Cell Metabolism
Short Article

Coordination of Triacylglycerol and Cholesterol Homeostasis by *DHR96* and the *Drosophila LipA* Homolog *magro*

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SUMMARY

Although transintestinal cholesterol efflux has been identified as an important means of clearing excess sterols, the mechanisms that underlie this process remain poorly understood. Here, we show that *magro*, a direct target of the *Drosophila DHR96* nuclear receptor, is required in the intestine to maintain cholesterol homeostasis. *magro* encodes a LipA homolog that is secreted from the anterior gut into the intestinal lumen to digest dietary triacylglycerol. Expression of *magro* in intestinal cells is required to hydrolyze cholesterol esters and promote cholesterol clearance. Restoring *magro* expression in the intestine of *DHR96* mutants rescues their defects in triacylglycerol and cholesterol metabolism. These studies show that the central role of the intestine in cholesterol efflux has been conserved through evolution, that the ancestral function of LipA is to coordinate triacylglycerol and cholesterol metabolism, and that the region-specific activities of *magro* correspond to the metabolic functions of its upstream regulator, DHR96.

INTRODUCTION

Coordinate regulation of lipid metabolism is central to human health, and disruption of this process leads to a range of metabolic disorders, including obesity and cardiovascular disease. Normal lipid homeostasis is maintained by balancing dietary lipid uptake and synthesis with lipid catabolism and excretion. Under normal feeding conditions, dietary lipids, such as triacylglycerol (TAG) and cholesterol esters, are broken down into free fatty acids, monoacylglycerols, and free sterols in the lumen of the intestine. These digested lipids can then be absorbed by the intestinal cells, where TAG is resynthesized and packaged together with cholesterol, cholesterol esters, and carrier proteins to form lipoprotein particles that are trafficked throughout the body. These lipids can be either utilized by cells or deposited in storage tissues, such as the adipose and liver. Under conditions of excess lipids, TAG and cholesterol esters are broken down and free fatty acids can be utilized for energy, whereas excess cholesterol is excreted from the body (Lusis and Pajukanta, 2008; van der Velde et al., 2010).

Nuclear receptors (NRs) are ligand-regulated transcription factors that play essential roles in multiple aspects of lipid homeostasis. Many NRs bind small lipophilic compounds, such as fatty acids, sterols, and other metabolic intermediates, and coordinate multiple aspects of metabolism by directing specific changes in gene expression. One example of this is LXRα (NR1H3), which binds oxysterols and promotes the modification and clearance of excess sterols (Kalaany and Mangelsdorf, 2006). In addition, LXRα is required to maintain proper TAG levels, at least in part through the regulation of SREBP-mediated fat synthesis (Schultz et al., 2000). Thus, LXRα activity is central to both TAG and cholesterol homeostasis, although much remains to be learned about the roles of specific LXR target genes in mediating these key metabolic functions.

We have been studying a *Drosophila* homolog of LXR, DHR96, as a simple system to understand the physiological and molecular roles for this family of NRs and their target genes. Biochemical and genetic studies of DHR96 have shown that it shares the central metabolic functions of its mammalian counterpart. DHR96 binds cholesterol and is required for normal cholesterol homeostasis, with *DHR96* null mutants exhibiting an ~20% increase in whole animal cholesterol levels due, at least in part, to increased *npc1b* expression (Horner et al., 2009; Bujold et al., 2010). In addition, *DHR96* mutants display an ~50% decrease in whole animal TAG levels that can be attributed to an inability to break down dietary TAG due to reduced expression of the intestinal lipase Magro *(CG5932)* (Sieber and Thummel, 2009). Interestingly, *magro* transcription responds to dietary cholesterol levels and this regulation is dependent on *DHR96* function, providing a potential link between cholesterol levels, DHR96, and TAG homeostasis (Horner et al., 2009; Bujold et al., 2010). Moreover, although Magro protein is most similar to mammalian gastric lipase (38% identity, 56% similarity), the second most similar protein is LipA (32% identity, 50% similarity), which has both TAG lipase and cholesterol esterase activities (Ameis et al., 1994). Genetic studies have demonstrated a central role for LipA in maintaining proper cholesterol levels in mice (Du et al., 2001). Similar phenotypes are seen in human *LipA* mutants suffering from cholesterol ester storage disease (CESD) and Wolman's disease (Burke and Schubert, 1972). These observations raise the possibility that *magro*, in addition to controlling TAG homeostasis, may regulate cholesterol homeostasis and

