acid residues with an average chain length of 16–18 carbons (long-chain). For all mass spectrometry data, lipid species with medium-chain fatty acid residues are indicated by green background; error bars indicate ±SD (* p<0.05, ** p<0.005, *** p<0.0001).

doi:10.1371/journal.pgen.1002828.g001
which of the different *Drosophila* lipoproteins were produced by the fat body or gut, we used reverse transcription PCR to determine the presence of *apolpp*, *apoltp*, and *cv-d* transcripts in these organs. *apolpp*, *apoltp*, and *cv-d* transcripts are readily detectable in the fat body (Figure S2). In contrast, none of them can be detected in the gut. Thus, the *Drosophila* larval gut does not produce any of these lipoproteins.

To ask what fraction of circulating Lpp, LTP and Cv-d was derived from the fat body, we blocked their production in this tissue by RNAi. Fat body-specific knock-down strongly reduces
levels of Lpp, LTP and Cv-d in the hemolymph, establishing this organ as the major source of circulating lipoproteins (Figure 2A–2C).

mtpl transcripts are readily detectable in the fat body, similar to what we observed for transcripts of the different apolipoproteins (Figure S2). To ask whether production of Lpp, LTP or Cv-d depended on MTP, we knocked down MTP in the fat body by RNAi and examined hemolymph lipoproteins. MTP RNAi causes the buildup of the uncleaved full-length apolipoprotein precursors apoLpp and apoLTP in the fat body, and strongly reduces hemolymph Lpp and LTP levels (Figure 2D). Thus, Drosohila MTP has a conserved function in the production of apoB-family lipoproteins in vivo. However, MTP RNAi does not reduce levels of Cv-d in circulation; thus, not all proteins with vitellogenin-N domains depend on MTP for their release to circulation. These data distinguish the vitellogenin-like lipoprotein Cv-d from canonical vitellogenins in other organisms, whose release is promoted by MTP [40,41].

Lpp and LTP Function Together to Mobilize Lipids from the Gut

Although Lpp originates in the fat body, we previously showed that its knock-down causes the buildup of neutral lipid in the gut [29]. To ask whether Lpp, or other lipoproteins, are recruited to the gut, we assessed lipoprotein levels in this organ by Western blotting. These experiments show that Lpp and LTP are readily detectable in the gut (Figure 2E), and loss of Cv-d does not cause the buildup of Cv-d in circulation; thus, not all proteins with vitellogenin-N domains depend on MTP for their release to circulation. These data distinguish the vitellogenin-like lipoprotein Cv-d from canonical vitellogenins in other organisms, whose release is promoted by MTP [40,41].

To ask whether either Lpp or LTP were required to export lipid from the gut to circulation, we studied mtpl mutant larvae, which arrest in the first larval instar and do not secrete these lipoproteins (Figure S3A–S3D). Loss of MTP increases the size and number of neutral lipid droplets in the anterior and posterior midgut and, more moderately, in the gastric caeca (Figure 3B). We wondered whether lipoprotein production in the fat body would be sufficient for the mobilization of lipids from the gut. To investigate this, we restored MTP activity specifically in the fat body using tissue-specific MTP RNAi. This strongly reduces their levels in the gut (Figure 2D). Thus, Lpp and LTP produced in the fat body enter circulation and are recruited to the gut.

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hemolymph. We conclude that LTP facilitates lipid export from the gut to Lpp. As the LTPs of other insects were shown to have similar lipid transfer activity [26], and also resemble Drosophila LTP in density and in the size of two apolipoprotein subunits [35,36], we suspect that these LTPs are scaffolded by orthologous apoB-family proteins.

*Manduca* LTP can facilitate lipid exchange between Lpp and the fat body. Stimulating adult fat bodies with adipokinetic hormone...
yields net transfer from the fat body to Lpp [28]. In contrast, in feeding Manduca larvae, net lipid flux is from Lpp to the fat body [27]. To ask whether LTP from Drosophila larvae promoted loading of Lpp with fat body lipids, we incubated hemolymph containing lipid-poor Lpp derived from LTP RNAi animals with either wild-type fat bodies or LTP RNAi fat bodies. In contrast to the gut, we did not observe net lipid transfer from fat body to Lpp, regardless of the presence of LTP (Figure S5A). Thus, LTP does not appear to promote the loading of Lpp with neutral lipids at the fat body in feeding larvae, similar to the situation in Manduca.

Previous work demonstrated that Manduca LTP could function as a carrier that shuttles lipids between donor and acceptor lipoproteins [43]. This led us to wonder whether Drosophila LTP acted as an intermediate in the transfer of lipids from the gut to Lpp. If so, then loss of Lpp might trap these lipids in LTP particles. To test this, we asked whether removing Lpp altered LTP density.

Figure 6. Lipid synthesis capacity of gut and fat body. (A), (B) Changes in gut (A) TAG and (B) DAG of lipid-fed and lipid-starved larvae upon Lpp RNAi, quantified by mass spectrometry (n = 3). Lpp knock-down causes neutral lipid accumulation in the gut, even when lipids are not provided with the diet. (C) Changes in hemolymph DAG upon gut-specific FAS RNAi, quantified by mass spectrometry (n = 3). DAG is normalized to hemolymph protein. FAS knock-down causes a decrease in hemolymph DAG. (D–G) Changes in fat body neutral lipids upon LTP or Lpp RNAi, quantified by mass spectrometry (n = 5). (D) total DAG; (E) DAG species; (F) total TAG; (G) TAG species. Knock-down of either lipoprotein decreases medium-chain DAG. (H) Larval fat body lipid droplets upon LTP or Lpp RNAi, visualized with Nile red. Lipoprotein knock-down does not reduce lipid droplet levels. Yellow: neutral lipid; red: phospholipid. Scale bars = 20 μm. (I) Changes in fat body PE species upon LTP or Lpp RNAi, quantified by mass spectrometry (n = 5). Acylglycerols are normalized to polar lipid. PE is represented as mol% of polar lipids.

doi:10.1371/journal.pgen.1002828.g006
Indeed, LTP shifts to lower density fractions in hemolymph from Lpp RNAi animals (Figure 4A, Figure S3B), suggesting that lipids are loaded onto LTP before being transferred to Lpp. While MTP transfers lipids to apoB in the secretory pathway of producing cells [2,3], LTP must function differently in the gut, since both Lpp and LTP are recruited there from circulation. To investigate the LTP/Lpp lipid transfer mechanism, we examined their subcellular localization in the lipid droplet-rich regions of the posterior midgut. Lpp accumulates both in the overlying muscle layer that surrounds the gut, and on the basal (outward facing) side of absorptive enterocytes (Figure 4C, 4D). In contrast, LTP is not detectable in muscle but accumulates strongly in basal regions of enterocytes. Its subcellular localization extends apically to abut the level where lipid droplets are found. There is little obvious subcellular colocalization of LTP and Lpp in the midgut.

