Chronic dysfunction of Stromal interaction molecule by pulsed RNAi induction in fat tissue impairs organismal energy homeostasis in *Drosophila*

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Obesity is a progressive, chronic disease, which can be caused by long-term miscommunication between organs. It remains challenging to understand how chronic dysfunction in a particular tissue remotely impairs other organs to eventually imbalance organismal energy homeostasis. Here we introduce RNAi Pulse Induction (RiPI) mediated by short hairpin RNA (shRiPI) or double-stranded RNA (dsRiPI) to generate chronic, organ-specific gene knockdown in the adult *Drosophila* fat tissue. We show that organ-restricted RiPI targeting Stromal interaction molecule (Stim), an essential factor of store-operated calcium entry (SOCE), results in progressive fat accumulation in fly adipose tissue. Chronic SOCE-dependent adipose tissue dysfunction manifests in considerable changes of the fat cell transcriptome profile, and in resistance to the glucagon-like Adipokinetic hormone (Akh) signaling. Remotely, the adipose tissue dysfunction promotes hyperphagia likely via increased secretion of Akh from the neuroendocrine system. Collectively, our study presents a novel *in vivo* paradigm in the fly, which is widely applicable to model and functionally analyze inter-organ communication processes in chronic diseases.

Energy homeostasis is pivotal to life. It is well-established that long-term positive energy balance drives obesity (reviewed in\(^1\)), which is defined as abnormal fat accumulation causative for a number of human diseases\(^2\).

The prevalence of obesity in children and adults has substantially increased around the world since 1980\(^3\). Moreover, over the past decades, no country has been successful in combating the obesity pandemic\(^4\). It is still under debate whether the increased supply of high calorie food\(^5\) combined with sedentary life style\(^6,7\) alone could explain the spread of obesity. But it is clear that the primary driver of obesity is the mismatch of energy intake and expenditure. Specifically, the maintenance of energy balance requires coordination of the major energy handling tissues such as liver (reviewed in\(^8\)) and adipose tissue (reviewed in\(^9\)) in mammals. Next to storing energy, adipose tissue also senses the energy status and communicates via adipokines to affect energy intake\(^10\) and expenditure\(^11\). Therefore, it is vital to understand how energy storage tissue regulates organismal energy balance both in an organ-specific manner and systemically.

Much like mammals, flies also have an energy storage tissue called fat body, which functions similar to liver and white adipose tissue (reviewed in\(^12\)). Importantly, the majority of extra energy in mammals and flies

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is deposited as triacylglycerols (TAGs) in adipose tissue intracellular organelles called Lipid Droplets (LDs) (reviewed in [13]). Notably, a number of lipid metabolism effectors and regulators has been found to be conserved from fly to man [14]. One of these regulatory pathways is the store-operated calcium entry (SOCE), a major determinant of the versatile second messenger intracellular Ca$^{2+}$ (iCa$^{2+}$). One core component of SOCE is the endoplasmic reticulum (ER) calcium sensor Stromal interaction molecule (abbreviated Stim in fly, STIM1 in mammals) [15].

In non-excitable cells, the canonical G-protein-coupled receptor (GPCR) signaling generates inositol 1,4,5-trisphosphate (IP3) in the cytosol [16], which stimulates a first wave of Ca$^{2+}$ release from ER stores to the cytoplasm by activating the calcium channel-receptor IP3R (reviewed in [17]). Stim/STIM1 senses the ER calcium depletion and re-localizes to ER-plasma membrane junctions, where it interacts with plasma membrane calcium channel proteins (called oll186-F in flies and Calcium release-activated calcium channel protein 1 (ORAI1) in mammals) to form active Ca$^{2+}$ release-activated Ca$^{2+}$ channels (CRACs), thereby ultimately facilitating the extracellular calcium entry [18]. With this iCa$^{2+}$ increase, SOCE regulates a plethora of cellular processes over a wide temporal spectrum (reviewed in [19]). We previously used acute knockdown of Stim in the fat body to identify SOCE as an adiposity regulator in flies [20]. A recent study confirmed that also mammalian STIM1 regulates lipid metabolism in liver and muscle. Moreover, the plasma membrane translocation of STIM1 required for normal SOCE is impaired in hepatocytes of obese mice, which causes further metabolic dysfunction [21]. While fat accumulation in response to acute SOCE interference is well established, the adverse consequences of chronic iCa$^{2+}$ malfunction are unknown as Stim/STIM loss-of-function is lethal in mice [22] and in flies [23].

Therefore, we developed a novel strategy called short-hairpin or double-stranded RNAi Pulse Induction (RiPI) to achieve tissue-specific, chronic knockdown of genes of interest exemplified by Stim in adult fly fat body. We demonstrate that the siRNAs generated by a short-term RiPI are long-lasting, which chronically reduces the expression of Stim mRNA in adult fly fat tissue and consequently leads to progressive severe obesity in flies. We further provide evidence that chronic knockdown of Stim not only causes the tissue-autonomous dysfunction of lipid mobilization in response to Adipokinetic hormone (Akh) and reduced insulin signaling in fly fat body, but also remotely controls hyperphagia. Our results suggest that chronic dysfunction of Stim/SOCEx in the energy storage tissue can dramatically modulate the systemic regulatory network, which drives the organismal energy imbalance.

**Results**

**Chronic in vivo gene knockdown by RNAi Pulse Induction (RiPI) in adult fly storage tissue.** To generate RiPI in adult *Drosophila* flies (Fig. 1A), we employed the GAL4 system combined with the temperature-sensitive TARGET system to control UAS-RNAi transgenes in a switchable fat body-specific manner (ts-FB-GAL4) [24]. As a proof of concept, we targeted the Adipokinetic hormone receptor (AkhR) gene, a well-characterized key regulator of lipid mobilization in the fat body. Adult flies at the age of six days, which carried the ts-FB-GAL4 and short hairpin (sh) *AkhR* RNAi transgene (UAS-sh*AkhRi*), were either subjected to a TARGET system-based RiPI (TRiPI) (34–48 hours at the permissive temperature 29 °C, *AkhR*–TRiPI Off) or kept continuously at the restrictive temperature 18 °C (*AkhR*–TRiPI On) (Fig. 1B). To exclude possible confounding effects of the temperature shift per se, we also examined control flies carrying the UAS-sh*AkhRi* transgene only under the two temperature regimes described above (*AkhR*–TRiPI On control and *AkhR*–TRiPI Off control) (Fig. 1B). To demonstrate that our conditional expression system is on-off switchable, we took advantage of ts-FB-GAL4 flies carrying a UAS-GFP reporter transgene (see Material & Methods). As expected, GFP mRNA was significantly up-regulated after the induction pulse (day 1) but was at control levels at day 10 after return to the restrictive conditions (Fig. 1C). Similarly, high levels of *AkhR* siRNA were detected right after the *AkhR*–TRiPI (21 fold higher than that of *AkhR*–TRiPI Off flies at day 0). Strikingly, however, *AkhR* siRNA levels still remained 13-fold higher compared to controls even 10 days after transgene switch-off (Fig. 1D). Consistently, at day 10 after the return to restrictive conditions, *AkhR* mRNA abundance was just around half compared to control flies; a similar gene knockdown level as observed immediately after the end of TRiPI (Fig. 1E). This chronic *AkhR* knockdown in the adipose tissue causes persistent body fat increase (Fig. S1A), which is characteristic for *AkhR* loss-of-function flies [25]. In contrast, we detected no body fat content increase in response to the same temperature shift regimen as Stim/STIM loss-of-function is lethal in mice [26] and in flies [27].