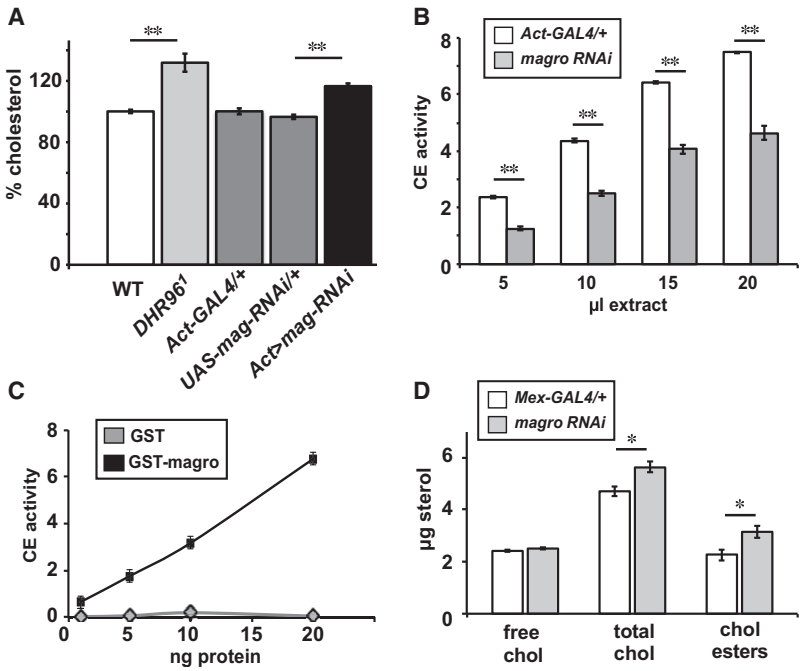

Figure 1. *magro* Maintains Proper Cholesterol Levels and Has Cholesterol Esterase Activity

(A) RNAi for *magro* results in elevated cholesterol levels similar to those seen in *DHR96* mutants. Wild-type (WT), *Act-GAL4/+*, and *UAS-magro-RNAi/+* control flies, along with *DHR96*[1] mutants and *Act-GAL4/UAS-magro-RNAi* animals (*Act>mag-RNAi*), were assayed for total cholesterol levels. The data were normalized to protein levels and are presented relative to a wild-type level of 100%.

(B) RNAi for *magro* results in reduced cholesteryl ester hydrolase activity (CE) in intestines. Intestines dissected from both *Act-Gal4/+* control and *Act-GAL4/UAS-magro-RNAi* (*magro* RNAi) animals were homogenized and increasing amounts of lysate were tested for cholesteryl ester hydrolase activity by assaying for the release of free cholesterol from a cholesterol acetate substrate. The y-axis shows µg/ml of free glycerol released.

(C) Purified GST-Magro protein has CE activity. Recombinant GST and GST-Magro were purified as described (Sieber and Thummel, 2009) and increasing amounts of protein were assayed for CE activity. The y-axis shows µg/ml of free glycerol released.

(D) Intestine-specific RNAi for *magro* results in elevated levels of esterified cholesterol. Free, esterified, and total cholesterol levels were measured from *Mex-GAL4/+* control and *Mex-GAL4/UAS-magro-RNAi* (*magro* RNAi) animals. Error bars represent ± SEM (*$p < 0.05$, **$p < 0.0001$).

DHR96 may function through *magro* to help coordinate TAG and cholesterol metabolism.

In this study we show that loss of *magro* function leads to an increase in cholesterol levels similar to that seen in *DHR96* mutants. We show that Magro, like LipA, has cholesterol esterase activity, and this enzyme is required in intestinal cells to maintain cholesterol homeostasis. In contrast, the TAG lipase activity of Magro arises from the anterior end of the gut and acts in the intestinal lumen to facilitate dietary fat uptake. Restoring *magro* expression in the intestine of the *DHR96* mutant is sufficient to rescue their lean phenotype and elevated levels of cholesterol. Our data support the model that *DHR96* functions through *magro* in the intestine to coordinate both dietary TAG breakdown and the clearance of excess sterols.

RESULTS

magro Is Required for Normal Cholesterol Homeostasis

The regulation of *magro* transcription by dietary cholesterol combined with its significant homology to *LipA* prompted us to test whether *magro* function is required for cholesterol homeostasis. *DHR96*[1] null mutants grown on a normal diet display elevated levels of cholesterol compared to genetically matched wild-type controls (Figure 1A), similar to the results seen when *DHR96* mutant larvae are subjected to a high cholesterol diet (Horner et al., 2009). Interestingly, ubiquitous RNA interference (RNAi)-mediated silencing of *magro* expression using *Act-GAL4*, as done previously (Sieber and Thummel, 2009), leads to a similar phenotype (Figure 1A). Taken together with our earlier work, which showed that both *DHR96* mutants and *magro* RNAi animals have significantly lower levels of TAG, these data suggest that *DHR96* functions through transcriptional regulation of *magro* to coordinate TAG and cholesterol homeostasis in *Drosophila*.

magro Regulates Cholesterol Homeostasis by Breaking Down Stored Cholesterol Esters