The subcellular localization of LTP raised the possibility that it was internalized by enterocytes; we therefore wondered whether endocytosis was required for lipid mobilization from the gut. To address this, we induced expression of dominant negative dynamin in enterocytes and monitored neutral lipid droplets at different times following induction. Within 3 h, neutral lipid droplets accumulate over a broader region of the posterior midgut, and their number within individual enterocytes increases (Figure 4E). By 6 h, most of the posterior midgut is filled with large lipid droplets, similar to the gut of lipoprotein-deficient larvae. Within the same time frame, LTP shifts its subcellular localization to accumulate at the cell boundaries of enterocytes (Figure 4F). In contrast, the subcellular distribution of Lpp appears unaltered. This suggests that endocytosis of LTP may be required for lipid mobilization from the gut. A model consistent with these data is that LTP is internalized by enterocytes, loaded with lipids in an endocytic compartment, and subsequently transfers its lipid cargo to Lpp.

We note that 24 h after induction of dominant negative dynamin, lipid droplets in the gut are strongly reduced (Figure S5C). Since dynamin blocks not only endocytosis, but also some plasma membrane delivery routes, we suspect that blocking endocytosis for longer periods of time might compromise delivery of proteins involved in lipid uptake.

The Gut Uses LTP to Load Lpp with Medium-Chain DAG and Sterols

The vast majority of lipids in the hemolymph are carried by Lpp, and Lpp RNAi reduces the amount of all hemolymph lipid species over 10-fold (Figure 5H). To ask which lipids depended LTP for their transfer to Lpp, we quantified changes in hemolymph lipids of LTP RNAi animals. LTP RNAi specifically reduces the levels of medium-chain DAG (DAG 26, DAG 28) and sterols (Figure 5H, Figure S3D) by about 70%. In contrast, levels of PE, the major polar Lpp lipid, are not changed. The amounts of several minor Lpp lipid classes (PC, TAG, sphingolipids) increase slightly. These data suggest that LTP specifically facilitates loading of DAG and sterols onto Lpp.

We reasoned that cargo transferred to Lpp by LTP might specifically accumulate in the gut upon either LTP or Lpp knockdown. We therefore asked whether DAG and sterol increased under these conditions. Wild-type guts contain both the medium-chain DAG found in Lpp and smaller amounts of long-chain DAG (Figure 5B, Figure S6) whose combined acyl chain length resembles those of cellular and Lpp phospholipids (Figure 5A). Upon LTP or Lpp RNAi, medium-chain DAG increases 5–8 fold with respect to polar lipids (Figure 5D, 5E). Long-chain DAG increases moderately, and contributes less to the total elevation in gut DAG. These data confirm that the gut uses LTP to export medium-chain DAG to Lpp. Although the gut must also be the source of Lpp sterol (Drosophila are sterol auxotrophs), sterols do not accumulate in this organ upon lipoprotein knock-down (Figure S7A, S7B). It is possible that sterol esterification may increase when export is blocked; however our current methods do not allow us to detect sterol esters.

Strikingly, loss of either LTP or Lpp also causes a strong increase in TAG in the gut (Figure 5F). Since the minor amounts of TAG normally present Lpp particles do not decrease upon LTP RNAi (Figure 5H), this cannot reflect a block in TAG export to Lpp. Interestingly, medium-chain TAG is most strongly elevated (Figure 5G). This suggests that some medium-chain fatty acids eventually exported as DAG can be stored as TAG, when export from the gut is blocked.

We wondered whether incorporation of medium-chain fatty acids into TAG was an obligate intermediate in the production of medium-chain DAG. To address this, we perturbed the two well-characterized lipolytic systems known to be required for hydrolysis of TAG in the Drosophila fat body – the Adipocyte Triglyceride Lipase homologue, Brummer (Bmm), and the Adipokinetic Hormone Receptor (AKHR) regulated lipase system [13,44]. We first quantified gut TAG and DAG species in bmm and akhr mutant larvae. Loss of either Bmm or AKHR causes TAG accumulation that is biased towards medium-chain species – similar to the effect of lipoprotein knock-down (Figure 5I, Figure S8A, S8B). Thus, the gut requires both lipolytic systems to mobilize TAG at a normal rate. Despite this, neither perturbation reduces medium-chain DAG in the hemolymph (Figure 5K, Figure S6C). Levels of medium-chain DAG in the gut actually increase slightly (Figure 5J, Figure S8D). We conclude that even under these conditions where TAG storage is favored over lipolysis, the gut can supply normal levels of DAG to Lpp.

To ask whether these lipolytic pathways might function redundantly to generate Lpp DAG, we knocked down Bmm and AKHR in enterocytes, alone and in combination, and measured resulting changes in hemolymph DAG. While neither knock-down alone reduces hemolymph DAG (similar to the effect of single mutants) simultaneous knock-down lowers hemolymph DAG by 15% (Figure S8E). These data suggest that some Lpp medium-chain DAG is derived from medium-chain TAG by Bmm and AKHR-dependent lipolysis. However, other mechanisms suffice to generate the majority of Lpp medium-chain DAG.

Lpp Medium-Chain DAG Is Derived Both from Dietary Lipids and De Novo Synthesis in the Gut

We were intrigued by the distinctive fatty acid composition of Lpp DAG. The combined acyl chain length in these DAG species (26–28 carbons) differs not only from that of phospholipids, but also from that of dietary lipids – both contain almost exclusively long-chain fatty acids (32–36 carbons) (Figure 1G, Figure 5A; M. Carvalho et al., submitted). These observations raise questions about the source of the medium-chain fatty acids in Lpp DAG. One possibility is that they are derived from dietary fatty acids by processing mechanisms such as chain length shortening. Alternatively, they may be generated de novo from non-lipid dietary components such as sugars.