Next, we targeted by RiPI the ER calcium sensor *Stromal interaction molecule* (*Stim*), a key component of the store-operated calcium entry (SOCE). *Stim* is a broadly expressed, essential gene in flies [28] and mice [29] and accordingly not accessible to conventional long-term knockout mutant analysis in adult animals. We have previously shown that short-term *Stim* knockdown in the fat body impairs gene function on the mRNA and protein level, which causes excessive lipid accumulation in fly fat cells [30]. Now we subjected six days old adult male flies to an adipose tissue-targeted *Stim*–TRiPI regimen (Fig. 1B) using a UAS-ds*Stim* RNAi transgene (Stim-RNAi1; Fig. S2A). Consistent with our finding on *AkhR*, the expression of *Stim* dsRNA was effectively switched on and off in response to the *Stim*–TRiPI regimen. *Stim* dsRNA went high immediately after the induction pulse (day 0) but was back to control levels at day 1 and day 10 after the return to the restrictive temperature (Fig. 2A). In contrast to the transient dsRNA pulse, the *Stim* mRNA abundance remained chronically decreased to about 50% at days 1 and 10 (Fig. 2B). In line with the chronic impairment of *Stim* function, *Stim*–TRiPI On flies accumulated significant body fat at day 1 after RiPI, and then progressively added fat (doubling at day 10) to reach remarkable
3.5 times the body fat content of Stim-TRiPI Off control flies at day 21 (Fig. 2C). This massive fly obesity development is a universal response to chronic Stim impairment, which is largely independent from fly age and sex. Next to young (Fig. 2C) and older (Fig. S2F) unmated male flies, also virgin female flies (Fig. S2C,G) and mated male flies of different ages (Fig. S2B,H) show significant body fat increase three weeks after Stim-TRiPI. By contrast, continuously mated female flies of different ages were resistant to Stim-TRiPI-dependent obesity (Fig. S2E,I) for currently unknown reasons.

To exclude RNAi off-target effects in the etiology of Stim-TRiPI-dependent obesity, we used a second RNAi transgenic line (Stim-RNAi2), which targets an independent sequence of Stim (Fig. S2A). Consistent with the previous results, the body fat content of Stim-RNAi2 flies increased significantly at day 11 after Stim-TRiPI and remained higher than controls at day 21 (Fig. S3A). Finally, we confirmed the causal role of Stim-TRiPI in fat accumulation by simultaneous expression of Stim-RNAi with either one of the two cDNA-based Stim transgenes: the RNAi-sensitive Stim-RA or the RNAi-resistant Stim-Rm transgene (for details see Material & Methods). Fat body-targeted expression of each of the transgenes causes body fat reduction in flies (Fig. S3B,C), which is characteristic for Stim gain-of-function15,25. RIPI on flies, which carry the Stim-dsRNAi and the RNAi-sensitive
cDNA-RA transgenes shows progressive fat accumulation as early as day 0 after the pulse induction (Fig. S3D). This fat accumulation profile is consistent with the interpretation that Stim-dsRNA downregulates both, the endogenous and the transgenic overexpression of Stim. In contrast, concomitant pulse induction of Stim-dsRNAi and the RNAi-resistant Stim-Rm transgene rescues the normal body fat phenotype at day 0 but not at day 11 or later.
21 (Fig. S3E). Again, these data are in agreement with the Stim-Ripl flies suffering from excessive fat accumulation, we asked whether these flies also display other phenotypes characteristic for mammalian obesity such as increased girth, elevated body weight, and reduced physical fitness. While Stim-TRiPI On and Stim-TRiPI Off flies are indistinguishable at day 1 (Fig. 3A), abdominal girth of day 21 Stim-TRiPI On flies is visibly inflated compared to Stim-TRiPI Off flies (Fig. 3B). Consistently, Stim-TRiPI On flies maintained about 22% body weight in three weeks to become 18% heavier than age-matched Stim-TRiPI Off flies (Fig. 3C) and a dry weight gain of Stim-TRiPI On flies is also observed (Fig. S3G). Consistent with inflated abdominal girth, and increased body weight, thin layer chromatography (TLC) data confirmed that the body TAG content of day 21 Stim-TRiPI On flies was 90% higher compared with day 21 Stim-TRiPI Off flies (Fig. 3D,E). Fly fat body tissue is functionally similar to mammalian adipose tissue and liver, which is a major organ for fat and glycogen reserve. To check if the fly adipose tissue increases, we examined fly fat body tissues in dissected abdomen and found more expanded fat tissue in day 21 Stim-TRiPI On flies than day 1 Stim-TRiPI Off flies and Off as well as day 21 Stim-TRiPI Off flies (Fig. 3EF, GG). At the cellular level, fat body tissue ex vivo staining showed that fat cell size is unaltered in day 1 Stim-TRiPI On flies (Fig. 3HJI), while it increased by around 46% at day 21 (Fig. 3JJ). In addition, the cumulative LD area per fat body cell of Stim-TRiPI On flies was around 2.3 fold higher compared to day 21 Stim-TRiPI Off flies (Fig. 3JK). These data indicate that the chronic dysfunction of Stim causes severe fly fat body cell hypertrophy.

Consistent with their increased fat reserves, obese Stim-TRiPI flies are more starvation resistant compared with control flies (Fig. S4A). Noteworthy, substantial post mortem lipid stores suggest a lipid mobilization impairment in Stim-TRiPI flies (Fig. S4B). Much in contrast to fat, the glycogen storage under ad libitum feeding and the glycogen mobilization upon starvation is comparable between Stim-TRiPI On and Stim-TRiPI Off control flies (Fig. S4C). By contrast, we found that day 10 Stim-DRiPI On flies displayed substantially higher level (around 1.9 fold) of circulating sugar levels compared to the value of day 10 Stim-TRiPI Off control flies (Fig. S4D). These results demonstrate that the metabolic dysregulation of body energy stores includes not only a defect in TAG mobilization but also hyperglycemia, which likely contributes to or at least correlates with the obesity phenotype.

We asked next, whether Stim-TRiPI-dependent obesity in flies correlates with physical fitness impairment. Indeed, the climbing ability of day 24 Stim-TRiPI flies was reduced by 40% compared to controls (Fig. S4D). Also, the median lifespan of Stim-DRiPI flies was significantly reduced by about 21% compared to corresponding control flies (Fig. S4E), which is reminiscent of the increased mortality correlated with human obesity. Collectively, these data show that the Stim-TRiPI model specifically affects the storage lipid metabolism and recapitulates hallmarks of mammalian obesity.

