Activin signaling mediates muscle-to-adipose communication in a mitochondria dysfunction-associated obesity model

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Mitochondrial dysfunction has been associated with obesity and metabolic disorders. However, whether mitochondrial perturbation in a single tissue influences mitochondrial function and metabolic status of another distal tissue remains largely unknown. We analyzed the nonautonomous role of muscular mitochondrial dysfunction in *Drosophila*. Surprisingly, impaired muscle mitochondrial function via complex I perturbation results in simultaneous mitochondrial dysfunction in the fat body (the fly adipose tissue) and subsequent triglyceride accumulation, the major characteristic of obesity. RNA-sequencing (RNA-seq) analysis, in the context of muscle mitochondrial dysfunction, revealed that target genes of the TGF-β signaling pathway were induced in the fat body. Strikingly, expression of the TGF-β family ligand, Activin-β (Actβ), was dramatically increased in the muscles by NF-κB/Relish (Rel) signaling in response to mitochondrial perturbation, and decreasing Actβ expression in mitochondrial-perturbed muscles rescued both the fat body mitochondrial dysfunction and obesity phenotypes. Thus, perturbation of muscle mitochondrial activity regulates mitochondrial function in the fat body nonautonomously via modulation of Activin signaling.

mitochondrial synchrony | Activin-β | complex I perturbation | NF-κB/Relish | lipid metabolism

Individual organs in a multicellular organism, besides performing their respective roles, must communicate with other organs to maintain systemic homeostasis. The central nervous system (CNS) in particular integrates information regarding the status of peripheral metabolic processes via hormonal signaling and directs energy homeostasis and feeding behavior (1). In addition, metabolic changes in a peripheral organ can affect the physiology of other peripheral organs (2, 3). The skeletal muscle system, which is newly recognized as playing endocrine-related roles, produces myokines after exercise to target other metabolic organs (liver, adipose tissue, pancreas, gut, and bone) and modulates systemic energy homeostasis (4).

Mitochondria are semiautonomous organelles that integrate multiple physiological signals. Growing evidence indicates that mitochondrial alterations in one organ leads to abnormalities in biological processes in distal organs through hormonal signaling (5, 6). In addition to exercise, which induces mitochondrial activity and improves muscle performance, mitochondrial perturbation-associated muscle injury is also sufficient to modulate functions of other organs and change systemic outcomes via myokine production. For example, in mammals, mitochondrial dysfunction due to disruption of autophagic function in skeletal muscles results in elevated production of muscular FGF21 that triggers browning of white adipose tissue and increases lipid mobilization (7). Further, in *Drosophila*, mild mitochondrial distress in adult muscles delays aging via an increase of muscular ImpL2 production and remote suppression of insulin signaling in the fat body and brain (8). Despite these examples, molecular mechanism(s) underlying muscle mitochondrial dysfunction-associated organ communication remains largely unknown. Characterization of this interorgan communication network may deepen our understanding of systemic diseases, such as aging and obesity.

In this study, we performed muscle-specific mitochondrial perturbation in *Drosophila* and demonstrate that this triggers simultaneous and nonautonomous mitochondrial dysfunction in the fat body. To characterize the affected signaling pathways, we analyzed transcriptome changes in the fat body by using RNA-sequencing (RNA-seq), leading us to implicate TGF-β signaling in muscle-to-fat-body communication. Finally, we show that production of Actβ, a TGF-β ligand, is increased in muscles with mitochondrial dysfunction and that muscle-derived Actβ triggers TGF-β signaling via Babo to decrease mitochondrial activity and increase lipid storage in the fat body. Altogether, our results identify Actβ as a mitochondrial-related hormone involved in "mitochondrial synchrony" regulation in both muscle and fat body.

Results

Muscle Mitochondrial Perturbation Results in Mitochondrial Dysfunction and Lipid Accumulation in the Fat Body. To examine whether perturbing mitochondrial function in muscles affects the function of other organs, we disrupted the activity of mitochondrial complex I NADH:ubiquinone oxidoreductase, which serves as the electron entry point in electron transport chain (ETC), specifically in larval muscles using RNAi. RNAi perturbation of the complex

Significance

Mitochondrial perturbation-associated dysregulation of one organ has been shown to nonautonomously affect the functions of other organs in both vertebrates and invertebrates. Using *Drosophila* as a genetic model organism, we characterized mitochondrial synchrony dysregulation across organs and uncovered that mitochondrial perturbation caused by complex I disruption in muscles remotely impairs mitochondrial function and lipid mobilization in the fat body, leading to obesity. We further identified that the TGF-β ligand Actβ, which is autonomously increased by muscular mitochondrial perturbation, mediates muscle-to-fat-body communication and synchronized mitochondrial dysregulation.

