Control of metabolic adaptation to fasting by dILP6-induced insulin signaling in *Drosophila* oenocytes

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Metabolic adaptation to changing dietary conditions is critical to maintain homeostasis of the internal milieu. In metazoans, this adaptation is achieved by a combination of tissue-autonomous metabolic adjustments and endocrine signals that coordinate the mobilization, turnover, and storage of nutrients across tissues. To understand metabolic adaptation comprehensively, detailed insight into these tissue interactions is necessary. Here we characterize the tissue-specific response to fasting in adult flies and identify an endocrine interaction between the fat body and liver-like oenocytes that regulates the mobilization of lipid stores. Using tissue-specific expression profiling, we confirm that oenocytes in adult flies play a central role in the metabolic adaptation to fasting. Furthermore, we find that fat body-derived *Drosophila* insulin-like peptide 6 (dILP6) induces lipid uptake in oenocytes, promoting lipid turnover during fasting and increasing starvation tolerance of the animal. Selective activation of insulin/IGF signaling in oenocytes by a fat body-derived peptide represents a previously unidentified regulatory principle in the control of metabolic adaptation and starvation tolerance.

Drosophila | metabolism | oenocytes | insulin | starvation

To maintain metabolic homeostasis during fasting periods, metazoans have to coordinate the mobilization of glycogen, lipids, and protein, ensuring an adequate energy supply across tissues. In addition to tissue-autonomous metabolic adjustments, endocrine signals are therefore critical components of the fasting response (1, 2). In mammals, the liver is central to adjusting intermediary metabolism during fasting. Starvation stimulates lipid accumulation in hepatocytes, which oxidize these lipids to provide energy in the form of ketone bodies for other tissues. Hepatocytes also respond to glucagon and insulin signals to control the expression of enzymes involved in glycogenolysis and gluconeogenesis, lipolysis, fatty acid oxidation, and ketogenesis, all regulated by a battery of transcription factors that include forkhead box O (Foxo), cAMP-response element binding (CREB), peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC1a), and hepatocyte nuclear factor 4a (HNF4a) (3–5). At the same time, the liver integrates a suite of endocrine signals derived from major energy storing and consuming tissues, such as the brain, the adipose tissue, and the muscle (1). Deregulation of these processes can lead to hepatic steatosis and is a major cause of metabolic diseases, including diabetes and metabolic syndrome (1, 3, 6).

Drosophila has emerged as a productive model organism in which to characterize the endocrine regulation of metabolic adaptation (7, 8). The central function of the liver in metabolic adaptation of flies is shared by the fat body and oenocytes. Whereas the fat body is an important glycogen and fat storage organ in flies, it also serves as an endocrine organ to coordinate metabolic homeostasis (9–12). Oenocytes have a critical role in lipid mobilization and turnover in larvae, accumulating lipids during starvation similar to mammalian hepatocytes and expressing genes with homology to liver-specific enzymes in mammals (13, 14). During fasting, oenocytes accumulate lipid droplets, a process that is required for mobilization of lipids from the fat body (14), suggesting a close interaction between fat body and oenocytes that is required for efficient lipid mobilization and turnover under fasting conditions. Signals that mediate this interaction remain unclear, and the role of oenocytes in adult metabolic homeostasis has not been explored.

The conserved insulin/IGF1 signaling (IIS) pathway is a central regulator of metabolic adaptation. IIS coordinates responses to nutritional changes and environmental stressors to regulate growth, proliferation, metabolism, and reproduction, influencing metabolic homeostasis and longevity (15–18). To perform these functions, insulin signaling activity has defined tissue-specific outcomes, regulating cellular metabolism according to the needs of each tissue (1, 19). In the mammalian liver, IIS activation by insulin stimulates glucose uptake, glycogenesis, and fatty acid synthesis (1, 19), whereas reduced IIS activity under fasting conditions results in activation of the transcription factor Foxo, which promotes glycogenolysis and beta oxidation in concert with the glucagon-regulated transcription factors CREB, PGC1a, and HNF4 (1, 16, 19, 20). In *Drosophila*, IIS activity is stimulated by a number of different insulin-like peptides (ILPs) that are independently regulated and can act in endocrine as well as local, paracrine fashion. A major source of dILPs are the median neurosecretory cells (mNSCs, also known as insulin-producing cells, IPCs) in the pars intercerebralis of the head, but expression of individual dILPs has also been reported in the gut, the ovary, glial cells, fat body, and abnormally growing imaginal discs (10, 11, 21–30). Whereas secretion of dILP2, -3, and -5 from mNSCs is regulated by nutritional cues, transcriptional regulation of these peptides depends on the stimulus (9, 21). Transcription of dILP2 is not affected by nutrient cues, but is repressed in response to environmental stressors (16, 17, 21, 31). Transcription of dILP3 and dILP5 in mNSCs, on the other hand, is significantly decreased in response to nutrient deprivation (21). *Drosophila* insulin-like peptide 6 (dILP6) has recently been found to control