Next, we asked how long-term knockdown of Stim changes systemic energy homeostasis. Consistent with previous acute RNAi expression studies, we observed that Stim-TRiPI On flies increased food intake by 90% immediately after pulse induction (Fig. 4A) and these flies stayed hyperphagic the following 10 days (Fig. 4B). Similarly, food intake was increased by around 50% in Stim-DRiPI On flies for eight days using this alternative RNAi induction system (Fig. S5A). These data suggest that hyperphagia contributes to extra fat accumulation. In line with this hypothesis are the results from restrictive pair-feeding experiments. In this paradigm, Stim-TRiPI On flies were offered slightly less than the food amount consumed by ad libitum fed Stim-TRiPI Off flies to largely match the dietary intake of experimental and control flies. Under pair-feeding, the fly body fat content of Stim-TRiPI On flies increased by moderate 127% during the first ten days, and reach 1.85-fold the value of age-matched Stim-TRiPI Off flies (Fig. 4C). The corresponding body fat values increase under ad libitum fed conditions are 343% during the first ten days (with a 30% increase already at day 1 after pulse induction) to reach 2.57-fold the value of Stim-TRiPI Off flies (Fig. 4C). To assess the contribution of hyperphagia-dependent lipogenesis we used dietary 14C glucose pulse-labeling to compare de novo lipid synthesis between Stim-DRiPI On and Off flies (Fig. S5B) as glucose intake is known to drive lipogenesis. Indeed, the 24 h 14C incorporation into neutral lipids of Stim-DRiPI On flies was almost five times higher compared to Stim-DRiPI Off flies (Fig. S5C), which substantially exceeds the corresponding incorporation into polar lipids (79%; Fig. S5D) or the food intake increase. These results suggest that besides hyperphagia, hyperactivity of TAG biosynthesis and/or reduced energy expenditure causes the excessive fat accumulation. Therefore, we used the respirometry-based metabolic rate estimation in fly to detect a 29% decrease in CO2 production at noon and a 17% decrease in the evening in the of Stim-DRiPI On flies compared to the corresponding control flies (Fig. S5E). Collectively, these data...
Figure 3. Chronic Stim dysfunction causes fat body cell hypertrophy and obesity in Drosophila. Images of day 1 (A) and days 21 (B) Stim-TRiPI flies display the expanded abdomen developed at day 21 of Stim-TRiPI On (encircled by red rectangle) as compared to Stim-TRiPI Off (encircled by light red rectangle) adult male flies. (C) Increased wet body weight of day 21 Stim-TRiPI On adult male flies as compared to Stim-TRiPI Off flies. (D) Major neutral lipid classes at day 0 and day 21 of Stim-TRiPI On and the Stim-TRiPI Off adult male flies, separated by thin layer chromatography illustrates specific accumulation of storage fat (triacylglycerol; TAG); MAG: Monoacylglycerol; DAG: Diacylglycerol; FA: Fatty acids; Standard refers to a defined mixture of FA, MAG, DAG, and TAG. (E) Integrated intensity quantification of the TAG bands in (D). Dissected flies show similar morphology of fat body tissue (white tissues encircled by green stippled lines) at day 1 but more fat tissue (the area is highlighted by blue arrow) at day 21 of Stim-TRiPI On (F, G; under the red line) as compared to age-matched Stim-TRiPI Off (F', G'; under the light red line) adult male flies. Scale bar in F, F', G, G' represents 0.5 mm. Visualization of lipid droplets (stained by LD540; red), cell membranes (stained by Cell Mask Deep red; white) and nuclei (stained by DAPI; blue) in abdominal fat body tissue of adult male flies at day 1 (H) and day 21 of Stim-TRiPI On (I) and the corresponding control male (Stim-TRiPI Off) flies at day 1 (H') and day 21 (I') shows an increased size of fat body cells (J) and increased lipid droplets (LD) area in fat cells (K) at day 21. Scale bars in H, H', I, and I' represent 50 μm. Data are presented as means ± standard deviations from 3–6 replicates. All data were analyzed by the two-tailed unpaired Student’s t-test. No. *p ≥ 0.05, *p < 0.05, **p < 0.01, ***p < 0.001.
demonstrate that hyperphagia, increased lipogenesis, and reduced energy expenditure contribute to fly obesity in response to chronic Stim knockdown by unknown molecular mechanisms.

Adipose tissue lipolysis dysfunction, Adipokinetic hormone resistance, and insulin signaling impairment caused by chronic impairment of Stromal interaction molecule. To address the tissue-autonomous mechanisms of chronic Stim knockdown, we performed differential transcriptome profiling by comparative RNAseq analysis on dissected fat body tissues of day 10 Stim-TRiPI On and Off flies (Fig. S6A–C). Unsurprisingly, gene ontology analysis revealed a complex regulatory response to chronic SOCE impairment suggesting the down-regulation of various metabolic processes (Fig. 5A). A group of significantly down-regulated genes comprises aralar1, Mdh1, Mdh2, Got1, and Got2 (Fig. 5B,C), all of which encode enzymes involved in NADH shuttling between mitochondria and cytoplasm (Fig. S6D). To understand the correlation between Akh signaling and genes involved in NADH shuttling, we co-analyzed the RNAseq dataset of fly larval fat body samples in response to Akh overexpression vs. control condition and our RNAseq dataset of adult fly fat body tissue under Stim-TRiPI On vs. Off condition. Intriguingly, 47 genes were up-regulated in larval fly fat body overexpressing Akh but down-regulated in Stim-TRiPI On adult fly fat body tissue: four of them are Mdh1, Got1, Got2 and aralar1, which could be considered as Akh signaling reporter genes (Fig. S7A). Moreover, Mdh1 mRNA is down-regulated as early as day 1 in Stim-TRiPI On flies (Fig. 5D) but not in Stim-TRiPI On control flies (Fig. S7B). Another gene contrarily expressed under the described conditions is Gprk2 (Fig. S7A), which is directly correlated with cAMP levels in fly tissue. Consistently, both RNAseq and RT-qPCR show that the AkhR mRNA level is up-regulated in the fat body of Stim-TRiPI On flies, however not the AkhR downstream signaling gene toh (Fig. 5E). Collectively, these finding suggest a differential impairment of Akh signaling in the fat body of obese Stim-TRiPI flies.