In order to determine whether Magro can act like LipA by cleaving cholesterol esters in addition to its TAG lipase activity, we assayed for cholesterol esterase activity in control and *magro* RNAi animals (Ameis et al., 1994). Although control intestinal lysates exhibit a high level of cholesterol esterase activity in a dose-dependent manner, the lysates from *magro* RNAi animals display an ~ 50% decrease in enzymatic function (Figure 1B). Moreover, purified recombinant GST-Magro efficiently cleaves a cholesterol ester substrate in vitro, demonstrating that these effects are a direct result of Magro enzymatic activity (Figure 1C). Taken together with our earlier biochemical studies, this result shows that Magro is a bifunctional enzyme that can act as both a TAG lipase and a cholesterol esterase (Sieber and Thummel, 2009).

If decreased intestinal cholesterol esterase activity causes the elevated cholesterol levels seen in the *magro* RNAi animals, then we should see an increase in stored cholesterol esters when *magro* expression is specifically silenced in the intestine. Consistent with this proposal, we see a significant increase in the levels of both total cholesterol and cholesterol esters in *Mex>magro* RNAi animals, whereas free cholesterol levels remain unchanged (Figure 1D). Elevated cholesterol levels can also be detected clearly in isolated intestines, supporting a role for *magro* in maintaining cholesterol levels in this tissue (Figure S1). These data support the model that Magro maintains cholesterol homeostasis through its ability to directly break down stored cholesterol esters in the intestine.

magro Is Expressed throughout the Digestive Tract

The similarity between lipase sequences complicates our ability to raise specific antibodies against Magro. Accordingly, we determined the pattern of Magro expression using a genomic

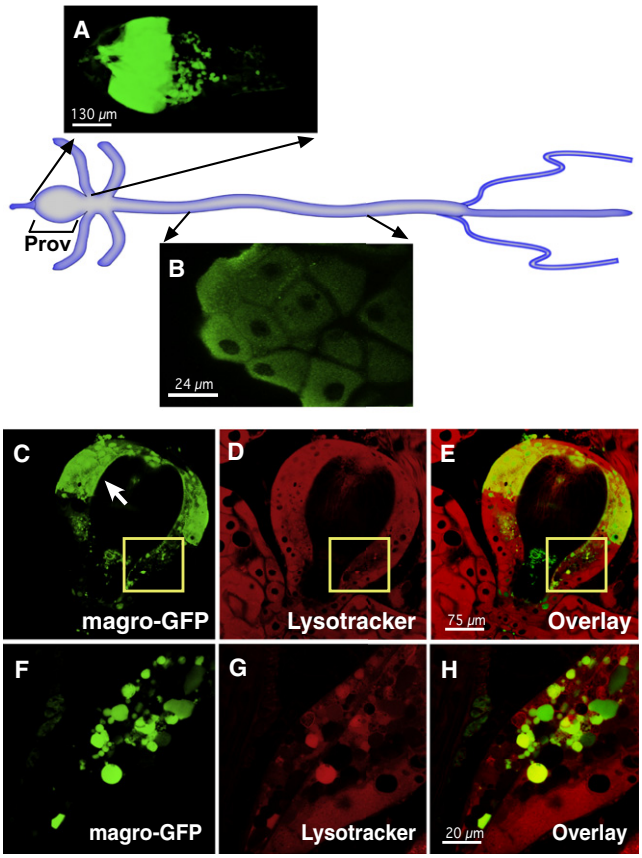

Figure 2. Magro Is Expressed in the Proventriculus and Midgut

(A) Magro-EGFP (green) expression in mid-third instar larvae is restricted to the intestine and accumulates to high levels in the anterior region of the proventriculus (Prov) (A, arrow in C). Antibody staining for EGFP also revealed lower levels of punctate expression in the cytoplasm of enterocytes.

(B) Higher resolution images of the proventriculus revealed Magro-EGFP in large vesicles that extend from the abundant expression at the anterior end of the proventriculus toward the junction with the midgut lumen (yellow boxes in C, D, and E, shown in panels F, G, and H). Lysotracker Red stains the large acidic vesicles that contain Magro-EGFP protein (D, E, G, and H).