Significance

This study characterizes the transcriptional response to fasting in adult flies in a tissue-specific manner, highlighting a central role for adult oenocytes in the regulation of lipid mobilization and supporting the proposed analogy between oenocytes and mammalian hepatocytes. A surprising and critical role for insulin signaling activity in the oenocyte fasting response is identified, and it is shown that the *Drosophila* insulin-like peptide 6 (dILP6), which is induced in the fat body in response to starvation, mediates this response. A new paracrine role for insulin signaling in regulating the interaction between adipose tissue and hepatocyte-like cells in the metabolic adaptation to fasting is thus identified.

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growth in nonfeeding states, and its transcription in the fat body is induced under starvation conditions (10, 11, 24). DILP6 is likely to be an endocrine mediator of lifespan extension by Foxo activity in the fat body (11).

Here, we have explored the metabolic adaptation to fasting in adult flies. Using tissue-specific expression profiling as well as a newly generated drug-inducible oenocyte-specific GeneSwitch driver, we identify a critical role for oenocyte-specific insulin signaling in metabolic adaptation of the adult. We further identify fat body-derived dILP6 as a signal that activates insulin signaling in oenocytes during starvation and promotes metabolic adaptation. Our results highlight the importance of oenocytes in the fasting response of adult flies and establish fat body-derived dILP6 as critical for the stimulation of lipid turnover in oenocytes.

Results

Starvation-Induced Tissue-Specific Transcriptome Profile Suggests an Important Metabolic Role for Oenocytes. To gain insight into tissue-specific responses to fasting in adult flies, we profiled transcriptional changes in manually dissected tissues of starved flies using RNA sequencing (RNAseq). (32). Previous studies have explored the transcriptional response to starvation in whole body samples of third instar larvae (33) or adult flies (34), as well as in isolated heads (35). These studies provided a general view of the transcriptional response to starvation, yet the fasting response of specific tissues has not been comprehensively compared to date. We analyzed the starvation response of young (3 d old) adult female flies that were starved for 6 h, thus providing insight into acute fasting responses. In these animals, we profiled the head (primarily brain and eye tissue), thorax (primarily muscle tissue), gut, fat body, and the abdominal cuticle from which the fat body had been removed surgically (this tissue thus contained oenocytes and epidermal cells). Hierarchical clustering of the resulting gene expression profiles revealed a significant tissue-specific diversity of transcriptome responses to starvation (Fig. 1*A* and Fig. S1*A*, see also Dataset S1 and *SI Experimental Procedures*). Whereas only 75 genes were induced in all starved tissues, a significant number of genes were induced after starvation only in the oenocyte-containing samples (1,648 genes), the brain (123 genes), gut (217 genes), fat body (683 genes), or muscle (262 genes).

To determine if these tissue-specific transcriptional programs would reveal functional differences between the starvation responses of individual tissues, we performed Gene Ontology analysis through FlyMine (www.flymine.org) (Fig. 1*B*). Among the genes induced in all tissues, genes characterized as being involved in proteolysis and carbohydrate metabolism were significantly enriched. Strikingly, however, the oenocyte-containing sample showed a much more significant enrichment of genes involved in carbohydrate and oxidative metabolism, indicating a selective role for this tissue in the metabolism of sugars and lipids.

The prevalence of these genes in the oenocyte-containing samples was specifically highlighted when the magnitude of the induction was taken into account. Among the top 50 most induced genes in these samples were several genes with classic catabolic and gluconeogenic function in hepatocytes: *amylase proximal* (*amy-p*), encoding an alpha-amylase involved in carbohydrate metabolism; *phosphoenolpyruvate carboxykinase* (*pepck*), which catalyzes the rate-limiting step in gluconeogenesis (36); *CG5966* (a triglyceride lipase); *lipophorin receptor 2* (*lpr2*), involved in capturing hemolymph lipids for transport into the cell (14); and *brummer* lipase (*bmm*), which has triglyceride activity and is a major regulator of lipid homeostasis in flies (34) (Fig. 1*C* and Fig. S1*B*). The induction of these genes in manually dissected oenocyte/epidermal samples of starved flies was confirmed by quantitative RT-PCR (qRT-PCR) (Fig. 1*D*).