Previous research showed that Akh/AkhR signaling is causative for lipid mobilization. Consistently, RNAseq indicates the transcriptional down-regulation of the paralogous lipase genes, doppelgänger of brummer (dob) and brummer (bmm), which encodes the fly homolog of mammalian adipose triglyceride lipase (ATGL) in response to chronic Stim impairment (Fig. S8A). Moreover, this analysis suggests a coordinated down-regulation of mitochondrial and peroxisomal β-oxidation pathways in obese Stim-TRiPI On flies (Fig. S8A). We confirmed the transcriptional down-regulation of lipid catabolic pathway genes by selected RT-qPCR analyses of bmm, carnitine O-palmitoyltransferase (CPT1), dACADVL, and dATP5B (Fig. S8A,C). In contrast, midway (mdy), which encodes the homolog of the key human lipid synthesis enzyme diacylglycerol acyltransferase 1 (DGAT1), was
Figure 5. Fat body transcriptome profile changes indicate impairment of Akh signaling in response to chronic Stim dysfunction. (A) Biological process Gene Ontology term analysis of genes down-regulated in response to chronic Stim impairment in the fat body results in enrichment of genes involved in metabolic processes such as carbohydrate, carboxylic acid, coenzyme, carboxylic acid, coenzyme metabolism, cofactor metabolism, Glucose 6-phosphate, organic hydroxyl compound, pyridine-containing compound and phosphorus metabolic processes, generation of precursor metabolites & energy. For details see supplementary information. (B) Volcano plot illustration of genes transcriptionally responsive to chronic Stim dysfunction, which were selected on the basis of their differential expression (log2-fold change > 0.5 or < -0.5) and p value adjusted with false discovering rate (FDR; value < 0.1) according to RNAseq data. Gene symbols of 15 most differentially regulated genes are labeled. Among them, the two repressed genes with highest confidence (Mdh1, Got2) were labelled in bold. (C) RT-qPCR confirms transcriptional down-regulation of the malate-aspartate shuttle genes aralar1, Mdh1, Mdh2, Got1 and Got2 at day 10 after Stim-TRiPI On as compared to Stim-TRiPI Off flies. (D) Among
only significantly up-regulated at day 1 but not at day 10 in Stim-TRiPI On fat body (Fig. S8D). Similarly, other key genes involved in lipid biosynthesis such as dFAS (fatty acid synthase gene), dACS (Acetyl Coenzyme A synthetase gene), dLipin (Mg²⁺-dependent PA phosphatase gene) were not significantly up-regulated at day 10 in Stim-TRiPI On fat body (Fig. S8D,E). Collectively, these data suggest that a partial Akh resistance as well as impairment of lipolysis and mitochondrial function mediate obesity progression in the fat body in response to chronic Stim knockdown.

To understand why Stim-DRiPI On flies have higher circulating sugar levels, we checked the expression of the Hex-C gene, a homolog of mammalian Glucokinase (GCK) likely involved in glucose clearance by fly fat body⁴³ and insulin signaling⁴⁴. Stim-TRiPI On flies show around 35% reduced expression of the Hex-C gene at day 10 (Fig. S9A), which might account for the hyperglycemia. Since insulin-deficient flies display hyperglycemia⁴² and insulin signaling in the fat body promotes circulating sugar clearance⁴⁶, we checked peripheral insulin signaling in Stim-TRiPI On flies. The insulin signaling target gene d4EBP's expression was not significantly up-regulated at day 1, and mildly but significantly up-regulated by around 25% at day 10 (Fig. S9B), which is repressed by the insulin signaling through active phosphorylated Akt⁴⁷. Besides, although insulin signaling was shown to promote fly fat cell proliferation and fat accumulation⁴⁸, we did not find a measurable increase of new DNA synthesis indicated by EdU staining in Stim-TRiPI On fly fat body tissues (Fig. S9C). Consistently, the phosphorylated Akt (at Ser308 but not Thr342)⁴⁸ level, the downstream readout of insulin signaling⁴⁹, shows a reduced trend by 37% in Stim-DRiPI On peripheral tissues (Figs S9D, S10A, S11A,B,C). Moreover, loss-of-function of the three central insulin peptide genes dIlp2,3,5, the master regulators of sugar homeostasis⁵¹,⁵², did not prevent the extra fat accumulation of Stim-DRiPI On flies (Fig. S9E). Consistent with our previous finding⁵⁰, insulin producing cells (IPCs) of Stim-TRiPI On flies also show reduced staining intensity of dIlp-2 by around 30% (Fig. S9F,G), indicative of increased dIlp-2 secretion⁵⁰. In line with this, RNAseq and RT-qPCR analysis identified the up-regulation of insulin secretion promoting gene CCHamide-2 (CCHa2)⁵⁴ by around 67% and dawdle (daw)⁵⁵ by around 35%, but down-regulation of insulin signaling inhibiting gene Ec dysone-inducible gene L2 (ImplL2)⁵⁶ by around 85% and insulin secretion suppression gene Limostatin (Lst)⁵⁷ by around 70% at the mRNA level (Fig. S10A). To measure circulating dIlp-2 directly, we combined the Stim-DRiPI system with dIlp-2HF (epitope-tagged dIlp-2 gene)⁵⁸ and found no significant difference on dIlp-2HF levels between obese Stim-DRiPI On and Stim-DRiPI Off flies (Fig. S10D). While the contradictory findings on circulating dIlp levels need future research attention, the results collectively show that insulin signaling is impaired in the fat body tissues of obese Stim-RIPI flies and partially dispensable for long-term body fat storage increase.

**Systemic Adipokinetic hormone signaling controls hyperphagia in response to chronic Stromal interaction molecule dysfunction in the fat body.** Adipokinetic hormone signaling does not only promote lipid catabolism in the fat body, but also regulates food intake with ectopic Akh expression causing hyperphagia⁵⁹,⁶⁰,⁶¹. Therefore, we asked if Stim dysfunction of fat body tissue promotes food intake via Akh signaling. We first quantified the Akh mRNA levels of day 1 and day 10 Stim-TRiPI On flies to find no expression differences compared to control flies (Fig. 6A). Since Akh signaling is controlled by Akh peptide secretion from the neuroendocrine corpora cardiaca (CC) cells⁶², reduced Akh immunostaining intensity in CC cells is used as a proxy for increased secretion of the hormone⁶³,⁶⁴. Therefore, we assessed Akh peptide levels by comparative immunohistochemistry on dissected CC cells of day 10 Stim-TRiPI On/Off flies using antibodies directed against Akh and the synaptic cytoskeletal protein Bruchpilot (served as neuronal marker for signal normalization) (Fig. 6B,C). Normalized Akh staining intensity in CC cells of Stim-TRiPI On flies was significantly reduced by around 27% compared to Stim-TRiPI Off flies (Fig. 6B,C) suggesting increased Akh secretion in response to chronic SOCE impairment in the fat body. To directly address the role of Akh in obese flies subject to chronic Stim fat body dysfunction, we compared the body fat content and the food intake of Akh heterozygous and homozygous mutant flies subjected to Stim-DRiPI On/Off (Fig. 6D,E). Loss-of-Akh completely suppressed obesity development (Fig. 6D) and hyperphagia (Fig. 6E) in Stim-DRiPI On flies.