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Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, https://www.ncbi.nlm.nih.gov/geo (accession no. GSE100214).

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I component *ND-75*/*NDUFS1* in muscle using the *dMef2-Gal4* driver resulted in a reduction in *ND-75* mRNA levels in *dMef2* > *ND-75-i* larval muscles by ∼80% (relative to controls bearing *white* [*w*] RNAi control, *dMef2* > *w-i*), whereas *ND-75* mRNA levels were not changed in the fat body (Fig. 1*A*). We previously reported that *ND-75* knockdown does not affect overall muscle integrity although it results in larvae with smaller muscles (8). However, complex I perturbation via *ND-75* knockdown in *dMef2* > *ND-75-i* muscle dramatically disrupted the integrity of mitochondria embedded in the myofibers compared with the well-organized, compact mitochondria observed in control *dMef2* > *w-i* larval muscles (Fig. 1*B*, arrowhead). Note that mitochondrial mass in *dmef2* > *ND-75-i* muscle was elevated in a compensatory manner (Fig. S1*A*). Consistent with mitochondrial disintegration, *dMef2* > *ND-75-i* larval muscles were associated with a decrease in both ADP-induced O_2 consumption of isolated mitochondria and ATP production of muscle tissue (Fig. 1 *C* and *D*). Further, larval muscle contraction was significantly reduced (Fig. S1*D*).

In addition to muscle mitochondrial abnormalities, we unexpectedly observed uneven and distorted mitochondrial morphology in the fat body in *dMef2* > *ND-75-i* larvae (Fig. 1*B*), although *ND-75* mRNA levels remained unchanged in the fat body (Fig. 1*A*). Both ADP-induced mitochondrial O_2 consumption and ATP production in the fat bodies of *dMef2* > *ND-75-i* larvae were significantly decreased (Fig. 1 *C* and *D*), indicating decreased mitochondrial activity in the fat body. Because mitochondrial activity is highly associated with lipid mobilization, which is mainly through lipid β-oxidation in the adipose tissue across species (9, 10), we tested whether mitochondrial dysfunction in the fat body impairs lipid homeostasis in *dMef2* > *ND-75-i* larvae. Thus, we performed quantitative lipidomic analysis to assess lipid composition changes in the fat body of *dMef2* > *ND-75-i* larvae. Interestingly, compared with the control, free fatty acid levels in *dMef2* > *ND-75-i* fat body were significantly increased (Fig. 1*E* and Dataset S1). Other lipid species, including TG (triglycerides) and DG (diglycerides), were also elevated in *dMef2* > *ND-75-i* fat body (Fig. 1*F* and Dataset S1). Consistent with these observations, Bodipy staining, which labels intracellular neutral lipids, revealed bigger lipid droplets and more lipid droplet numbers in the fat body of *dMef2* > *ND-75-i* larvae (Fig. 1*G*). Biochemical assays also confirmed an increase in TG storage in *dMef2* > *ND-75-i* fat body and whole animals, compared with controls (Fig. 1 *H* and *I*). Increased lipid storage might be caused by muscle-associated changes in feeding. However, we were able to exclude this possibility, because *dMef2* > *ND-75-i* larvae ate less (Fig. S1*C*), a change in activity expected to decrease lipid storage. Taken together, our data demonstrate that mitochondrial perturbation caused by complex I disruption in the muscle remotely impairs mitochondrial activity in the fat body and results in lipid accumulation.