Oenocytes Are Critical Tissues for Mounting Effective Starvation Response. Confirming the role of oenocytes in sugar and lipid metabolism, genes that were significantly more highly expressed in starved oenocytes than in any other tissues primarily encode proteins involved in lipid and carbohydrate metabolism (Fig. S2*A*). A prominent example is *pepck*. It is induced to a higher extent (nearly 12-fold) in the oenocyte-containing abdominal cuticle samples compared with thorax and head, and there is no induction in fat body or gut. Furthermore, the absolute expression (reads per kilobase per million mapped reads, RPKM) of

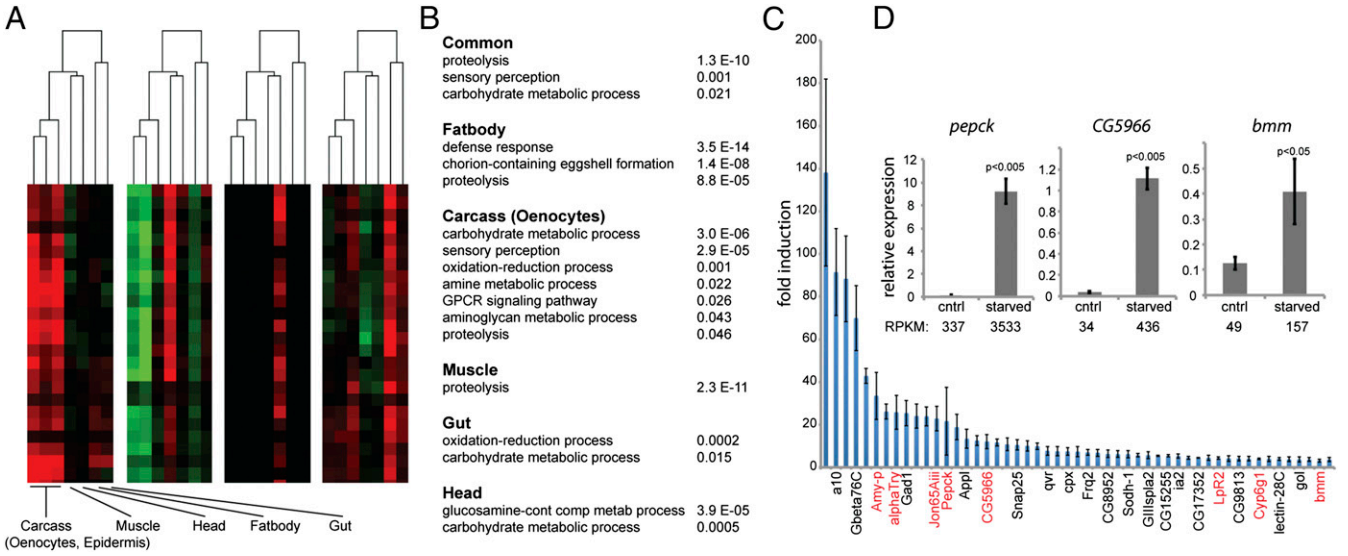

Fig. 1. Tissue-specific transcriptome responses to fasting. (*A*) Examples of heat maps from RNAseq libraries showing induction (red) or repression (green) of distinct gene groups in specific tissues of female, wild-type (w^{1118}) flies after 6 h of starvation. (*B*) Gene Ontology enrichment analysis of genes induced by starvation in distinct tissues. (*C*) Average fold induction of the top 50 genes most strongly induced in oenocytes/epidermal cells. Average/SD is shown from RNAseq results of three independent samples. See fully labeled chart in Fig. S1*B*. (*D*) Quantitative RT-PCR to validate the induction of *pepck*, *CG5966*, and *bmm* in oenocyte/epidermal samples of female flies after 6 h of starvation. Expression values are normalized to *actin5C*. Averages and SEM are shown. *P* values are from Student *t* test ($n \geq 3$). See also Figs. S1 and S2, Dataset S1, and *SI Experimental Procedures*.

pepck is more than threefold higher in the oenocyte-containing samples than in any other tissues. Similar differential induction and expression levels were observed for the inositol oxygenase CG6910, succinate CoA ligase Skap, the lipophorin receptor Lpr2, galactokinase, the Acyl-CoA-dehydrogenase CG12262, the redox enzyme Sodh-1, the fatty Acyl CoA-reductase CG13091, and the triglyceride lipases CG5966 and bmm. The specific induction and high expression of these genes in oenocyte-containing tissue samples further support the notion that adult oenocytes play a critical role in coordinating metabolic adaptation to fasting and are consistent with their high expression reported in "carcass" samples in FlyAtlas (37).