Since Stim-TRiPI On flies have higher circulating sugar levels than control flies, the increased Akh secretion cannot be triggered by lower circulating sugar levels as reported previously⁶⁶. Thus, we propose that increased Akh secretion in Stim-RIPI On flies might be regulated by unknown fat body tissue secreted factors. RNAseq analysis on Stim-TRiPI fat body tissues identified 16 differentially expressed genes encoding proteins belong to "secreted" group (supplementary data file 2). Interestingly, Lst, CCHam2, daw, Est-6, and PGRP-SD were also transcriptionally regulated in fly larval fat body overexpressing Akh (Fig. S10B), which qualifies them as fat body-derived signal candidates to regulate Akh secretion. Collectively, these results support the model that the chronic impairment of Stim activity in the fat body remotely triggers Akh secretion from the CC cells by currently unknown mechanisms, which in turn stimulates food intake and thereby contributes to obesity progression.
Figure 6. Stim dysfunction in the fat body exerts systemic control of hyperphagia and obesity possibly via Akh secretion from neuroendocrine cells. (A) Akh mRNA expression levels are unresponsive to Stim-TRiPI On as compared to Stim-TRiPI Off flies in adult male flies at day 1 and 10 after RNAi induction (n = 3–6). (B) Reduced Akh peptide storage in corpora cardiaca cells (red) visualized by immunohistochemistry on adult male flies at day 10 under control condition (Stim-TRiPI Off; B”) or at day 10 after Stim-TRiPI On (B”). Note that anti-Bruchpilot antibody NC82 was used to counterstain the corpora cardiaca cells (B’ and B”). Scale bars in B represent 50 μm. (C) Quantification of Akh reduction in corpora cardiaca cells of day 10 Stim-TRiPI On adult male flies as compared to corresponding control adult male Stim-TRiPI Off flies (n = 7–12). (D) Stim-DRiPI On causes progressive obesity in heterozygous but not in homozygous Akh mutant males. Relative (rel.) fat content is represented as fold change normalized with respect to the value of day 1 Stim-DRiPI Off flies in Akh heterozygous (n = 6). (E) Loss-of-Akh suppresses the hyperphagia of Stim-DRiPI On male flies. No difference between average daily food intake from day 1 to day 8 in Akh mutant male flies with (Stim-DRiPI Off) or without (Stim-DRiPI Off) Stim dysfunction in the fat body (n = 32). Data are presented as means ± standard deviations. All data were analyzed by the two-tailed unpaired Student’s t-test. No *p ≥ 0.05, *p < 0.05, **p < 0.01, ***p < 0.001.
Discussion

Chronic knockdown of target genes by RNAi Pulse Induction (RiPI). Here we present in vivo evidence for chronic targeted gene knockdown following RiPI in the adult Drosophila fat body. We found that a short pulse induction of siRNA targeting the AkhR gene generates persisting siRNAs, which causes significant down-regulation of AkhR for at least 10 days. Persistence of RNAi has been associated with RNA-dependent RNA polymerase (RdRP)-mediated siRNA amplification in C. elegans63,65 and in human cells44. In Drosophila, however, it remains controversial whether the genome encodes a functional RdRP. Therefore, slow degradation of the transgene-derived siRNAs might confer the chronic gene knockdown. In fact, RNAi effector double-stranded siRNAs (21nt and 24nt) are more stable than the 18nt double-stranded RNAs in the human cytosolic extract47. Moreover, in human HEK293T cells, the anti-sense strand of siRNA is more resistant to intracellular nucleases compared to the sense strand of the siRNA duplex, which is likely due to the incorporation of anti-sense siRNAs into the activated RNA induced silencing complex (RISC)48,49. Therefore, the involvement of RISC might allow the slow degradation of siRNAs in adult Drosophila fat body cells. The slow decline of the siRNA level is apparently sufficient to chronically knockdown the endogenous gene expression of AkhR and Stim, which causes progressive body fat increase. This mode of action is further supported by the fact that pulsed overexpression of RNAi resistant Stim-mRNA only transiently rescues the fat content increase due to Stim-TRiPI. Consistently, long-term gene silencing (at least 11 days) is also observed in adult flies after injection of low concentrations of dsRNAs50. Similarly, in an EGFP-transgenic mouse model, the inhibition of the reporter expression lasts as long as two months after siRNA injection4. In summary, we show here that in vivo RNAi generates long-lasting RNAi, which allows chronic knockdown of target genes in a tissue-specific manner. We propose that RiPI is a versatile tool to study causative relationships and temporal sequences in inter-organ communication processes.

A Drosophila obesity model triggered by chronic Stromal interaction molecule dysfunction in the fly adipose tissue. Using RiPI, we established a Drosophila obesity model based on chronic, adipose tissue-directed knockdown of Stim, which shares remarkable similarity to characteristics of human obesity. First, the visibly enlarged abdomen of the obese flies corresponds to increased waist circumference, which gains importance as meaningful parameter to assess android adiposity3. Similarly, body fat accumulation causes significant weight gain, another readout to quantify obesity in rodents23 and human24. Second, the excessive fat accumulation correlates with climbing deficits of the obese flies, with physical fitness reduction being another hallmark of human adiposity65. Moreover, obese Stim-TRiPI flies have reduced life span, which is reminiscent of the higher mortality rates in human obesity patients26. Third, we demonstrate that early-onset hyperphagia drives the positive energy balance in Stim-TRiPI flies. Consistently, increased food intake is the major driver of human obesity2. Hyperphagia is linked to increased dietary glucose conversion into storage fat in obese Stim-TRiPI flies. Notably, increased food intake and elevated glucose conversion into storage lipids has also been reported after silencing obesity blocking neurons in the fly central brain77. With hyperphagia being an important contributor, obesity development in Stim-RiPI flies is not monocausal. It is noteworthy that the rise in fat storage in Stim-DRiPI substantially exceeds the food intake increase. Moreover, matching the food intake of Stim-TRiPI On and Off flies still results in body fat accumulation. Importantly, there is a significantly reduced metabolic rate of Stim-DRiPI flies. Finally, the observed hyperglycemia at day 10, physical fitness reduction at day 24 and shortened life span of Stim-TRiPI On flies are associated with obesity development, similar to type 2 diabetes (T2D)72, exercise intolerance79 and mortality80, which are also highly correlated with human obesity. In summary, chronic knockdown of Stim in the adult fat body causes fly obesity by a number of physiological factors culminating in organellar energy imbalance similar to mammalian adiposity.

Chronic knockdown of Stromal interaction molecule causes adipose tissue dysfunction. Our study highlights the critical roles played by Stim in interaction with Akh/AkhR signaling and insulin signaling in the fly fat body tissue. Reduced expression of Mdk1 and Gprk2 suggests impaired Akh/AkhR signaling in the fat body of Stim-TRiPI flies. Mammalian MDH1 has been linked to glycolysis in cells with mitochondrial dysfunction81, obese Stim-TRiPI On flies display normal glycogen storage and mobilization during starvation. Similar findings are also observed in AkhR, AkhA and AkhR mutant fly larvae52 and adult flies, albeit their capability to mobilize glycogen is weakly impaired41. A possible explanation is that flies employ corazonin, a starvation-responsive pathway complementary to Akh, to utilize glycogen41. In addition to storage glycogen, the reduced expression of genes involved in lipolysis predicts an impairment of starvation-induced storage lipid mobilization. Indeed, obese Stim-TRiPI flies display an abnormal lipid mobilization profile under starvation and die with residual fat resources. Similarly, impaired lipid mobilization is also observed in flies with loss-of-function mutation in the TAG lipase gene bmm42 or in flies lacking either InsP3R3 or AkhR36. Consistently, loss-of-function of STIM1/2 in mammalian cells, also impairs lipolysis via down-regulation of cAMP46. Moreover, decreased catecholamine-stimulated lipolysis has been identified in human obese individuals35. Collectively, our results show that fat body tissue of obese Stim-TRiPI flies is resistant in response to Akh signaling, which drives the obesity development.