To determine the contribution of dietary lipids to Lpp medium-chain DAG, we compared levels of hemolymph DAG in lipid-fed and lipid-starved animals. Lipid starvation increases the density of hemolymph Lpp (Figure S9A). Thus, lipid-starved animals produce similar amounts of Lpp, but these particles contain less lipid. Furthermore, lipid starvation reduces the ratio of medium-chain DAG to polar lipids in Lpp by about 2-fold (Figure S9B). However, lipid starvation affects Lpp density and DAG content...
Figure 7. Lpp supplies lipids to peripheral organs. (A–D) Changes in wing disc neutral lipids upon LTP or Lpp RNAi, quantified by mass spectrometry (n = 5). (A) total DAG; (B) DAG species; (C) total TAG; (D) TAG species. Knock-down of either lipoprotein decreases medium-chain DAG and TAG species. Lpp knock-down in addition decreases long-chain TAG. (E) Lipid droplets of wing discs upon LTP or Lpp RNAi, visualized with Nile red. Lpp knock-down decreases wing disc lipid droplets more strongly than LTP knock-down. Yellow: neutral lipids; red: phospholipids. Scale bars = 50 μm. (F–I) Changes in brain neutral lipids upon LTP or Lpp RNAi, quantified by mass spectrometry (n = 5). (F) total DAG; (G) DAG species; (H) total TAG; (I) TAG species. Lpp knock-down decreases DAG and TAG, whereas LTP knock-down does not affect brain neutral lipids. (J),(K) Changes in PE species in (J) wing disc and (K) brain, quantified by mass spectrometry (n = 5). The major Lpp PE species (PE 32:1, PE34:1) decrease upon Lpp knock-down in the wing disc, but not in the brain. Acylglycerols are normalized to polar lipid. PE is represented as mol% of polar lipids.
doi:10.1371/journal.pgen.1002828.g007
more mildly than LTP RNAi, which reduces the ratio of DAG to polar lipid by about 3-fold. This raises the possibility that only part of the medium-chain DAG loaded onto Lpp by LTP is derived from dietary lipids.

To explore the contribution of endogenous synthesis, we asked to what extent neutral lipid accumulation in the gut of Lpp RNAi larvae depended on dietary lipids. We knocked down Lpp in lipid-fed and lipid-starved larvae, and quantified neutral lipids in the gut. Although lipid starvation slightly reduces the amount of TAG and DAG in wild-type guts, these lipids accumulate dramatically when Lpp levels are reduced - both in lipid-fed and in lipid-starved larva (Figure 6A, 6B). On both diets, neutral lipid species containing medium-chain fatty acids increase most strongly in response to Lpp RNAi (Figure S9C). This supports the idea that part of the medium-chain DAG present in Lpp derives from endogenous synthesis in the gut.

*Drosophila* Fatty Acid Synthase (FAS) can synthesize medium-chain fatty acids [45], raising the possibility that it generates fatty acids for the medium-chain DAG present in Lpp. To investigate the contribution of fatty acid synthesis in the gut to Lpp DAG, we knocked down FAS in this organ, and quantified hemolymph DAG. FAS RNAi decreases Lpp DAG by 30% (Figure 6C). Therefore, even when fatty acids are supplied by the diet, a significant proportion of Lpp DAG contains fatty acids derived from endogenous synthesis in the gut.

**The Fat Body Autonomously Maintains Its TAG Stores within a Narrow Range**

The insect fat body is a major site of lipid synthesis, storage and export [25]. How does the balance of lipid import and export affect the lipid composition of this organ? We first examined the contribution of lipid delivery from the gut by blocking this transport route through RNAi-mediated LTP knock-down. Fat bodies of LTP RNAi animals contain much less sterol than those of wild-type, consistent with the sterol auxotrophy of *Drosophila* (Figure S7A, S7C). Interestingly, LTP RNAi fat bodies also contain much less medium-chain DAG (Figure 6D, 6E). Thus, the fat body does not maintain medium-chain DAG levels when export from the gut is blocked.

To what extent is delivery of lipids from the gut required to build TAG stores in the fat body? The wild-type fat body contains large amounts of TAG with predominantly long-chain fatty acids – unlike the gut, which contains similar amounts of medium-chain and long-chain TAG (Figure 5C, Figure S6). Removing lipids from the diet does not reduce the amount of TAG stored in the fat body (Figure S9D). Thus, the fat body does not maintain medium-chain DAG levels when export from the gut is blocked.

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neutral lipids in brain cells significantly. Thus, normal TAG storage in the brain requires Lpp-mediated lipid delivery, but it is not limited by the LTP-mediated loading of Lpp with DAG in the gut.

**Phospholipid Composition of the Wing Disc and Gut Is Influenced by Lpp-Mediated Lipid Delivery**

To what extent do different tissues synthesize their own membrane lipids? Do they also rely on Lpp to deliver some membrane lipids? Because *Drosophila* cannot synthesize sterols, it is unsurprising that sterol accumulation in peripheral tissues depends on Lpp and LTP (Figure S7A) [18]. To investigate whether Lpp was important for delivery of other membrane lipids, we quantified the polar lipid composition of tissues from LTP and Lpp RNAi animals. Wing disc and gut from Lpp RNAi animals contain about 20% less PE 32:1 and PE 34:1 than those of wild-type (Figure 7J, Figure S10B). Interestingly, these PE species are not only major membrane constituents, but are also precisely those species that are most abundant in Lpp (Figure S10A). In contrast, we did not observe a reduction in any species of PC, which is abundant in membranes, but only a minor component of Lpp (Figure S10C). These observations suggest that wing disc and gut cells cannot completely compensate for the loss of Lpp-derived PE species by increasing endogenous PE synthesis. They further raise the possibility that Lpp PE species might be directly incorporated into cell membranes without remodeling.

In contrast to wing disc and gut, the brain can maintain normal levels of all phospholipids including PE 32:1 and PE 34:1 even when Lpp levels are strongly reduced by RNAi (Figure 7K, Figure S10C, S10D). Thus, while the brain requires Lpp delivery to store normal levels of TAG, its phospholipid composition is autonomously controlled.

**Discussion**

How disturbed lipoprotein metabolism affects lipid composition in individual organs is insufficiently understood. *Drosophila* could provide a useful model in which to study this problem, but its lipoproteins had not been well characterized. Here, we outline the basic features of the lipoprotein metabolism of *Drosophila* larvae, and its relevance for tissue-specific fat storage and membrane lipid composition (Figure 8).

The major inter-organ lipid transport routes in *Drosophila* are executed by a single lipoprotein – Lpp, which is scaffolded by the apoB homologue apoLpp. Its major polar lipid constituents are long-chain PE and sterols, and its major neutral lipid is medium-chain DAG. Lpp lipidation takes place in two consecutive steps, which require distinct lipid transfer proteins, MTP and LTP, and take place in different organs – fat body and gut. ApoLpp is translated and lipoproteinized in the fat body by an MTP-dependent mechanism, resulting in the formation of dense Lpp particles rich in PE. These are recruited to the gut, where they are further loaded with DAG and sterols through the activity of LTP. Thus, although Lpp originates in the fat body, it is loaded both with fat body and gut lipids.