To study the role of adult oenocytes in starvation responses in more detail, we generated an inducible oenocyte-specific driver. A sequence of 800 nucleotides of the promoter E (promE800) from the *desaturase1* gene had been reported to drive expression of transgenes specifically in adult oenocytes (13), and we used this sequence to generate a GeneSwitch construct (38) for a mifepristone (RU486)-inducible oenocyte-specific driver. Transgenic flies carrying this construct specifically express UAS-linked GFP in oenocytes in response to RU486 exposure (Fig. S2*B*). We used this driver to genetically ablate oenocytes in males and females by expressing the proapoptotic gene *hid* (Fig. S2*B*). This selective ablation of oenocytes resulted in significant starvation sensitivity in both males and females, confirming the critical role of oenocytes in metabolic homeostasis (Fig. S2*C*; these animals show normal feeding behavior, Fig. S2*D*). We further tested whether knockdown (by RNAi) of selected metabolic genes, identified as oenocyte specific in the transcriptome analysis, would influence starvation sensitivity. We selected *lpr2* and *pepck*, as both are well-characterized genes involved in starvation responses (14, 36) and found that oenocyte-specific knockdown of either of them caused a marked increase in starvation sensitivity (Fig. S2*C*; using a ubiquitous driver, we confirmed that these RNAi constructs effectively knockdown the intended targets, Fig. S2*E*). Genes that are up-regulated specifically in oenocytes thus serve an essential role in mounting an effective starvation response in the whole organism.

IIS in Oenocytes Is Crucial to Maintain Starvation Resistance and Systemic Lipid Reserves. To test whether IIS modulates the starvation response of oenocytes, we profiled the transcriptome of manually dissected cuticle/oenocyte samples in which IIS activity was suppressed specifically in oenocytes by expression of a dominant-negative insulin receptor (InR^{DN}) (39); ubiquitous expression of this construct results in strong induction of Foxo target genes, confirming that endogenous IIS activity is suppressed (Fig. S3*A*), under the control of promE800GS. Many of the genes induced to a significantly weaker extent in oenocytes of these animals than in wild-type flies encode proteins involved in lipid and carbohydrate metabolism (including the lipases CG5966 and magro; Fig. 2*A*). As expected, this group also included known downstream targets of IIS, for example the *target of brain insulin* (tobi) (40). These results were confirmed independently for selected genes by RT-PCR (Fig. 2*B*).

The requirement for IIS activity in the transcriptional response to starvation suggested that, in contrast to other tissues, IIS signaling is active in oenocytes of starved flies. Indeed, the PI3Kinase reporter tub::GFP-PH (41) shows increased membrane localization in oenocytes of starved flies, whereas membrane localization of the same reporter decreases in surrounding abdominal fat body of starved flies (Fig. S3*B*). Based on these results, we hypothesized that IIS in oenocytes might affect systemic metabolic adaptation to fasting conditions. We tested this notion by assessing the levels of triglycerides, glycogen, glucose, and trehalose in starved flies and found that inhibiting IIS activity in oenocytes did not influence steady-state levels or fasting-induced decreases in systemic trehalose or glycogen, but significantly

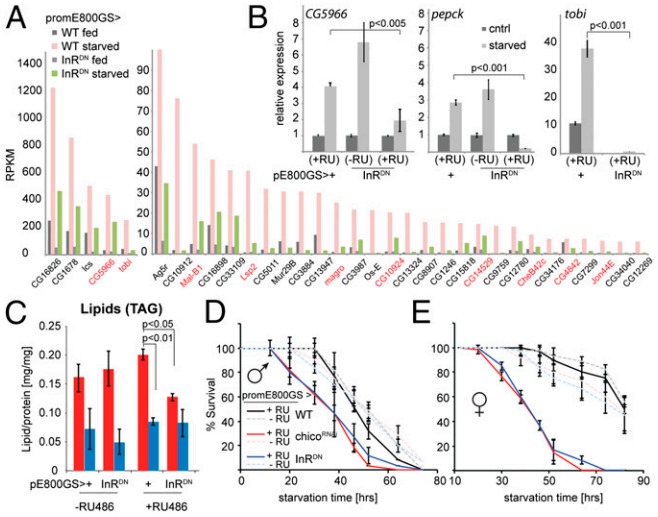

Fig. 2. IIS in oenocytes is required for starvation resistance and lipid turnover. (*A*) Group of genes with highest expression in starved oenocyte/epidermal samples that are also significantly less (more than threefold less) expressed in oenocyte samples expressing InRDN. Highlighted are known insulin-induced genes (*tobi*), as well as genes encoding proteins involved in carbohydrate (Mal-B1, CG10924), protein (Jon44E, CG14529), and lipid metabolism (CG5966, magro). (*B*) qRT-PCR testing InR-dependent induction of selected genes. Young flies (5 d old) were exposed to RU486 (+RU) or vehicle (−RU)-containing food for 1 wk, then starved for 6 h. Expression values are normalized to *actin5C*. Averages and SEM are shown. *P* values are from Student *t* test ($n \geq 3$). (*C*) Triacylglyceride (TAG) levels in male flies of the indicated genotypes (exposed to RU486 or vehicle for 1 wk, starved for 36 h). See Fig. S3*C* for data on glucose, glycogen, and trehalose. Average and SEM are shown; *P* values are from Student *t* test ($n \geq 3$). (*D* and *E*) Starvation sensitivity of flies with reduced IIS activity in oenocytes. Flies were exposed to RU486 or control food for 3 d before starvation. Averages and SDs of four cohorts of 15 flies each are shown.