magro-EGFP transgenic line. This construct contains ~7 kb of genomic DNA spanning the *magro* locus with 5 kb of upstream promoter sequences and the EGFP gene fused in-frame to the 3′-end of the *magro* protein coding region. Magro-EGFP is expressed specifically in the intestine with the highest levels of protein accumulation in the anterior region of the proventriculus (Figure 2A). The proventriculus is a bulb shaped structure at the anterior end of the gut that consists of three distinct cell layers (Figure S2). Magro-EGFP is expressed in the anterior half of the outermost layer of cells in the proventriculus (Figures 2C, arrow; and S2). In addition, protein is clearly visible in large vesicles that lie posterior to this region of expression (Figures 2C–2E, yellow boxes). These are acidic vesicles that stain positive for Lysotracker Red (Figures 2F–2H), consistent with the acid lipase activity of Magro and LipA. Interestingly, visualization of vesicles using CD8-EGFP in this region reveals that they move in a posterior direction toward the intestine, providing a potential mechanism to deliver digestive enzymes, such as Magro, into the intestinal lumen (Figure S3). Lower levels of Magro-EGFP protein can also be seen in the major cell type of the intestine, the enterocytes, in a punctate cytoplasmic pattern (Figure 2B). Interestingly, we do not observe Magro-EGFP in large Lysotracker-positive vesicles in these cells. Taken together, these observations suggest that Magro is trafficked differently in different cell types of the digestive tract, raising the possibility that the cholesterol esterase and TAG lipase activities of this enzyme are regionally specified.

magro Acts within the Intestinal Lumen to Maintain Triacylglycerol Homeostasis

As a first step toward determining whether the enzymatic activities of Magro localize regionally within the intestine, we raised *DHR96* mutant and *magro* RNAi animals on a diet supplemented with pancreatin. This enzymatic mixture of pancreatic enzymes, including TAG lipase and cholesterol esterase, should specifically restore TAG lipase and cholesterol esterase activity in the lumen of the gut. Although pancreatin supplementation had no effect on TAG levels in control animals, this diet restored wild-type levels of TAG in both *DHR96* and *magro* RNAi animals (Figures 3A and 3B). Conversely, pancreatin supplementation had no impact on the elevated cholesterol levels in these animals (Figure 3C and 3D). These results are consistent with those seen when wild-type flies are treated with Orlistat (tetrahydrolipstatin), which acts as a competitive inhibitor of secreted lipases and cholesterol esterases inside the lumen of the intestine (Borgström, 1988). Although Orlistat treatment is sufficient to decrease whole animal TAG levels, as seen previously (Sieber and Thummel, 2009), it has no significant effect on total cholesterol levels (Figure S4). These data confirm our earlier studies indicating that Magro functions in the intestinal lumen to maintain appropriate levels of TAG, and demonstrate that its effects on cholesterol homeostasis are conferred within the cells of the intestinal tract.

magro Functions in the Proventriculus to Promote the Breakdown of Dietary Triacylglycerol

The apparent vesicular trafficking of Magro protein from the proventriculus toward the body of the midgut provides a mechanism to explain its delivery into the lumen of the intestine. If this model is correct, then disrupting *magro* function specifically in the proventriculus should have an effect on TAG levels but little or no effect on cholesterol homeostasis. To test this hypothesis, we used the *bab1-GAL4* driver, which is expressed highly in the proventriculus and weakly near the midgut-hindgut junction (Figure 4A) (Cabrera et al., 2002). Using this construct to direct *magro* RNAi resulted in a significant decrease in whole animal TAG levels (Figure 4B), similar to that seen with the panintestinal *Mex-GAL4* driver (Sieber and Thummel, 2009). In contrast, proventriculus-specific *magro* RNAi has no effect on total cholesterol levels (Figure 4C). We conclude that the TAG metabolic functions of Magro are restricted to the proventriculus, whereas its cholesterol regulatory function resides outside the proventriculus in the intestine.

DHR96 Regulates Major Aspects of Lipid Metabolism through Its Target Gene *magro*

If decreased *magro* expression is physiologically relevant to the cholesterol accumulation phenotype seen in *DHR96* mutants,

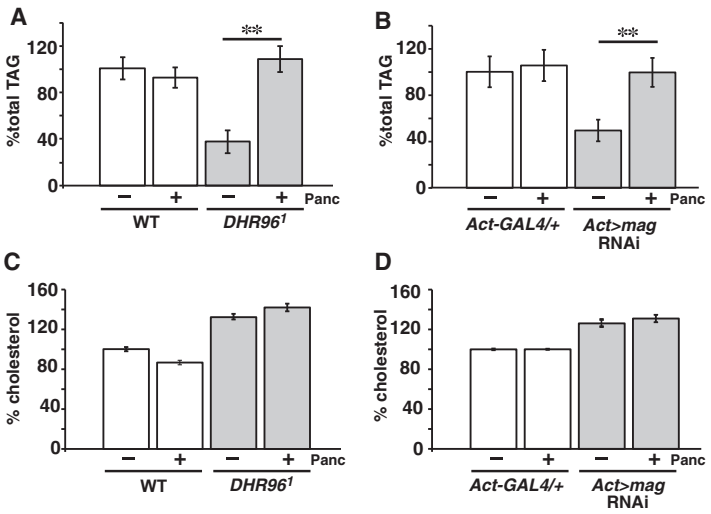

Figure 3. Magro Enzymatic Activity Is Not Required in the Intestinal Lumen to Maintain Cholesterol Homeostasis