Lipidation of mammalian apoB, like that of *Drosophila* apoLpp, proceeds in two distinct steps, formation of primordial phospholipid-rich lipoprotein particles, and subsequently acquisition of bulk neutral lipid [2]. However, this process occurs entirely in the secretory pathway of producing cells. MTP has been proposed to be required both for initial transfer of phospholipids, and for the recruitment of TAG to the ER lumen for incorporation into lipoproteins [47,48,49,50]. Interestingly, *Drosophila* MTP has been shown to promote the secretion of apoB-containing lipoproteins.
from COS cells, and to transfer phospholipids, but not TAG, between liposomes [31,51]. This suggested that MTP acquired the ability to transfer TAG in the vertebrate lineage. Our experiments show that Drosophila MTP is required for the production of the two Drosophila apoB-family lipoproteins Lpp and LTP in vivo; they further show that MTP is insufficient to load Lpp with normal quantities of DAG, the major neutral lipid of Lpp. These data support the idea that MTP originally evolved to promote the assembly of phospholipid-rich apoB-family lipoproteins.

The novel Drosophila apoB-family lipoprotein LTP shares many properties with the Lipid Transfer Particle purified from the hemolymph of several insects, including Manduca and Locusta [35,36]. The scaffolding proteins of Drosophila LTP, apoLTP and apoLTPII, are generated from a single precursor, apoLTP. Orthologous apoB-family proteins of other insects are therefore plausible candidates for the scaffolding proteins of their LTP particles. Insect LTPs were shown to contain a third, small protein subunit, apoLTPIII [36,52]. Our biochemical experiments do not address whether Drosophila LTP might contain an apoLTPIII subunit, because LTP is of such low abundance that silver staining barely detects the much larger apoLTP. Sequence analysis of apoLTP does not suggest the existence of a protease cleavage site that could give rise to a protein of the size of apoLTPIII, and neither apoLTP nor apoLTPII antibodies detect an additional protein of this size. Thus, if apoLTPIII exists in Drosophila, it is not likely to be derived from the apoLTP precursor.

The function of LTP as a lipid transfer protein rather than a carrier of bulk hemolymph lipid uncovers surprising evolutionary plasticity of the apoB lipoprotein family. Insect LTPs have been studied in vitro in a wide range of systems [38]. In different contexts, they have been shown to facilitate the exchange of DAG and phospholipids between Lpp and fat body or gut [26,27,28], and even between insect and human lipoproteins of different densities [52,53,54]. Our studies of feeding Drosophila larvae indicate that only a subset of the lipid transfer activities of LTP may be relevant under specific metabolic conditions in vivo. LTP moves DAG and sterols from the larval gut onto Lpp. However, it does not facilitate significant net transfer of fat body lipids to Lpp. Consistent with this, radiolabeling experiments showed that the rate of DAG transfer from larval Manduca fat body to Lpp exceeds the rate of the reverse process [27]. This may reflect a dominance of nutritional lipid uptake and fat storage in feeding larvae.

Although we have been unable to identify a Drosophila HDL-like lipoprotein, we note that LTP and Lpp share some functional features with mammalian HDL, despite being scaffolded by unrelated apolipoproteins. Together, Lpp and LTP mediate efflux of sterols from the gut to circulation. Conceivably, other tissues that recruited both lipoproteins might efflux sterol for reverse transport.

While it is clear that dietary lipids contribute to Lpp DAG, the gut does not directly incorporate dietary fatty acids into DAG destined for export. The long-chain fatty acids that predominate in the diet strikingly differ from the medium-chain fatty acids in Lpp DAG (M. Carvalho et al., submitted). A possible explanation is that the gut remodels dietary fatty acids, conceivably via limited β-oxidation. Interestingly, the gut is also a lipogenic organ and a significant fraction of the medium-chain fatty acids found in Lpp DAG derives from de novo fatty acid synthesis in this organ. In more primitive animals, such as Caenorhabditis elegans, lipid uptake, storage and lipogenesis all occur in the gut [53]. More complex animals, including Drosophila, have developed separate organ systems for lipid storage and lipogenesis. However, our data show that this separation of functions is not absolute in the fly. Rather, other nutrients such as amino acids or sugars might be partially converted to lipid by the gut, instead of being transported intact into circulation. It would be interesting to ask what circumstances favor this conversion. Intriguingly, de novo lipogenesis has been observed in the mammalian gut, especially under conditions of insulin resistance, and has been proposed to contribute to the postprandial dyslipidemia observed in this state [56]. Drosophila may be a useful model to explore this problem.

Gut and fat body differ in how they respond to blockage of lipid export to Lpp. Enterocytes vastly and rapidly expand their normally moderate stores of medium-chain DAG and TAG. This occurs even in the absence of dietary lipids, when exported lipids are derived from endogenous fatty acid synthesis. Thus, the gut has a flexible capacity for lipid storage. In contrast, the larval fat body maintains its neutral lipid stores within tight limits. When lipoprotein transport is blocked, endogenous lipid synthesis from other dietary components may suffice to build the large TAG stores of this organ. Furthermore, even though the fat body normally supplies the entire animal with large amounts of lipoproteins, TAG stores hardly increase when Lpp is not produced. Homeostatic mechanisms must maintain fat body TAG levels. In this way, the fat body differs from the gut, which accumulates fat when lipoprotein export is blocked, similar to mammalian gut and liver [5].

Peripheral tissues cannot maintain normal TAG levels in the absence of Lpp. The wing disc depends on Lpp for a large fraction of its fat stores. Interestingly, our work indicates that lipid delivery from the fat body and gut differentially contributes to wing disc neutral lipids. TAG species containing medium-chain fatty acids depend on LTP and Lpp-mediated DAG mobilization from the gut. TAG species containing long-chain fatty acids also depend on Lpp-mediated lipid delivery, but are less affected by a blockage of DAG export from the gut. As Lpp is produced in the fat body, this suggests that long-chain TAG in wing discs may be derived from lipids supplied by the fat body. The most abundant source of long-chain fatty acids in Lpp is PE, which raises the possibility that wing discs use Lpp phospholipids to build cellular fat stores. Consistent with this, cultured murine hepatocytes convert a significant fraction of LDL or HDL-derived PC to TAG [57,58], although the in vivo relevance of this pathway remains to be explored. However, Lpp still contains reduced amounts of medium-chain DAG when LTP-mediated lipid loading is impaired. Thus, long-chain fatty acids in wing disc TAG might also derive from elongation of medium-chain fatty acids. Interestingly, although medium-chain DAG is the most abundant lipid transported through circulation, tissues store only minor amounts of neutral lipid containing medium-chain fatty acids. This would be consistent with the idea that tissues either elongate these fatty acids or subject them to β-oxidation.