reduced the steady-state concentration of systemic triglycerides, and impaired the fasting-induced decrease in systemic triglycerides and free glucose (Fig. 2*C* and Fig. S3*C*). Furthermore, inhibiting IIS activity in oenocytes also reduced new lipid synthesis, as determined by measuring incorporation of 14C into the triacylglyceride fraction in animals fed 14C-labeled glucose (42) (Fig. S3 *C* and *D*). We confirmed that this effect was not due to changes in feeding behavior: animals expressing $chico^{RNAi}$ or InR^{DN} in oencytes showed no significant difference in uptake of a food dye compared with wild-type controls (Fig. S3*E*). Interestingly, animals in which *pepck* was knocked down in oenocytes exhibited the same reduction in steady-state levels of systemic triglycerides and in fasting-induced triglyceride turnover as animals in which IIS was impaired, further highlighting the role of *pepck* induction in the oenocyte-specific regulation of metabolic homeostasis (Fig. S3*F*). Increasing InR expression in oenocytes, on the other hand, resulted in a moderate increase in systemic triglyceride levels, both in fed and in starved conditions (Fig. S3*F*). Interestingly, these effects of InR in oenocytes may be Foxo independent, as we were unable to detect Foxo in adult oenocytes (whereas Foxo is readily detectable and responds to starvation by nuclear translocation in surrounding abdominal fat body of adult flies; Fig. S3*G*).

These results suggested that oenocyte-specific IIS activity and IIS target gene induction is critical for systemic lipid homeostasis and for the mobilization of stored lipid under fasting conditions. Supporting this idea, we found that both male and female flies were significantly more sensitive to starvation when IIS pathway components were disrupted specifically in oenocytes. Starvation sensitivity was observed both when InR^{DN} was expressed in

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oenocytes, as well as when the insulin receptor substrate Chico was knocked down by RNAi (Fig. 2 *D* and *E*).

IIS Is Required in Oenocytes to Accumulate Lipid Droplets During Fasting. Our results indicate that IIS activity in oenocytes is critical to coordinate lipid mobilization. As part of their role in lipid turnover, oenocytes accumulate lipid droplets during starvation (14, 43), and we assessed this phenotype by asking whether IIS activity in oenocytes acts cell autonomously to influence the lipid turnover function of adult oenocytes. We generated both loss-of-function conditions (expressing InR^{DN} or $chico^{RNAi}$) and gain-of-function conditions (overexpressing wild-type InR) for IIS activity in oenocytes and observed lipid droplets under steady-state and fasting conditions using Nile Red staining. As described for larval oenocytes, starvation induced a significant increase in the number and size of lipid droplets detected inside adult oenocytes (Fig. 3*A* and Fig. S4*A*). In oenocytes with increased InR expression, lipid droplets were significantly more numerous and larger than in wild-type oenocytes even under fed conditions, whereas starvation dramatically enhanced these phenotypes (Fig. 3*B*). Expression of InR^{DN}, or knockdown of *chico*, on the other hand, significantly reduced the accumulation of lipid droplets under starvation conditions (Fig. 3 *C* and *D*). Similarly, knockdown of the IIS target *pepck* impaired starvation-induced lipid uptake in oenocytes (Fig. S4*B*). IIS activity is thus required and sufficient to promote lipid uptake in oenocytes. This function is consistent with the IIS-dependent induction of lipophorin receptor 2 (LpR2) in oenocytes of fasting flies. Lipophorin functions as the main lipid transport protein in the hemolymph of both larvae and adults (44–46), and lipophorin receptor is involved in the uptake of neutral lipids in various tissues (44, 47). Interestingly, inhibiting IIS activity in oenocytes is sufficient to cause metabolic imbalance in flies even under fed conditions (Fig. 2*C*), whereas changes in lipid vesicle accumulation in eonocytes are not observed in these conditions (Fig. 3). It is likely that this discrepancy reflects the fact that metabolic parameters determined in Fig. 2*C* are the consequence of chronic perturbation of the IIS pathway, whereas lipid vesicle accumulation in oenocytes of starved animals (Fig. 3) is an acute response. Further studies are needed to explore the exact mechanism by which chronic changes in IIS activity in oenocytes influence metabolic homeostasis.