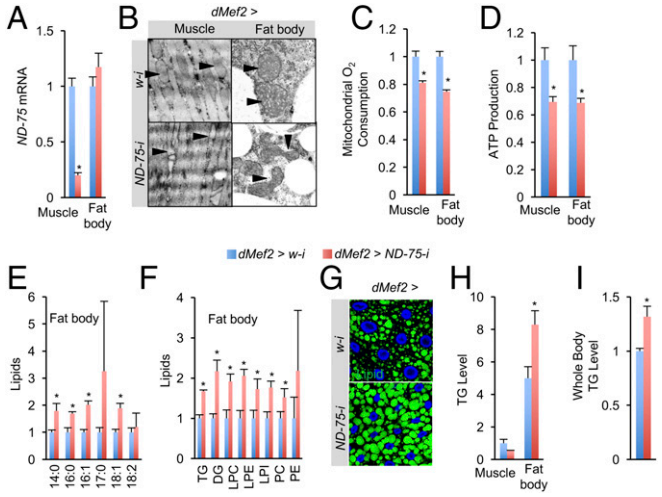

Fig. 1. Muscle mitochondrial complex I perturbation results in mitochondrial dysfunction and lipid accumulation in the fat body. (*A*) Relative *ND-75* mRNA levels in the muscles and fat bodies of *dMef2* > *w-i* (*dMef2-Gal4*/*UAS-w-i*) and *dMef2* > *ND-75-i* (*dMef2-Gal4*/*UAS-ND-75-i*) larvae ($n = 3$, 10 pooled tissues per replicate). (*B*) Representative electronic microscope images of mitochondria morphology in larval muscles (embedded in myofiber) or fat body. Arrowheads indicate individual mitochondria. (*C* and *D*) O_2 consumption rates in isolated mitochondria after ADP treatment (*C*, $n = 3$, 20 pooled tissues per replicate) and ATP levels (*D*, $n = 3$, 5 pooled tissues per replicate) in larval tissues. (*E* and *F*) Lipidomic analysis indicating composition changes of short-chain lipids (*E*) and other lipids (*F*), including triglyceride (TG), diglyceride (DG), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylinositol (LPI), phosphatidylcholine (PC), and lysophosphatidylethanolamine (PE), in the larval fat bodies. (*G* and *H*) Bodipy staining of intracellular neutral lipid in larval fat body cells (*G*) and TG levels in larval tissues (*H*, $n = 3$, 5 pooled tissues per replicate). (*I*) TG levels in the third instar larvae ($n = 3$, 5 pooled larvae per replicate). Data are presented as means ± SEM $^*P < 0.05$.

Hormone/ligand-induced signaling is essential for interorgan communication. We next sought to characterize the mechanism by which muscle mitochondrial perturbation remotely impairs fat body function. We hypothesized that mitochondrial-injured muscles produce ligands that regulate biological processes in the

In addition to ND-75, we also tested the effects of RNAi reagents targeting other complex I components. As observed with ND-75, knockdown of *ND-49*/*NDUFS2* or *ND-42*/*NDUFA10* in the muscle also decreased mitochondrial activity in both muscle and fat body and resulted in fat body lipid accumulation (Fig. S1 *B*–*G*).

To examine mitochondrial dysfunction in the fat body, we perturbed the components of the ETC (electron transfer chain) or lipid β-oxidation process using RNAi. Surprisingly, perturbation of ETC complex components dramatically depleted whole animal lipid storage (Fig. S2), probably due to disruption of fat body integrity. However, knockdown of components involved in lipid β-oxidation significantly increased TG storage in the fat body (Fig. S2), consistent with the result obtained for complex I perturbation in the muscle. Thus, our results indicate that muscle mitochondrial perturbation remotely suppresses mitochondrial activity and lipid mobilization in the fat body via modulation of lipid β-oxidation.