Moreover, our study supports the possibility to model T2D in adult flies. Obese Stim-TRiPI flies show reduced expression of the glucose clearance gene Hex-C, whose mammalian homolog was also suppressed in T2D patients86. Besides, we also provide evidences to support that obese Stim-TRiPI flies have hyperglycemia, impairment of insulin signaling in fat body tissue, and larger lipid droplets. Similar features were also described in fly larvae reared on high sugar diet36,47, which resemble mammalian insulin resistance84. Regarding unchanged circulating dillp-2 level in obese Stim-DRiPI flies, insulin-like peptide secretion might be interfered by the knockdown of Stim in the insulin producing cells of Stim-DRiPI flies mediated by ubiquitous driver daGS, more investigation on circulating insulin levels of obese Stim-DRiPI flies by specific driver need to be done in future. Interestingly, the indicators of insulin signaling impairment mentioned above occur at later stage of Stim-RIPI obesity development, and accordingly are possibly the consequence of Stim-TRiPI on mediated-fat gain, which also supports the concept that obesity compromises insulin signaling.
Metabolic fat body dysfunction drives organismal energy imbalance. Apart from the specific role of the fat body in storage lipid handling and glucose clearance, we show that chronic knockdown of Stim in this organ remotely promotes Akh secretion from the fly CC neuroendocrine cells, which leads to hyperphagia. Our RNAseq and gene expression analysis indicate a list of genes encoding candidate hormone or secreted proteins. Among them, CCHA2, daw, and Lst have been shown to function as hormones to regulate insulin-like peptide secretion\(^\text{34,35,37}\). In addition, CCHA2, daw, Lst are also regulated by Akh overexpression in opposite direction. Whether differential expression of these genes mentioned above mediates the (mis)communication between the fat body and the CC cells is currently unknown. Nevertheless, the communication between the fat body and the CC cells is essential for the food intake increase as well as further obesity development induced by long-term knockdown of Stim. Interestingly, a study provided evidence that muscle tissue in flies communicates with the CC cells to control Akh secretion via the myokine Unpaired2 (Upd2)\(^38\). Upd2 had been previously shown to act as adipokine, which signals the fed state from the fat body. Unlike mammalian leptin, Upd2 remotely acts on insulin-producing cells in the central brain to regulate insulin secretion but not food intake\(^39\). Recently, Akh mRNA expression was shown to be regulated by a gut-neuronal relay by midgut-secreted peptide Buriscon α in response to nutrients\(^40\). Given the fact that the transcription of Akh is unaffected in Stim-RiPI On flies, identification of the adipokine, which regulates the Akh release directly or indirectly to affect food intake in the Stim-RiPI fly obesity model requires future research efforts.

In conclusion, our work introduces RNAi Pulse Induction as a novel in vivo paradigm for chronic, tissue-specific gene interference. RiPI makes essential genes accessible to long-term functional analysis in the adult fly, as exemplified here by establishing a Drosophila obesity model caused by chronic knockdown of Stim in the adult fat body. Moreover, this study reveals, that the fat body integrates the tissue-autonomous and the systemic branches of Akh signalling; by regulation of lipid mobilization via SOCE in the fat body, and possibly by remote-control of Akh secretion from the CC cells. Recently, the evolutionarily conserved role of SOCE in controlling energy metabolism has attracted the interest of mammalian studies\(^46,27\). While Akh is structurally not conserved to humans, there is a growing number of remotely-controlled orexigenic peptide hormones in mammals with apsorin being one of the latest additions\(^92,93\). Collectively, our findings in the fly add further evidence to the existence of conserved regulatory principles in animal energy homeostasis control emanating from SOCE signalling in fat storage tissues.

Material and Methods

Fly stocks and husbandry. The following Drosophila fly stocks were used in this study: StimRNAi\(^94\), ts-FB-Ga4\(^41\), da-GS\(^95\), dIlp2\(^–\)3,5 mutant\(^96\), Akh\(^3\), Ilp2\(^{gd2HH}\)^\(^2\). Balancer line 2 (\#7198), IlpR RNAi\(^2\)(\#51710) were obtained from the Bloomington Drosophila Stock Center (BDSC, Harvard TRIP library fly line\(^36\)); w\(^1118\) (\#60000) and Stim RNAi\(^2\)(\#47073), AkhR RNAi\(^2\)(\#9546) from the Vienna Drosophila Resource Center (VDRC; GD and KK library)\(^36\). Stim RNAi resistant cDNA transgenic and Stim cDNA RA transgenic fly lines were generated as described in supplementary information. Details on the genotypes of the fly stocks used in this study are listed in Table S1.

For density-controlled fly propagation, approximately 200 embryos were transferred into a medium food vial and 100 flies were collected 24–48 h after adult eclosion. These flies were kept in under 12:12 Light:Dark cycle and 60–70% humidity with food exchange every other day. Unless stated otherwise, all experiments were carried out on adult male flies. Details on the fly food recipes, and RNAi pulse induction regimens in adult flies are described in supplementary information.

Quantification of body fat, glycogen, and protein levels. Unless stated differently, three to six replicates of each condition (typically five adult male flies per replicate) were collected. A coupled-colorimetric assay for TAG equivalents was carried out as previously described\(^26\). Glycogen level was determined with method described in Gálková et al.\(^31\). Protein level for normalization of body fat and glycogen content was quantified with Bicinchoninic acid assay (BCA) as described in Gálková et al.\(^31\).

Fly body weight measurement. Three to six replicates of five adult males each were transferred into a 1.5 mL Eppendorf tube and the total weight determined using a Sartorius Microbalance MC5 (Sartorius AG, Göttingen, Germany). For dry weight, the open tubes containing flies were incubated at 65 °C for 24 hours. The empty tube weights were previously determined by averaging the values of three different measurements and subtracted from the total weight to determine the wet and dry weight of the flies. The experiment was repeated twice.

Food intake and physiological assays. To quantify adult fly food intake, a capillary feeding (CAFE) assay was performed according to Ja et al.\(^38\) with slight modifications\(^94\). At least 36 flies per condition were assayed for food intake analysis. The following physiological assays were carried out based on previously described methods with minor modifications: starvation resistance assay\(^92\), startle-induced climbing assay\(^41\), and metabolic rate assay\(^37\); for further details see supplementary information.

Ex vivo adult fly fat body tissue imaging, lipid and cell area quantification. Three to five flies per condition were analyzed. The experiment was done as described in Baumbach et al.\(^25\). Further details refer to supplementary information.