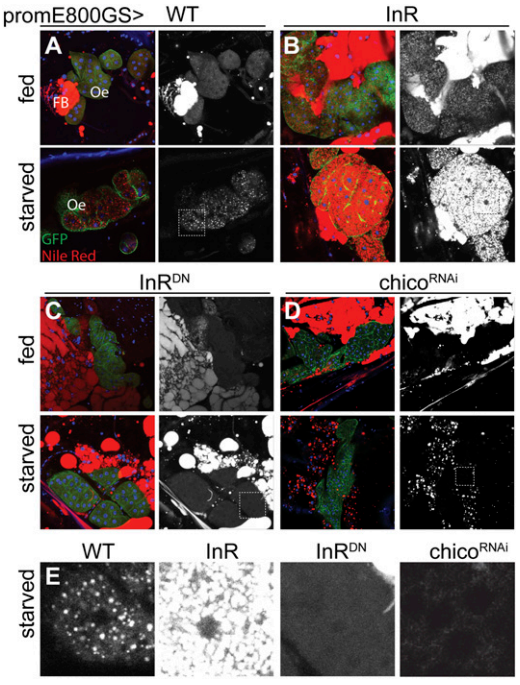

Fig. 3. IIS is required in oenocytes to accumulate lipid droplets after starvation (24 h). (*A*) Oenocytes from wild-type female flies accumulate lipid droplets after 24 h of starvation as seen by Nile Red staining. (*B*) InR overexpressing oenocytes accumulate lipid droplets even when fed, and show increased accumulation after starvation. (*C* and *D*) Oenocytes in which the IIS pathway is disrupted (InR^{DN}, $chico^{RNAi}$) exhibit little or no lipid droplets after starvation. (*E*) Enlarged images of oenocytes of the four genotypes after starvation. (All left panels) Nile Red (red), promE800GS > GFP (green), and DAPI (blue). (All right panels and *E*) Nile Red in white. FB, fat body; Oe, oenocytes.

DILP6 in Fat Body Is Required to Accumulate Lipid Droplets in Oenocytes and Maintain Starvation Resistance. Insulin is an anabolic hormone that promotes the storage of macronutrients under ad libitum conditions (1, 15). Accordingly, insulin like peptide expression and secretion commonly decreases during fasting in flies (9, 21). In contrast, our results suggested that IIS activation is specifically required in oenocytes during starvation to maintain starvation resistance and mobilize lipid reserves. This surprising finding suggested that, under starvation conditions, oenocytes receive a signal that specifically activates IIS in these cells (consistent with the increase in membrane-bound GFP-PH in oenocytes of starved flies, Fig. S3*B*). We hypothesized that one of the dILPs whose expression is not reduced by starvation might mediate this signal. DILP6 is one such ligand. It is expressed in the fat body both in larvae and adults (10, 11, 24), and is induced after starvation (11). Because the fat body and oenocytes are closely associated in the adult abdomen, the induction of dILP6 after starvation suggested that it might serve as a paracrine signal that coordinates the fasting response between fat body and oenocytes. To test this idea, we used the fat body-specific driver ppl::Gal4 in combination with tub::$Gal80^{ts}$ (13) to overexpress or knock down dILP6 ($dILP6^{RNAi}$) specifically in the fat body in a temperature-sensitive manner (Fig. 4; we confirmed the activity of both constructs by RT-PCR, Fig. S4*G*). This perturbation resulted in increased or decreased phosphorylation of Akt in oenocytes, respectively (Fig. S4*C*), confirming that fat body-derived dILP6 signals to oenocytes to regulate IIS activity. Overexpression of dILP6 using either ppl::Gal4 or the RU486-inducible fat body and gut driver S_1106::GS further resulted in transcriptional induction of the IIS targets *CG5966* and *pepck* in manually dissected oenocyte/epidermal samples (Fig. S4*D*). Accordingly, when dILP6 was overexpressed, increased lipid droplet accumulation was observed in oenocytes even under fed conditions (Fig. 4 *A* and *B*). In animals expressing $dILP6^{RNAi}$, in turn, starvation-induced accumulation of lipid droplets in oenocytes was significantly reduced (Fig. 4*C*). We confirmed these *dilp6* loss-of-function phenotypes using the loss-of-function allele $dilp6^{41}$ (29) (Fig. 4*D*). The effects of overexpressing dILP6 in fat body are not caused by autocrine stimulation of IIS activity in fat body, as coexpressing dILP6 and InR^{DN} in this tissue caused the same increase in lipid uptake in oenocytes as expressing dILP6 alone (Fig. S4*E*).

These results strongly support the idea that fat body-derived dILP6 influences lipid turnover in oenocytes. We further assessed the starvation resistance of flies in which dILP6 expression was knocked down in adult fat body (avoiding developmental phenotypes caused by loss of dILP6) and found that their starvation tolerance significantly decreased when $dILP6^{RNAi}$ was expressed in the fat body (Fig. 4*E*). As in animals in which IIS activity is impaired directly in oenocytes, we also observed a steady-state reduction in systemic triglycerides (especially in males, where this reduction was more pronounced than in females) and an impaired ability to mobilize stored lipids during starvation (Fig. 4*F*). Note that this sexual dimorphism is also reflected in the differential sensitivity of males and females to starvation (Fig. 4*E*), and