The brain also requires Lpp-mediated lipid delivery to build its TAG stores. Interestingly, the brain stores normal levels of TAG when gut lipid mobilization is inhibited. While this does not exclude the possibility that the brain may directly acquire lipids from the gut under normal conditions, it indicates that TAG levels in this organ are more resistant to fluctuations in nutritional conditions than those in the wing disc.

In addition to providing fatty acids for neutral lipid storage, lipoproteins also influence the phospholipid composition of wing disc and gut: Lpp knock-down specifically reduces those PE species that are most abundant in Lpp. This suggests that Lpp might directly deliver PE to the cellular membranes of wing disc and gut. It further raises the possibility that phospholipid synthesis in other tissues could have organism-wide effects on membrane lipid composition. Since PE-rich Lpp particles are assembled in the fat body, this tissue is a likely source of these lipids. However, the
brain does not depend on Lpp to maintain its normal membrane phospholipid composition. Furthermore, our previous work suggested that the brain is more resistant to sterol depletion than other tissues [59]. In general, these data indicate that the lipid composition of the brain is more tightly and autonomously controlled than that of other tissues.

In mammals, cellular lipid synthesis and lipid supply from circulation are coordinated through the SREBP pathway [60,61]. Since Drosophila SREBP is regulated by PE instead of sterols [62], it will be interesting to explore whether altered PE levels in Lpp-deprived wing discs might activate SREBP signaling and increase lipid synthesis or lipoprotein uptake. If true, coordination of cellular lipid synthesis with lipid supply through lipoproteins is an evolutionarily conserved function of the SREBP pathway.

Lipoproteins transport large amounts of lipids through circulation – including many of the polar and neutral lipid species present in cells. Our data indicate that in Drosophila, individual organs utilize lipoprotein-derived lipids not only for fat storage but also for membrane homeostasis. ApoB-deficient human patients, and patients with dyslipidemia suffer from various abnormalities in peripheral tissues. Our data suggest that it may be worthwhile to explore whether altered PE levels in Lpp-deprived larvae, diluted in Grace’s insect medium. Subsequently, lipoprotein density was determined by isopycnic centrifugation and immunoblotting.

**Induction of Dominant Negative Dynamin**

A dominant negative allele of dynamin (shibire K44A) was expressed in enterocytes in a time-controlled manner with MyoA-GAL4, Tubulin-GAL80TS.

**Shotgun Lipidomics Mass Spectrometry**

Lipids from hemolymph and tissue homogenates were extracted and analyzed by shotgun mass spectrometry in positive ion mode as described in [59]. Sterols were quantified according to [64]. For supporting mass spectrometry data, see Figure S11.

For more detailed protocols, fly strains, and the generation of mtp and apoltp mutants, RNAi transgenes and antibodies see Text S1.

**Supporting Information**

**Figure S1 Drosophila lipoproteins and their lipid content. (A)** Specificity of apolipoprotein antibodies. An immunoblot of hemolymph from feeding third instar larvae was consecutively probed for the individual proteins. The apoLTPI antibody recognizes two proteins of similar molecular weight. ApoLTPI harbors two closely spaced furin consensus sequences (see Figure 1A), indicating that the two forms of apoLTPI are generated by alternative use of either cleavage site. Note that MTP likewise exists in two isoforms of similar size (see Figure 2D, 2E); however, we could not identify putative protease consensus sites in the MTP sequence. (B) Coomassie staining of hemolymph proteins fractionated on an isopycnic Optiprep gradient. Lpp is the only detectable protein present in the lower-density fractions 1–9. Note that NuPAGE MES buffer was used for electrophoresis of this gradient, whereas electrophoresis of the gradients shown in Figure 1C, 1D was performed with Tris-glycine buffer. (C) Immunoblot showing that apoLpp, apoLT, Cv-d and MTP proteins are present in embryos, third instar larvae and adult flies. (D) Distribution of lipid classes in the hemolymph density gradient from Figure 1E. Shown is the % lipid of each lipid class present in each fraction. (E) Ceramide-Phosphorylethanolamine (CerPE) present in different fractions of the hemolymph density gradient of Figure 1E. Shown is the total amount of CerPE present in each gradient fraction from 1 μl hemolymph. Note that CerPE 2:2 and 2:3 (2 double bonds, 2 or 3 hydroxyl groups) partially co-fractionate with LTP, whereas CerPE 3:2 (1 double bond, 2 hydroxyl groups) is confined to the Lpp fractions.

(TIF)

**Figure S2** Apolipoprotein transcripts are not detectable in the gut. Reverse transcription PCR showing that apolipoprotein transcripts can be detected in the fat body, but not in the gut of third instar larvae. Primer pairs were designed to span small introns to preclude contamination with genomic DNA. Note that actin transcripts can be readily detected in cDNA preparations of both fat body and gut.

(TIF)

**Figure S3** Phenotypes of apoltp, apoltp and mtp mutants. (A) Schematic representation of the mtp null allele mtp<del>del</del>. (B) Immunoblot of larvae homozygous for mtp<del>del</del>. Mutant animals lack any detectable MTP protein. (C) Wild-type and mtp<del>del</del> larvae
4 days after egg laying. Wild-type animals have reached the third larval instar. Animals homozygous for mtp<sup>ex1A</sup> arrest in the first larval instar. (D) Immunofluorescence of the posterior midgut from first instar mtp<sup>ex1A</sup> mutant larvae showing that mtp mutant guts lack detectable Lpp and LTP. Basolateral membranes are marked with discs large (Dlg). Scale bars = 20 μm. (E) Immunoblot of first instar mtp<sup>ex1A</sup> larvae in which lipoprotein production was rescued by fat body-specific expression of MTP with Lpp-GALA. ApoLpp cleavage is impaired in mtp<sup>ex1A</sup> larvae, but restored through fat body-specific expression of MTP. (F) Fat body-specific expression of MTP with Lpp-GALA in mtp<sup>ex1A</sup> larvae rescues intestinal lipid mobilization. Lipid droplets of first instar posterior midguts are visualized with Nile red. Yellow: neutral lipids; red: phospholipids. Scale bars = 20 μm. (G) Immunofluorescence showing that Lpp is produced in yolk cells of stage 14 embryos, but then spreads throughout the whole embryo. Lpp expression is visualized with Lpp-GALA-driven membrane GFP (CD8-GFP). Nuclei are visualized with DAPI. Scale bars = 50 μm. (H) Knock-down efficiency of Lpp and LTP in third instar larvae, 4 days after induction of RNAi. ApoLI and apoLTPI levels in whole larval extracts were quantified by immunoblotting. Note that Lpp RNAi entails a concomitant partial reduction of LTP. Error bars indicate ± SD (n = 5). (I) Schematic representation of the apol/L alleles apol<sup>tx1A</sup> and apol<sup>tx1B</sup>. (J) Immunoblot of second instar larval homeozygous for apol<sup>tx1A</sup> and their hemolymph. Mutant animals show strongly reduced apoLTPI and apoLTPII levels, with apoLII and MTP being unaffected. (K) Immunoblot of first instar larval homeozygous for apol<sup>tx1B</sup>. Mutant animals show strongly reduced apoLTPII levels, with apoLI and MTP being unaffected. (L) Wild-type, apol<sup>tx1A</sup> and apol<sup>tx1B</sup> larvae 4 days after egg laying. Wild-type animals have reached the third larval instar. Animals homeozygous for apol<sup>tx1A</sup> arrest in the second larval instar, animals homeozygous for apol<sup>tx1B</sup> arrest in the first larval instar. (M) Intestinal lipid droplets of second instar apol<sup>tx1A</sup> mutant larvae visualized with Nile red. Yellow: neutral lipids; red: phospholipids. Mutant larvae strongly accumulate lipid droplets in the anterior midgut (not shown) and posterior midgut. Scale bars = 50 μm. Moderate lipid accumulation also occurs in the gastric caecae. Scale bars = 20 μm. (TIF)