Thin layer chromatography (TLC). Four replicates (five adult males per replicates) were collected for TLC, which was done as described in Baumbach et al.\(^25\) with minor modifications. Experiments were repeated twice. Details are described in supplementary information.
RT-qPCR. Three to six independent biological replicates were used. RT-SYBR qPCR was done as described in Bellet et al.11. RT-Taqman qPCR followed the instructions of the kit manufacturer. Details including primer sequences on quantitative RT-qPCR are described in supplementary information.

RNAseq analysis of adult fly fat body tissues. Abdominal fat body tissues of adult male flies at day 10 and 11 after Stim-TriPI On/Off induction were dissected in cold PBS buffer, snap frozen in liquid nitrogen, and immediately stored at −80°C. Dissected fat body tissues of about 20 flies were pooled as one biological replicate per condition of each Stim-TriPI On/Off experiment. Three independent biological replicates of fat body tissues were used for total RNA isolation using a commercial RNA extraction kit (Norgen BIOTEK, Cat. #: 36200). Typical RNA samples contained at least 1 µg of RNA with concentrations around 50 ng/µL. RNA sample quality analysis, library preparation, sequencing, sequence data trimming, mapping, and reads counting were performed by the Max Planck-Genome-centre Cologne, Germany (http://mpgc.mpipz.mpg.de). Further details on RNAseq quality control, gene differential expression analysis and gene ontology analysis are described in supplementary information.

Western blot analysis. Three independent biological replicates were used for the experiments. Primary antibodies: rabbit anti-Phospho-Akt Ser505 (1:500, NEB, Cat. #405 S), rabbit anti-Phospho-Akt Thr342 (1:500, phosphoSolutions, Cat. #: 104–342), rabbit anti-Akt (1:500, NEB, Cat. #9272 S) and mouse anti-β-tubulin (Developmental Studies Hybridoma Bank (DSHB); Cat. #: E7); secondary antibodies: goat anti-rabbit-HRP (1:2000, Pierce, Cat. #: 31640) and goat anti-mouse-HRP (1:10000, Pierce Cat. #31430). For further details on tissue lysis buffer and Western blot analysis see supplementary information.

Akh and dlp-2 immunostaining and confocal imaging. Akh and dlp-2 immunohistochemistry was performed as described26 with modifications described in supplementary information.

Circulating sugar level measurement. Circulating sugar level of adult flies was determined as described43 with modifications described in supplementary information.

Statistical analysis. Unless stated differently, data were analysed by two-tailed paired Student’s t-tests using Microsoft Excel 2011. Statistical significance of the differences between samples is represented by asterisk: *p < 0.05, **p < 0.01, ***p < 0.001. All error bars represent standard deviations of the mean.