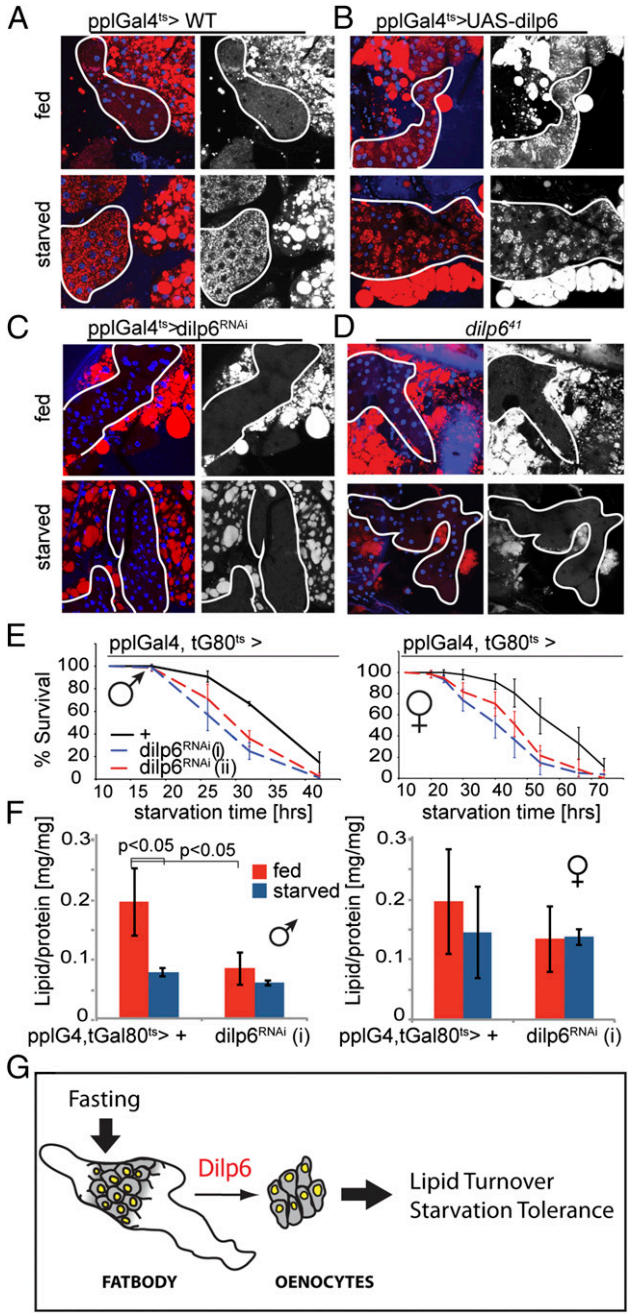

Fig. 4. DILP6 in fat body is required to accumulate lipid droplets in oenocytes, for lipid turnover, and for starvation resistance. (*A* and *C*) DILP6 knockdown in the fat body (pplG80 > dilp6^{RNAi}) results in less accumulation of lipid droplets in oenocytes during fasting. (*B*) DILP6 overexpression in the fat body (pplG80 > UASdILP6) leads to lipid droplet accumulation in oenocytes even in fed flies. (*D*) DILP6 mutant flies (*dilp6^{41}*) also fail to accumulate lipid droplets in oenocytes during fasting. Oenocytes are circled. Nile Red (red), promE800GS > GFP (green), and DAPI (blue). (*Right*) Nile Red in white. (*E*) DILP6 knockdown in the fat body also increases starvation sensitivity both in males and females. Here, two separate dILP6^{RNAi} lines from the Vienna *Drosophila* RNAi Center were used (TF IDs 102465 and 45218). The fraction of surviving flies is represented as averages and SDs of four to five cohorts of 15–20 flies each. (*F*) DILP6 down-regulation in the fat body leads to lower systemic TAG levels, which cannot be mobilized further under fasting both in males (*i*) and females (*ii*). Averages and SDs of TAG levels normalized to total protein are shown. *P* values are from Student *t* test ($n \geq 3$). (*G*) Our results suggest a role for fat body-derived dILP6 in the control of lipid uptake in oenocytes under fasting conditions. This interaction regulates fasting-induced lipid turnover and is thus critical for starvation tolerance.

may be a consequence of the differential physiology of male and female flies.

Overexpression of dILP6 in fat body and gut (using S_1106::GS), however, did not induce starvation tolerance, but rather increased starvation sensitivity compared with wild-type flies (Fig. S4*F*; note that S_1106 shows inducible expression of UAS-linked transgenes in the fat body, but constitutive expression in the gut) (48). Constitutively increasing dILP6/IIS signaling in oenocytes thus results in negative metabolic consequences systemically, indicating that the acute induction of lipid turnover by dILP6 from fat body during fasting has to be limited to a short-term response to achieve metabolic homeostasis.

dILP6 from fat body is thus critical for IIS-mediated lipid accumulation in oenocytes, stimulating starvation-induced lipid turnover and starvation tolerance (Fig. 4*G*).