**Figure S4** Phenotypes of RNAi against the vitellogenin-like protein Cv-d. (A) Lipid droplets in the posterior midgut, fat body and wing disc of Cv-d RNAi third instar larvae visualized with Nile red. Yellow: neutral lipids; red: phospholipids. Cv-d knock-down does not obviously perturb lipid droplets in any organ. Scale bars = 50 μm. (B) Unesterified sterols of the gut, fat body and wing disc of Cv-d RNAi third instar larvae visualized with Filipin. Sterols can still be detected in all organs upon Cv-d knock-down. Scale bars = 50 μm. (TIF)

**Figure S5** Lipid mobilization from fat body and gut. (A) Immunoblot of isopycnic KBr gradients from lipid transfer experiments between fat bodies and LTP RNAi hemolymph containing HA-Lpp. Lpp density does not decrease upon incubation with fat bodies, regardless the presence of LTP. Note that the density of fractions in gradient 3 are shifted with respect to those of gradient 1 and 2. (B) Immunoblot of isopycnic KBr gradients from wild-type or Lpp RNAi hemolymph. Note that LTP shifts to lower-density fractions, when Lpp levels are reduced. (C) Lipid droplets in guts from second instar larvae at different time points after the induction of shibire K44A (dominant negative dynamin) in enterocytes, visualized with Nile red. Within a few hours after induction of shibire K44A, neutral lipid droplets accumulate to a similar extend as in the gut of lipoprotein-deficient larvae. However, guts are almost completely devoid of lipid droplets 24 h after induction. Yellow: neutral lipids; red: phospholipids. Scale bars = 50 μm. See also Figure 4E. (D) Changes in hemolymph DAG upon LTP or Lpp RNAi, quantified by mass spectrometry. DAG species are normalized to hemolymph protein. Lipid species with medium-chain fatty acid residues are indicated by green background. Error bars indicate ± SD (control n = 6; LTP n = 6; Lpp RNAi n = 7; free sterols n = 4. * p<0.05, ** p<0.005, *** p<0.0001). (TIF)

**Figure S6** Neutral and polar tissue lipid content. Chain length distribution of fatty acid residues in TAG and DAG species in larval tissues, quantified by mass spectrometry. TAG and DAG species are normalized to polar lipids. Due to the high TAG content of the fat body, TAG levels in gut, wing disc and brain are additionally depicted in a separate panel. Lipid species with medium-chain fatty acid residues are indicated by green background. Error bars indicate ± SD (n = 5). (TIF)

**Figure S7** Lipoproteins are required for the export of sterols from the gut. (A) Unesterified sterols of the posterior midgut, fat body, wing disc and brain of LTP or Lpp RNAi third instar larvae, visualized with Filipin. Knock-down of LTP or Lpp causes a strong reduction in fat body, wing disc and brain sterols, but does not reduce sterols in the gut. Scale bars = 50 μm. (B), (C) Changes in unesterified sterols in (B) gut and (C) fat body upon LTP or Lpp RNAi, quantified by mass spectrometry. Free sterols are normalized to polar lipid. Error bars indicate ± SD (n = 3; * p<0.05). (TIF)

**Figure S8** The role of lipolysis in the mobilization of neutral lipids from the gut. (A) Changes in intestinal TAG species of bmm and akhr mutant larvae, quantified by mass spectrometry. TAG species are normalized to polar lipid. (B) Lipid droplets in posterior midguts of bmm and akhr mutant second larval stages, visualized with Nile red. Yellow: neutral lipids; red: phospholipids. Scale bars = 20 μm. (C) Changes in hemolymph DAG species of bmm and akhr mutant larvae, quantified by mass spectrometry. DAG species are normalized to hemolymph protein. (D) Changes in intestinal DAG species of bmm and akhr mutant second larval stages, quantified by mass spectrometry. DAG species are normalized to polar lipid. (E) Changes in hemolymph DAG species upon intestinal Bmm RNAi, AKHR RNAi or Bmm+AKHR RNAi, quantified by mass spectrometry. RNAi was driven with MysIA-GAL4. DAG species are normalized to hemolymph protein. Lipid species with medium-chain fatty acid residues are indicated by green background. Error bars indicate ± SD (n = 3). (TIF)

**Figure S9** The contribution of dietary lipids and intestinal lipogenesis to Lpp medium-chain DAG. (A) Immunoblot of isopycnic KBr gradients of hemolymph prepared from lipid-fed or lipid-starved wild-type larvae, or lipid-fed LTP RNAi larvae. Lipid starvation increases the density of Lpp particles; LTP RNAi increases Lpp density even further. (B) Neutral/polar lipid ratio of hemolymph from lipid-starved and LTP RNAi larvae, quantified by mass spectrometry. Lipid starvation reduces the neutral/polar Lpp lipid ratio. LTP RNAI reduces the neutral/polar Lpp lipid ratio even further. Error bars indicate ± SD (n = 3). (C) Changes in intestinal DAG and TAG species of lipid-fed and lipid-starved Lpp RNAi larvae, quantified by mass spectrometry. Acylglycerols are normalized to polar lipid. Note that Lpp RNAI strongly increases...
intestine, neutral lipids, regardless the presence of lipids in the diet. Lipid species with medium-chain fatty acid residues are indicated by green background. Error bars indicate \( \pm SD \) \((n = 3)\). (D) Changes in fat body TAG upon lipid starvation, quantified by mass spectrometry. TAG levels normalized to polar lipid slightly increase in the absence of dietary lipid. Error bars indicate \( \pm SD \) \((n = 5)\).