References
1. Spiegelman, B. M. & Flier, J. S. Obesity and the regulation of energy balance. Cell 104, 531–543 (2001).
2. Hill, J. O., Wyatt, H. R. & Peters, J. C. Energy balance and obesity. Circulation 126, 115–132 (2012).
3. Garicy, J. S. Obesity and Related Diseases. Churchill Livingstone (1988).
4. Ng, M. et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 384, 766–781 (2014).
5. Vandervijver, S. et al. Increased food energy supply as a major driver of the obesity epidemic: a global analysis. Bull World Heal. Organ 93, 446–456 (2015).
6. Livingstone, M. E. et al. How active are we? Levels of routine physical activity in children and adults. Proc. Natl. Sci. 62, 681–701 (2003).
7. Church, T. S. et al. Trends over 5 decades in U.S. occupation-related physical activity and their associations with obesity. PLoS One 6, e19657 (2011).
8. Trefis, E., Gannon, M. & Wasserman, D. H. The liver. Curr. Biol. 27, R1147–R1151 (2017).
9. Rosen, E. D. & Spiegelman, B. M. What we talk about when we talk about fat. Cell 156, 20–44 (2014).
10. Halaas, J. L. et al. Weight-reducing effects of the plasma protein encoded by the obese gene. Science. 269, 543–546 (1995).
11. Scheltem, D. A. Insights into energy balance from doubly labeled water. Int. J. Obes. (Lond.) 32, S72–S75 (2008).
12. Arrese, E. L. & Soulages, J. L. Insect fat body: energy, metabolism, and regulation. Annu. Rev. Entomology 55, 207–225 (2010).
13. Kühnlein, R. P. The contribution of the Drosophila model to lipid droplet research. Prog. Lipid Res. 50, 348–356 (2011).
14. Pospisilik, J. A. et al. Drosophila genome-wide obesity screen reveals hedgehog as a determinant of brown versus white adipose cell fate. Cell 140, 148–160 (2010).
15. Baumbach, J. et al. A Drosophila in vivo screen identifies store-operated calcium entry as a key regulator of adiposity. Cell Metab. 19, 331–343 (2014).
16. Roos, J. et al. STIM1, an essential and conserved component of store-operated Ca2+ channel function. J. Cell Biol. 169, 435–445 (2005).
17. Bootman, M. D. Calcium signalling. Cold Spring Harb. Perspect. Biol. 4, a011171 (2012).
18. Clapham, D. E. Calcium Signaling. Cell 131, 1047–1058 (2007).
19. Liou, J. et al. STIM is a Ca2+ sensor essential for Ca2+ -store- depletion-triggered Ca2+ influx. Curr. Biol. 15, 1235–1241 (2005).
20. Zhang, S. L. et al. STIM1 is a Ca2+ sensor that activates CRAC channels and migrates from the Ca2+ store to the plasma membrane. Nature 437, 902–905 (2005).
21. Feske, S. et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature 441, 179–185 (2006).
22. Vig, M. et al. CRACM1 Is a plasma membrane protein essential for store-operated Ca2+ entry. Science 312, 1220–1223 (2006).
23. Zhang, S. L. et al. Genome-wide RNAi screen of Ca2+ influx identifies genes that regulate Ca2+ release-activated Ca2+ channel activity. Proc. Natl. Acad. Sci. USA 103, 9357–9362 (2006).
24. Soboloff, J., Rothberg, B. S., Madesh, M. & Gill, D. L. STIM proteins: dynamic calcium signal transducers. Nat. Rev. Mol. Cell Biol. 13, 549–565 (2012).
25. Baumbach, J. et al. Gqg, Go1 and Plc21C control Drosophila body fat storage. J. Genet. Genomics 41, 283–292 (2014).
26. Maus, M. et al. Store-Operated Ca2+ Entry controls induction of lipolysis and the transcriptional reprogramming to lipid metabolism. Cell Metab. 25, 698–712 (2017).
27. Arruda, A. P. et al. Defective STOREM-mediated store operated Ca2+ entry in hepatocytes leads to metabolic dysfunction in obesity. Elife 6, e29968 (2017).
28. Pathak, T., Trivedi, D. & Hasan, G. CRISPR-Cas induced mutants identify a requirement for dSTIM in larval dopaminergic cells of Drosophila melanogaster. G3 Genes/Genomes/Genetics 7, 923–933 (2017).
29. Brand, A. H. & Perrimon, N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118, 401–415 (1993).
30. McGuire, S. E. et al. Spatiotemporal rescue of memory dysfunction in Drosophila. Science 302, 1765–1768 (2003).
31. Beller, M. et al. PERILIPIN-dependent control of lipid droplet structure and fat storage in Drosophila. Cell Metab. 12, 521–532 (2010).
32. Klepetal, P. et al. Thermal stress depletes energy reserves in Drosophila. Sci. Rep. 6, 33667 (2016).
33. Grönke, S. et al. Dual lipolytic control of body fat storage and mobilization in Drosophila. PLoS Biol. 5, e137 (2007).
34. Bauman, G. et al. P(Switch), a system for spatial and temporal control of gene expression in Drosophila melanogaster. Proc Natl Acad Sci USA 98, 12602–12607 (2001).
35. Tricoire, H. et al. The steroid hormone receptor EcR finely modulates Drosophila lifespan during adulthood in a sex-specific manner. Mech. Ageing Dev. 130, 547–552 (2009).
36. Musselman, L. P. et al. Role of fat body lipogenesis in protection against the effects of caloric overload in Drosophila. J. Biol. Chem. 288, 8028–8042 (2013).
37. Vatsenko, A. S. et al. Measurement of metabolic rate in Drosophila using respirometry. J. Vis. Exp. 88, e51681 (2014).
38. Song, W. et al. Midgut-derived activin regulates glucacon-like action in the fat body and glycemic control. Cell Metab. 25, 386–399 (2017).
39. Cheng, S., Maier, D. & Hipfner, D. R. Drosophila G-protein-coupled receptor kinase 2 regulates cAMP-dependent Hedgehog signaling. Development 94, 85–94 (2012).
40. Bisch, S. et al. Opposing effects of dietary protein and sugar regulate a transcriptional target of Drosophila insulin-like peptide signaling. Cell Metab. 7, 321–332 (2008).
41. Gálékóvá, M. et al. Energy homeostasis control in Drosophila adipokine hormone mutants. Genetics 201, 665–683 (2015).
42. Grönke, S. et al. Brummer lipase is an evolutionary conserved fat storage regulator in Drosophila. Cell Metab. 1, 323–330 (2005).
43. Barry, W. E. & Thummel, C. S. The Nutrient-responsive hormone CCHamide-2 controls growth by regulating insulin-like peptides in the brain of Drosophila melanogaster. Development 140, 208–12 (2014).
44. Scanga, S. E. et al. The conserved PI3K/PTEN/Akt signaling pathway regulates both cell size and survival in Drosophila. Oncogene 19, 3971–7 (2000).
45. Grönke, S. et al. Molecular evolution and functional characterization of Drosophila insulin-like peptides. PLoS Genet. 6, e1000857 (2010).
46. Park, S. et al. A genetic strategy to measure circulating Drosophila insulin reveals genes regulating insulin production and secretion. PLoS Genet. 10, e1004554 (2014).
47. Gao, X. J. et al. A transcriptional reporter of intracellular Ca2+ in Drosophila. Nat. Neurosci. 18, 917–925 (2015).
48. Sano, H. et al. The nutrient-responsive hormone CCHamide-2 controls growth by regulating insulin-like peptides in the brain of Drosophila melanogaster. PLoS Genet. 11, 1–26 (2015).
49. Ghosh, A. C. & Connor, M. B. O. Systemic Activin signaling independently regulates sugar homeostasis, cellular metabolism, and pH balance in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 111, 5729–5734 (2014).
50. Hoeßler, B. et al. Imp-L2, a putative homolog of vertebrate IGF-binding protein 7, counteracts insulin signaling in Drosophila and is essential for starvation resistance. J. Biol. 7, 10 (2008).
51. Alfa, R. W. et al. Suppression of insulin production and secretion by a decenin hormone. Cell Metab. 21, 323–333 (2015).
52. Gálékóvá, M. et al. The obesity-related adipokine hormone controls feeding and expression of neuropeptide regulators of Drosophila metabolism. Eur. J. Lipid Sci. Technol. 119, 1600138 (2017).
53. Tourjane, N. et al. Coupled sensing of hunger and thirst signals balances sugar and water consumption. Cell 166, 855–866 (2016).
54. D’Aniello, J. R. & Birnbaum, M. J. Regulation of fat cell mass by insulin in Drosophila melanogaster. Mol. Cell. Biol. 29, 6341–6352 (2009).
55. Schleisch, S. et al. DENV-3C-1 promotes translation re-initiation downstream of uORFs to control tissue growth. Nature 512, 208–12 (2014).
56. Zarnik, N. et al. Brummer lipase is an evolutionary conserved fat storage regulator in Drosophila. Cell Metab. 1, 323–330 (2005).
57. Dzitoyeva, S., Dimitrijevic, N. & Manev, H. Intra-abdominal injection of double-stranded RNA into anesthetized adult Drosophila triggers RNA interference in the central nervous system. Mol. Psychiatry 6, 665–670 (2001).
58. Liu, J. et al. Stability study of unmodified siRNA and relevance to clinical use. Oligonucleotides 18, 345–354 (2008).
59. Smith, U. Abdominal obesity: a marker of ectopic fat accumulation. J. Clin. Investig. PMC 125, 1790–1792 (2015).
60. Ingalls, A. M., Dickie, M. M. & Smell, G. D. Obese, a new mutation in the house mouse. J. Hered. 41, 317–318 (1950).
61. Gray, D. S. & Fujisaka, K. E. N. Use of relative weight and body mass index for the determination of adiposity. J. Clin. Epidemiology 44, 545–549 (1991).
62. Roda, C. et al. Lifestyle correlates of overweight in adults: a hierarchical approach (the SPOTLIGHT project). Int. J. Behav. Nutr. Phys. Act. 13, 114 (2016).
63. Pischon, T. et al. General and abdominal adiposity and risk of death in Europe. Eur. J. Med. 359, 2105–2120 (2008).
64. Al-Anzi, B. et al. Obesity-blocking neurons in Drosophila. Neuron 63, 329–341 (2009).
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Perkins, L. A. et al. The transgenic RNAi project at Harvard medical school: resources and validation. Genetica 201, 843–852 (2015).

Dietz, G. et al. A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature 448, 151–156 (2007).

Hildebrant, A., Bickmeyer, I. & Kühnlein, R. P. Reliable Drosophila body fat quantification by a coupled colorimetric assay. eLife, e23796 (2011).

Ja, W. W. et al. Prandiology of Drosophila and the CAFE assay. Proc Natl Acad Sci USA 104, 8253–8256 (2007).

Author Contributions

R.P.K. conceived the study; Y.X. and R.P.K. designed the experiments; Y.X. performed all experiments except some of ex vivo fat body imaging, TLC, and body weight experiments done by A.F.B. and circulating sugar experiments by C.H. G.T. and T.R. contributed to circulating insulin experiments. Y.X. and C.H. analyzed the data; Y.X. and R.P.K. wrote the manuscript. All authors revised the manuscript.

Additional Information

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