Discussion

Our results highlight the critical role of oenocytes in maintaining metabolic homeostasis in adult flies and identify a dILP6-mediated signaling interaction between the fat body and oenocytes, which is critical for the mobilization of stored lipids and for overall starvation tolerance of the animal. The role of oenocytes in metabolic adaptation resembles the function of the mammalian liver, as has been predicted from studies in larvae (14). The various insulin-like peptides of flies influence growth and stress responses in different contexts, providing a complex, spatiotemporally defined regulatory system to sustain local cell growth, proliferation, cell survival, and metabolism (10, 11, 21–30). So far, the molecular codes that ensure specificity in this signaling network remain unclear. It is possible that the affinity of the one fly insulin receptor is different for different peptides, allowing regulation of insulin signaling activity on the basis of unique spatiotemporal concentration patterns of individual peptides. Alternatively, so far undefined coreceptors of the insulin receptor may exist that provide specificity. Finally, the spatial separation of peptides produced by the intestine, the nervous system, and the fat body may indicate a functional separation into locally acting and systemically acting peptides with different diffusibility. Additional studies are required to explore these mechanistic questions in more detail. Our results strongly indicate that fat body-derived dILP6 signals to oenocytes to activate IIS. It is conceivable, however, that fat body-deriver dILP6 acts more indirectly, i.e., by stimulating the release of dILPs from other tissues that then activate IIS in oenocytes under starvation conditions.

Whereas activation of IIS in oenocytes is required for the induction of a battery of metabolic genes, it remains unclear how exactly these genes are transcriptionally regulated. It is possible that Foxo repression by insulin signaling results in a derepression of genes encoding metabolic enzymes; yet we have been unable to detect Foxo protein in oenocytes (as opposed to in fat body, where Foxo can clearly be observed) (31) (Fig. S3*G*). Other insulin-responsive transcription factors may thus be critical for this regulation.

Finally, it remains to be established if the direct activation of lipid uptake in oenocytes by a fat body-derived insulin-like signal has parallels in vertebrates. Because the general signaling mechanisms regulating metabolic homeostasis are conserved between insects and vertebrates, it can be anticipated that similar endocrine mechanisms may exist in vertebrates and are involved in human metabolic diseases. Supporting this view, starvation induces similar lipid uptake and gene expression in the mammalian liver, and an endocrine role for adipose tissue in regulating liver function and metabolism has been established (2, 6, 49). Furthermore, both hyper- and hypoinsulinemia can induce lipid accumulation in the liver and has been implicated in nonalcoholic fatty liver disease (50).

Experimental Procedures

See *SI Experimental Procedures* for further details.

Fly Lines and Handling. The following strains were obtained from the Bloomington *Drosophila* Stock Center: w1118, OreR, UAS-mCD8: GFP, UAS-InR^{DN} (K14902A), UAS-InR (BL 8262), tub-Gal80ts, and Dilp6[41]. Chico^{RNAi} (transformant ID 101329) and dilp6^{RNAi} (transformant ID 102465) lines were obtained from the Vienna *Drosophila* RNAi Center. UAS-Hid was provided by J. C. Billeter, University of Groningen, Groningen, Netherlands; pplGal4 by M. Pankratz, Universität Bonn, Bonn; and UAS-dILP6 by M. Tatar, Brown University, Providence, RI. Unless otherwise noted, transgenic flies were generated and maintained in the w^{1118} genetic background on standard cornmeal/yeast/molasses *Drosophila* medium at 25 °C on a 12-h circadian cycle.

Neutral Lipid Staining and Confocal Microscopy. Dorsal abdominal fillets of female flies containing both fat body and oenocytes were dissected in PBS and fixed in 4% (wt/vol) paraformaldehyde/PBS for 30 min at room temperature before staining with Nile Red (Sigma) and DAPI.

Starvation Tolerance and Metabolic Measurements. Starvation experiments were performed placing 15–20 flies in each vial with water-soaked filter paper. Mortality rates were determined by counting the number of dead flies at fixed time intervals.

For glucose, trehalose, glycogen, and triglyceride measurements, cohorts of five to seven male or female flies were homogenized in 200 μL PBST, followed by a brief centrifugation step to remove cuticle. Sugars (trehalose, glycogen, and glucose) in these homogenates were measured using the Glucose (HK) Assay kit (Sigma), lipids were measured using Stanbio Triglyceride LiquiColor Test reagent, and proteins using the Biorad Bradford reagent. All measurements were normalized to total protein concentration.

RNAseq Analysis. Head, thorax, gut, fat body, and dorsal abdominal fillet (containing oenocytes and epidermal cells) from fed or 6-h starved females were manually dissected, and RNA was extracted from each tissue as outlined above. RNAseq libraries were generated and sequenced by the University of Rochester Medical Center Genomics Core Facility using an Illumina HiSeq sequencer.

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