**Figure S10** Consequences of lipoprotein knock-down on the phospholipid composition of cellular membranes. (A) Changes in hemolymph PE species caused by LTP or Lpp RNAi, quantified by mass spectrometry. Individual PE species are normalized to total hemolymph protein. Note that Lpp RNAi strongly reduces all hemolymph PE species. (B) Changes in PE species in the gut caused by Lpp RNAi, quantified by mass spectrometry. Individual PE species are represented as mol\% of total polar lipids. Note that no PC species is significantly decreased in cellular membranes upon either LTP or Lpp RNAi. (C) Changes in PE species in fat body, gut, wing disc and brain caused by LTP or Lpp RNAi, quantified by mass spectrometry. Individual PE species are represented as mol\% of polar lipids. Note that no phospholipid PE species is significantly decreased upon either LTP or Lpp RNAi. Error bars indicate \( \pm SD \) \((n = 6)\) for LTP or Lpp RNAi experiments: WP JLS. Analyzed the data: WP JLS SE. Contributed reagents/materials/analysis tools: WP JLS MB MC AM AS. Wrote the paper: WP SE.

**References**

1. Vance DE, Vance JE (2008) Biochemistry of lipids, lipoproteins and membranes: Elsevier.
2. Olofsson SO, Asp L, Boren J (1999) The assembly and secretion of apolipoprotein B-containing lipoproteins. Curr Opin Lipidol 10: 341–346.
3. Hussain MM, Shi J, Dreizen P (2003) Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly. J Lipid Res 44: 22–32.
4. Gregg RE, Wetterauer JR (1994) The molecular basis of abetalipoproteinemia. Curr Opin Lipidol 5: 81–86.
5. Berriot-Vuillaume N, Agerberck LP, Samson-Bouna M, Wetterauer JR (2000) The role of the microsomal triglyceride transfer protein in abetalipoproteinemia. Annu Rev Nutr 20: 663–697.
6. Glass CK, Witztum JL (2001) Atherosclerosis. the road ahead. Cell 104: 503–516.
7. Eckel RH, Grundy SM, Zimmet PZ (2005) The metabolic syndrome. Lancet 365: 1415–1428.
8. Goldberg JL, Brown MS (1977) The low-density lipoprotein pathway and its relation to atherosclerosis. Annu Rev Biochem 46: 997–930.
9. Spector AA, Mathur SN, Kaduce TL, Hyman BT (1980) Lipid nutrition and stock maintenance. We gratefully acknowledge Bruce Edgar and Ronald Kühnlein for providing fly stocks. We thank Sven Ssyork for help with transgenesis and stock maintenance. We are grateful to Elisabeth Knust, Kai Simons, and Teymuraz Kurzchalia for comments on the manuscript.

**Author Contributions**

Conceived and designed the experiments: WP SE. Performed the experiments: WP JLS. Analyzed the data: WP JLS SE. Contributed reagents/materials/analysis tools: WP JLS MB MC AM AS. Wrote the paper: WP SE.

15. Birse RT, Choi J, Reardon K, Rodriguez J, Graham S, et al. (2010) High-fat-diet-induced obesity and heart dysfunction are regulated by the TOR pathway in Drosophila. Cell Metab 12: 533–544.
16. Smolenaaars MM, Madsen O, Rodenburg KW, Van der Horst DJ (2007) Insect lipid transfer protein superfamily. J Lipid Res 48: 489–502.

**Figure S11** Supporting mass spectrometry data. (A) Changes in DAG species in hemolymph, fat body, gut, wing disc and brain upon LTP or Lpp RNAi, quantified by mass spectrometry. Shown are both number of double bonds and combined acyl chain length. Hemolymph DAG is normalized to polar lipids. Error bars indicate \( \pm SD \) \((n = 8)\) for hemolymph and brain; \( \pm SD \) \((n = 6)\) for LTP n = 7, Lpp RNAi n = 7, organs: n = 5. (B) Changes in TAG species in hemolymph, fat body, gut, wing disc and brain caused by LTP or Lpp RNAi, quantified by mass spectrometry. Shown are both number of double bonds and combined acyl chain length. Hemolymph TAG is normalized to protein; tissue TAG is normalized to polar lipids. Error bars indicate \( \pm SD \) \((n = 8)\) for LTP n = 6, Lpp RNAi n = 7; organs: n = 5. (C) Total tissue polar lipid count in different LTP and Lpp RNAi mass spectrometry experiments. Similar amounts of a given tissue were extracted and quantified for each condition. Error bars indicate \( \pm SD \) \((n = 5)\).
31. Sellers JA, Hou L, Athar H, Hassain MM, Shelness GS (2003) A Drosophila microsomal triglyceride transfer protein homolog promotes the assembly and secretion of human apolipoprotein B. Implications for human and insect transport and metabolism. J Biol Chem 278: 20367–20373.

32. Shoulders CC, Narcisi TM, Read J, Chester A, Bent DJ, et al. (1994) The abetalipoproteinemia gene is a member of the vitellogenin family and encodes an alpha-helical domain. Nat Struct Biol 1: 285–286.

33. Babini PJ, Bogerd J, Koosman FP, Van Marrewijk WJ, Van der Horst DJ (1999) Apolipoprotein II/I, apolipoprotein B, vitellogenin, and microsomal triglyceride transfer protein genes are derived from a common ancestor. J Mol Evol 49: 150–160.

34. Kutty RK, Kutty G, Kannadhat R, Duncan T, Koosnin EV, et al. (1996) Molecular characterization and developmental expression of a retinoid- and fatty acid-binding glycoprotein from Drosophila. A putative lipophorin. J Biol Chem 271: 20641–20649.

35. Ryan RO, Wells MA, Law JH (1986) Lipid transfer protein from Manduca sexta hemolymph. Biochem Biophys Res Commun 136: 260–263.

36. Hirayama Y, Chino H (1990) Lipid transfer particle in locust hemolymph: purification and characterization. J Lipid Res 31: 793–799.

37. Chen J, Honeyager SM, Schleeve J, Arvanosov A, Laughon A, et al. (2012) Crossveinless d is a vitellogenin-like lipoprotein that binds Bmps and HSPGs, and is required for normal BMP signaling in the Drosophila wing. Development 139: 2170–2176.