Wild-type (WT) and *Act-GAL4/+* controls, along with *DHR96*[1] mutants and *Act-GAL4/UAS-magro-RNAi* (*Act>mag*-RNAi) animals, were raised on medium supplemented with 2 mg/mls pancreatin, an enzymatic mixture of pancreatic TAG lipase and cholesterol esterase. (A–D) Mature adults were collected and assayed for TAG (A and B) and cholesterol (C and D) levels. The data were normalized to protein levels and are presented relative to a wild-type level of 100%. Error bars represent ± SEM (**$p < 0.0001$).

then restoring *magro* function in these animals should rescue their defect in cholesterol homeostasis. Consistent with this hypothesis, expressing wild-type *magro* specifically in the intestine of the *DHR96* mutant is sufficient to reduce the cholesterol levels in these animals (Figure 4D). This rescue, however, is not complete, which is consistent with the multiple levels of cholesterol metabolism that are regulated by DHR96 (Horner et al., 2009; Bujold et al., 2010). Moreover, specific expression of *magro* in the proventriculus of *DHR96* mutants effectively rescues their lean phenotype (Figure 4E) but has no significant effect on their elevated cholesterol levels (Figure 4F). Taken together with our other data, these results indicate that the region-specific enzymatic activities of Magro correspond to the major lipid metabolic functions of DHR96. DHR96 regulation of *magro* expression in the proventriculus maintains an appropriate level of TAG lipase activity in the intestinal lumen to facilitate the breakdown of dietary lipid whereas DHR96 regulation of *magro* in the body of the intestine promotes the clearance of excess sterols.

DISCUSSION

Magro and LipA Share Conserved Functions in Maintaining Lipid Homeostasis

Relatively little is known about the mechanisms that regulate cholesterol metabolism in *Drosophila*. Most studies have focused on DHR96 and the Niemann-Pick (NPC) disease gene homologs, which play important roles in dietary cholesterol absorption and intracellular cholesterol trafficking (Niwa and Niwa, 2011). In this paper, we identify the intestine as a key tissue for maintaining cholesterol homeostasis, and we define a central role for the LipA homolog, Magro, in mediating this function. Like LipA, Magro has dual enzymatic activities, cleaving both TAG and cholesterol esters, consistent with their common fatty acid ester bonds (Ameis et al., 1994). Mouse *LipA* mutants display a lack of stored fat in the form of white adipose tissue along with excess cholesterol esters (Du et al., 2001), reflecting the major defects in *magro* RNAi animals. Similar phenotypes are seen in human *LipA* mutants suffering from CESD and Wolman's disease (Burke and Schubert, 1972). Patients with Wolman's disease also have digestive dysfunction, which may be related to the defects in lipid uptake that we observe in *magro* RNAi animals. In addition to these shared phenotypes, however, mammalian *LipA* mutants display massive accumulations of lipid in the liver, spleen, and intestine—defects that are not apparent in *magro* RNAi animals (Du et al., 2001). Nonetheless, the parallels between Magro and LipA function in flies and humans establish *Drosophila* as a system to further our understanding of CESD and Wolman's disease and define the ancestral function for this class of acid lipases, demonstrating their central role in the intestine to coordinate TAG and cholesterol homeostasis.

Interestingly, the dual enzymatic functions of Magro appear to arise from distinct regions of the intestine. Disruption of *magro* function specifically in the proventriculus blocks its TAG lipolytic activity but does not affect cholesterol levels in these animals (Figures 4B and 4C). In contrast, *magro* RNAi throughout the intestine affects both TAG and cholesterol homeostasis (Figure 1D). These region-specific activities are consistent with our dietary supplementation studies with pancreatin and Orlistat (Figures 3 and S4). They are also consistent with the expression pattern of Magro-EGFP protein, providing insights into how the dual functions of this enzyme are manifested. Magro-EGFP is expressed abundantly in the large outer columnar cells in the anterior half of the proventriculus (Figures 2A and S2). We also see a stream of large acidic vesicles that originate from this region and move in a posterior direction toward the lumen of the intestine (Figures 2F–2H and S3). This apparent vesicular trafficking of Magro is consistent with the cells at the anterior end of the proventriculus having secretory functions, depositing peritrophic matrix components into the lumen that lies between the outer and inner cell layers of the proventriculus (King, 1988) (Figure S2). The peritrophic matrix is a meshwork of chitin and glycoproteins that provides a protective lining within the gut, much like the mucosal layer of the mammalian intestine (Hegedus et al., 2009). The observation that the Magro-EGFP vesicles reside in the same region of the proventriculus as the developing peritrophic matrix suggests that they are synthesized and exported into the lumen of the gut in a similar manner. This also raises the possibility that the digestive enzymes that are embedded in the peritrophic matrix may originate from vesicular trafficking in the proventriculus. Like *magro*, many genes with predicted digestive functions, including glucosidases, mannosidases, and endopeptidases, are regulated by DHR96 and expressed in the intestine (Sieber and Thummel, 2009). Several genes that contribute to the peritrophic matrix are also regulated by DHR96.