38. Ryan RO, van der Horst DJ (2000) Lipid transport biochemistry and its role in energy production. Annu Rev Entomol 45: 233–260.

39. Banaszak L, Sharrock W, Timmins P (1991) Structure and function of a lipoprotein: lipovitellin. Annu Rev Biophys Biophys Chem 20: 221–246.

40. Shibata Y, Branicky R, Landaverde IO, Hekimi S (2003) Redox regulation of SREBP processing and membrane lipid production by the liver. J Biol Chem 278: 27793–27800.

41. Rava P, Ojakian GK, Shelness GS, Hussain MM (2006) Phospholipid transfer activity of microsomal triglyceride transfer protein is sufficient for the assembly and secretion of apolipoprotein B lipoproteins. J Biol Chem 281: 11019–11027.

42. Raabe M, Veniant MM, Sullivan MA, Zlot CH, Bjorkgren J, et al. (1999) Analysis of the role of microsomal triglyceride transfer protein in the liver of tissue-specific knockout mice. J Clin Invest 103: 1287–1298.

43. Wang Y, Tran K, Yao Z. (1999) The activity of microsomal triglyceride transfer protein is essential for accumulation of triglyceride within microsomes in McA-RH7777 cells. A unified model for the assembly of very low density lipoproteins. J Biol Chem 274: 27793–27800.

44. Raabe M, Veniant MM, Sullivan MA, Zlot CH, Bjorkgren J, et al. (1999) Analysis of the role of microsomal triglyceride transfer protein in the liver of tissue-specific knockout mice. J Clin Invest 103: 1287–1298.

45. Ryan RO, Senthilathipan KR, Wells MA, Law JH (1983). Facilitated daisyglycerol exchange between insect hemolymph lipoproteins. Properties of Manduca sexta lipid transfer particle. J Biol Chem 263: 14140–14145.

46. Ryan RO, Wessler AN, Price HM, Ando S, Yokoyama S (1990) Insect lipid transfer particle catalyzes bidirectional vectorial transfer of dicylglycerol from lipophorin to human low density lipoprotein. J Biol Chem 265: 10531–10535.

47. Silver ET, Szabo DG, Ryan RO (1990) Lipid transfer particle-induced transformation of human high density lipoprotein into apolipoprotein A-I-deficient low density particles. J Biol Chem 265: 22487–22492.

48. Mullane BC, Ashrafi K (2009) C. elegans fat storage and metabolic regulation. Biochim Biophys Acta 1791: 474–478.

49. Hsieh J, Hayashi AA, Webb J, A чел (2008) Postprandial dyslipidemia in insulin resistance: mechanisms and role of intestinal insulin sensitivity. Atheroscler Suppl 9: 7–13.

50. Horton JD, Goldstein JL, Brown MS (2002) SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 109: 1125–1131.

51. Blacklock BJ, Smillie M, Ryan RO (1992) Insect lipid transfer particle can facilitate net vectorial lipid transfer via a carrier-mediated mechanism. J Biol Chem 267: 14033–14037.

52. Gronke S, Muller G, Hirsch J, Fellert S, Andreuss A, et al. (2007) Dual lipolytic control of body fat storage and mobilization in Drosophila. PLoS Biol 5: e137. doi:10.1371/journal.pbio.0050137.

53. Ryan RO, Wessler AN, Price HM, Ando S, Yokoyama S (1990) Insect lipid transfer particle catalyzes bidirectional vectorial transfer of dicylglycerol from lipophorin to human low density lipoprotein. J Biol Chem 265: 10531–10535.

54. Mullaney BC, Ashrafi K (2009) C. elegans fat storage and metabolic regulation. Biochim Biophys Acta 1791: 474–478.

55. Mullaney BC, Ashrafi K (2009) C. elegans fat storage and metabolic regulation. Biochim Biophys Acta 1791: 474–478.

56. Hsieh J, Hayashi AA, Webb J, A чел (2008) Postprandial dyslipidemia in insulin resistance: mechanisms and role of intestinal insulin sensitivity. Atheroscler Suppl 9: 7–13.

57. Horton JD, Goldstein JL, Brown MS (2002) SREBP: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 109: 1125–1131.

58. Nohruth A, Zhang SC (2009) Coordination of lipid metabolism in membrane biogenesis. Annu Rev Cell Dev Biol 25: 539–560.

59. Dobrosotskaya IY, Seegmiller AC, Brown MS, Goldstein JL, Rawson RB (2002) Regulation of SREBP processing and membrane lipid production by phospholipids in Drosophila. Science 296: 879–883.

60. Shapiro JP, Law JH (1983) Facilitated daisyglycerol exchange between insect hemolymph lipoproteins. Properties of Manduca sexta lipid transfer particle. J Biol Chem 263: 14140–14145.

61. Dobrosotskaya IY, Seegmiller AC, Brown MS, Goldstein JL, Rawson RB (2002) Regulation of SREBP processing and membrane lipid production by phospholipids in Drosophila. Science 296: 879–883.

62. Rava P, Ojakian GK, Shelness GS, Hussain MM (2006) Phospholipid transfer activity of microsomal triglyceride transfer protein is sufficient for the assembly and secretion of apolipoprotein B lipoproteins. J Biol Chem 281: 11019–11027.

63. Nohturfft A, Zhang SC (2009) Coordination of lipid metabolism in membrane biogenesis. Annu Rev Cell Dev Biol 25: 539–560.

64. Shibata Y, Branicky R, Landaverde IO, Hekimi S (2003) Redox regulation of SREBP processing and membrane lipid production by the liver. J Biol Chem 278: 27793–27800.

65. Shibata Y, Branicky R, Landaverde IO, Hekimi S (2003) Redox regulation of SREBP processing and membrane lipid production by the liver. J Biol Chem 278: 27793–27800.

66. Shibata Y, Branicky R, Landaverde IO, Hekimi S (2003) Redox regulation of SREBP processing and membrane lipid production by the liver. J Biol Chem 278: 27793–27800.

67. Shibata Y, Branicky R, Landaverde IO, Hekimi S (2003) Redox regulation of SREBP processing and membrane lipid production by the liver. J Biol Chem 278: 27793–27800.

68. Shibata Y, Branicky R, Landaverde IO, Hekimi S (2003) Redox regulation of SREBP processing and membrane lipid production by the liver. J Biol Chem 278: 27793–27800.

69. Shibata Y, Branicky R, Landaverde IO, Hekimi S (2003) Redox regulation of SREBP processing and membrane lipid production by the liver. J Biol Chem 278: 27793–27800.