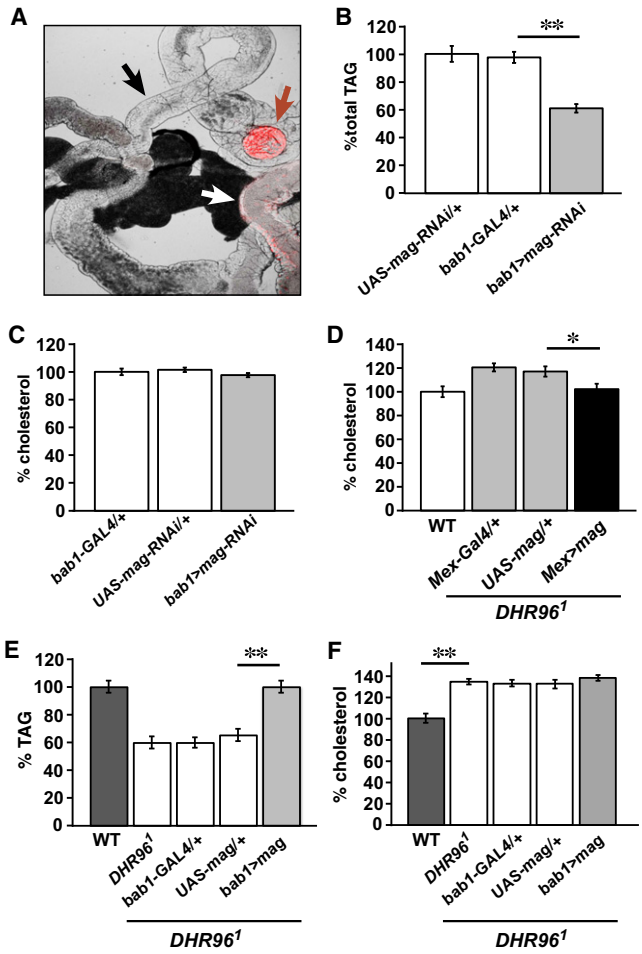

Figure 4. DHR96 Regulation of *magro* in Distinct Regions of the Intestine Coordinates TAG and Cholesterol Homeostasis

(A) *bab1-GAL4* drives high levels of *UAS-NLS-DsRed* expression in the proventriculus (red arrow) with no detectable expression in the body of the midgut (black arrow) and low levels near the midgut-hindgut junction (white arrow).

(B and C) Proventriculus-specific RNAi for *magro* results in reduced levels of TAG but does not affect cholesterol. *bab1-GAL4/+* and *UAS-magro-RNAi/+* control flies, along with *bab1-GAL4/UAS-magro-RNAi* (*bab1>mag-RNAi*) animals were assayed for TAG (B) and cholesterol (C). The data were normalized to protein levels and are presented relative to a wild-type level of 100%.

(D) Midgut-specific expression of *magro* is sufficient to partially rescue the elevated cholesterol levels in *DHR96* mutants. Wild-type (WT) and *DHR96* mutants carrying the *Mex-GAL4* driver alone, *UAS-magro* transgene alone, or both *Mex-GAL4* and *UAS-magro* (*Mex>mag*) to express *magro* in the midgut were assayed for total cholesterol levels. The data were normalized to protein levels and are presented relative to a wild-type level of 100%.

(E and F) Proventriculus-specific expression of *magro* is sufficient to rescue the lean phenotype of *DHR96* mutants, but has no effect on its elevated cholesterol levels. Wild-type (WT) and *DHR96* mutants carrying the *bab1-GAL4* driver alone, *UAS-magro* transgene alone, or both *bab1-GAL4* and *UAS-magro* (*bab1>mag*) to express *magro* in the proventriculus were assayed for TAG (E) and cholesterol (F). The data were normalized to protein levels and are presented relative to a wild-type level of 100%. Error bars represent ± SEM (*$p < 0.01$ **$p < 0.0001$).

It would be interesting to determine whether the proventriculus synthesizes and secretes these proteins in a coordinated manner.

In addition to its abundant expression in the proventriculus, Magro-EGFP is also present at lower levels throughout the intestine, visible as punctate cytoplasmic staining in the enterocytes (Figure 2B). This expression pattern provides a possible mechanism to explain the role of Magro in maintaining cholesterol homeostasis. We propose that Magro acts as a cholesterol esterase in the enterocytes, breaking down stored cholesterol to facilitate its elimination from the intestine. This model is consistent with the neutral lipid stores that are known to reside in the *Drosophila* intestine, second only to the fat body. It is also consistent with recent evidence that LipA acts as cholesterol esterase in macrophage foam cells to promote ABCA1-mediated cholesterol efflux (Ouimet et al., 2011). These data suggest that LipA and Magro may break down stored cholesterol esters upstream of reverse cholesterol transport and transintestinal cholesterol efflux to promote the clearance of excess sterols. Tissue-specific studies of *LipA* function in the pancreas and intestine may provide a clearer understanding of its relationship to the apparent exocrine role of Magro in the proventriculus and its ability to promote cholesterol clearance in the intestine.

Finally, our data provide new directions in understanding the roles of LXR family members in lipid metabolism. Both *DHR96* mutants and *magro* RNAi animals display reduced levels of TAG and elevated levels of cholesterol, and restoring *magro* expression in the intestines of *DHR96* mutant animals largely rescues these defects, establishing *magro* as a key target for DHR96 regulation (Figures 1 and 4C) (Sieber and Thummel, 2009). These functions for DHR96 parallel those of its mammalian homolog, LXR. LXR activation, specifically in the intestine, results in a dramatic increase in fecal sterol excretion that correlates with increased expression of the *ABCG5/ABCG8* sterol transporter (van der Veen et al., 2009; Lo Sasso et al., 2010). This observation suggests that LXR promotes reverse cholesterol transport in this tissue, which represents the best characterized mechanism for eliminating excess cholesterol from the body. Reverse cholesterol transport involves HDL-mediated transport of cholesterol from peripheral tissues to the hepatobiliary tract, leading to the removal of excess sterol by biliary excretion from the body. However, genetic studies of key components in biliary cholesterol excretion, such as *abcb4* mutants and *abcg5/abcg8* double mutants, have challenged the importance of reverse cholesterol transport for cholesterol excretion and have led to the proposal that the intestine plays a more direct role in this process (van der Velde et al., 2010). These studies shift the focus of cholesterol efflux toward the intestine and implicate a central role for LXR in regulating intestinal cholesterol clearance, not only through regulation of reverse cholesterol transport but also through novel targets involved in transintestinal cholesterol efflux (Kruit et al., 2005). In addition, our evidence that intestinal cholesterol esterase activity is critical for clearing excess sterol from the body suggests that acid lipases such as LipA may function downstream from LXR to maintain cholesterol homeostasis. Although there is no direct evidence that LXR regulates *LipA* expression, a recent study showed that elevated levels of oxidized LDL can repress *LipA* expression in endothelial cells, an effect that can be reversed

by treatment with LXR agonists (Heltianu et al., 2011). Further studies are required to determine whether the regulatory links between LXR, LipA, and cholesterol homeostasis have been conserved through evolution and whether *Drosophila* can be used as a simple model system to better define the mechanisms of transintestinal cholesterol efflux.

EXPERIMENTAL PROCEDURES

Fly Stocks

The following stocks were used in this study: *Canton S*, *DHR96*^{1} (King-Jones et al., 2006), *Mex-Gal4* (Phillips and Thomas, 2006), *Act-Gal4/CyO* (Bloomington # 25374), *UAS-magro* (Sieber and Thummel, 2009), *UAS-DHR96* (Horner et al., 2009), and *bab1-GAL4/TM3* (Cabrera et al., 2002). Flies were maintained on standard Bloomington Stock Center medium with malt at 25°C.

Metabolite Assays

Newly eclosed adult male flies were aged 5–7 days prior to use for all experiments. TAG and cholesterol assays were conducted as described (Horner et al., 2009; Sieber and Thummel, 2009) All results shown are derived from 12 samples of 5 animals collected from each genotype under each condition, and repeated at least 3 times. A representative experiment is shown in each figure.

Statistical Analyses

Statistical analysis was done for each experiment using an unpaired two-tailed Student's t-test with unequal variance. All quantitative data are reported as the mean ± SEM. The n and SEM for each data point is derived from the 12 samples of 5 animals collected from each genotype under each condition.

SUPPLEMENTAL INFORMATION

Supplemental Information include Supplemental Experimental Procedures and four figures and can be found with this article online at doi:10.1016/j.cmet.2011.11.011.

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REFERENCES

Ameis, D., Merkel, M., Eckerskorn, C., and Greten, H. (1994). Purification, characterization and molecular cloning of human hepatic lysosomal acid lipase. Eur. J. Biochem. *219*, 905–914.

Borgström, B. (1988). Mode of action of tetrahydrolipstatin: a derivative of the naturally occurring lipase inhibitor lipstatin. Biochim. Biophys. Acta *962*, 308–316.

Bujold, M., Gopalakrishnan, A., Nally, E., and King-Jones, K. (2010). Nuclear receptor DHR96 acts as a sentinel for low cholesterol concentrations in Drosophila melanogaster. Mol. Cell. Biol. *30*, 793–805.

Burke, J.A., and Schubert, W.K. (1972). Deficient activity of hepatic acid lipase in cholesterol ester storage disease. Science *176*, 309–310.

Cabrera, G.R., Godt, D., Fang, P.Y., Couderc, J.L., and Laski, F.A. (2002). Expression pattern of Gal4 enhancer trap insertions into the bric à brac locus generated by P element replacement. Genesis *34*, 62–65.

Du, H., Heur, M., Duanmu, M., Grabowski, G.A., Hui, D.Y., Witte, D.P., and Mishra, J. (2001). Lysosomal acid lipase-deficient mice: depletion of white and brown fat, severe hepatosplenomegaly, and shortened life span. J. Lipid Res. *42*, 489–500.

Hegedus, D., Erlandson, M., Gillott, C., and Toprak, U. (2009). New insights into peritrophic matrix synthesis, architecture, and function. Annu. Rev. Entomol. *54*, 285–302.

Heltianu, C., Robciuc, A., Botez, G., Musina, C., Stancu, C., Sima, A.V., and Simionescu, M. (2011). Modified low density lipoproteins decrease the activity and expression of lysosomal acid lipase in human endothelial and smooth muscle cells. Cell Biochem. Biophys. *61*, 209–216.

Horner, M.A., Pardee, K., Liu, S., King-Jones, K., Lajoie, G., Edwards, A., Krause, H.M., and Thummel, C.S. (2009). The Drosophila DHR96 nuclear receptor binds cholesterol and regulates cholesterol homeostasis. Genes Dev. *23*, 2711–2716.

Kalaany, N.Y., and Mangelsdorf, D.J. (2006). LXRS and FXR: the yin and yang of cholesterol and fat metabolism. Annu. Rev. Physiol. *68*, 159–191.

King, D.G. (1988). Cellular organization and peritrophic membrane formation in the cardia (proventriculus) of Drosophila melanogaster. J. Morphol. *196*, 253–282.

King-Jones, K., Horner, M.A., Lam, G., and Thummel, C.S. (2006). The DHR96 nuclear receptor regulates xenobiotic responses in Drosophila. Cell Metab. *4*, 37–48.

Kruit, J.K., Plösch, T., Havinga, R., Boverhof, R., Groot, P.H., Groen, A.K., and Kuipers, F. (2005). Increased fecal neutral sterol loss upon liver X receptor activation is independent of biliary sterol secretion in mice. Gastroenterology *128*, 147–156.

Lo Sasso, G., Murzilli, S., Salvatore, L., D'Errico, I., Petruzzelli, M., Conca, P., Jiang, Z.Y., Calabresi, L., Parini, P., and Moschetta, A. (2010). Intestinal specific LXR activation stimulates reverse cholesterol transport and protects from atherosclerosis. Cell Metab. *12*, 187–193.

Lusis, A.J., and Pajukanta, P. (2008). A treasure trove for lipoprotein biology. Nat. Genet. *40*, 129–130.

Niwa, R., and Niwa, Y.S. (2011). The fruit fly Drosophila melanogaster as a model system to study cholesterol metabolism and homeostasis. Cholesterol *2011*, 1–6.

Ouimet, M., Franklin, V., Mak, E., Liao, X., Tabas, I., and Marcel, Y.L. (2011). Autophagy regulates cholesterol efflux from macrophage foam cells via lysosomal acid lipase. Cell Metab. *13*, 655–667.

Phillips, M.D., and Thomas, G.H. (2006). Brush border spectrin is required for early endosome recycling in Drosophila. J. Cell Sci. *119*, 1361–1370.

Schultz, J.R., Tu, H., Luk, A., Repa, J.J., Medina, J.C., Li, L., Schwendner, S., Wang, S., Thoolen, M., Mangelsdorf, D.J., et al. (2000). Role of LXRs in control of lipogenesis. Genes Dev. *14*, 2831–2838. 10.1101/gad.850400.

Sieber, M.H., and Thummel, C.S. (2009). The DHR96 nuclear receptor controls triacylglycerol homeostasis in *Drosophila*. Cell Metab. *10*, 481–490.

van der Veen, J.N., van Dijk, T.H., Vrins, C.L., van Meer, H., Havinga, R., Bijsterveld, K., Tietge, U.J., Groen, A.K., and Kuipers, F. (2009). Activation of the liver X receptor stimulates trans-intestinal excretion of plasma cholesterol. J. Biol. Chem. *284*, 19211–19219.

van der Velde, A.E., Brufau, G., and Groen, A.K. (2010). Transintestinal cholesterol efflux. Curr. Opin. Lipidol. *21*, 167–171.