Modulation of TGF-β Signaling in the Fat Body upon Muscle Mitochondrial Perturbation. We have shown that muscle-derived ImpL2 results in inhibition of systemic insulin signaling in adult flies (8), suggesting that ImpL2 might also play a role in muscle-to-fat-body communication in larvae. However, *ImpL2* knockdown in *ND-75*–deficient larval muscles (*dMef2* > *ND-75-i* + *ImpL2-i*) was not sufficient to alleviate TG accumulation caused by complex I perturbation (Fig. S3*A*), suggesting that additional mechanisms exist in the muscle-to-fat-body regulation. To explore the potential mechanisms, we analyzed changes in the fat body transcriptome in *dMef2* > *ND-75-i* larvae by RNA-seq. Following statistical analyses, 1,592 genes (13% of the transcriptome) were significantly changed (fold change > 1.5) with high confidence; of these, 1,080 are up-regulated and 512 are down-regulated (Fig. 2*A* and Dataset S2). Gene Ontology (GO) term enrichment analysis of the differentially expressed gene sets revealed that genes involved in amino acid, lipid, carbohydrate, and energy metabolism were preferentially affected (Fig. 2*B* and Dataset S3). Interestingly, most differentially expressed genes in the GO category "lipid catabolic process" were significantly down-regulated, whereas most genes in "fatty acid metabolic process," "lipid biosynthetic process," and "Acetyl-CoA metabolic process" were up-regulated (Fig. 2*B*). In addition, more than half of the genes that encode mitochondrial proteins, including components of complex I, II, III, IV, V, TCA, and β-oxidation, were up-regulated in *dMef2* > *ND-75-i* larval fat body (Fig. 2 *C* and *D*). Altogether, these results suggest that mitochondrial dysfunction in the *dMef2* > *ND-75-i* larval fat body is not caused by overall down-regulation of mitochondrial genes.

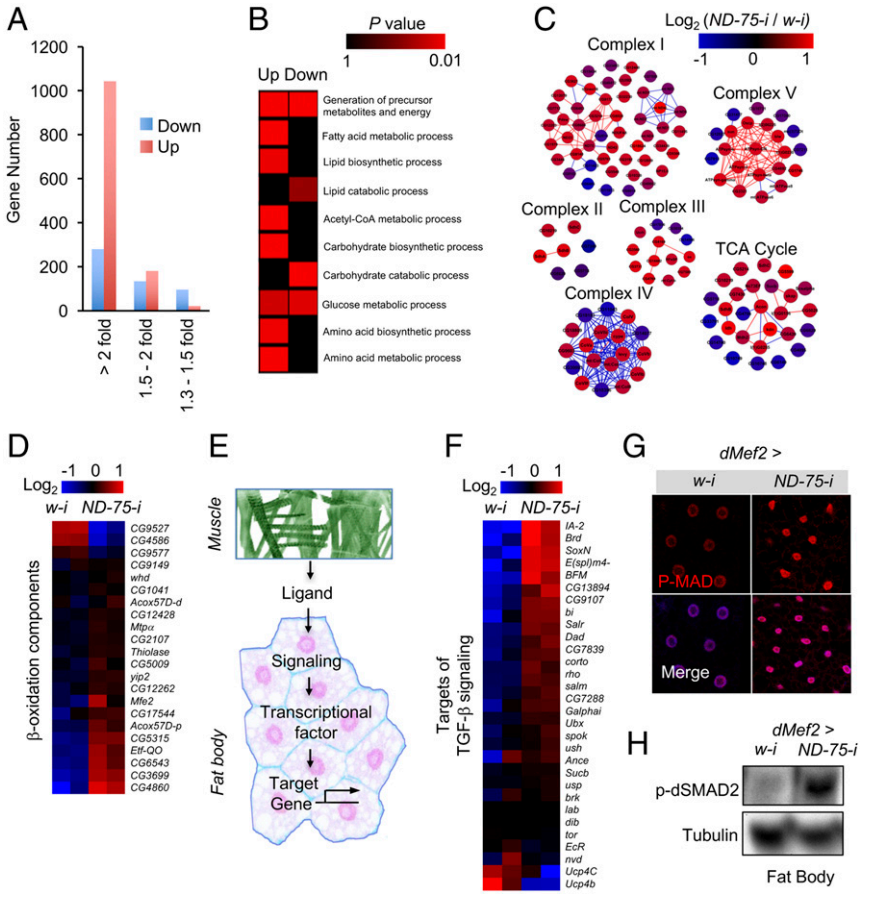

Fig. 2. Transcriptome analysis reveals that muscle mitochondrial perturbation enhances TGF-β signaling in the fat body. (*A*) Gene expression changes in *dMef2 > ND-75-i* larval fat bodies, compared with *dMef2 > w-i*. (*B*) Gene set enrichment analysis indicates that genes involved in metabolic processes are significantly enriched among up and/or down-regulated genes (colors indicate enrichment *P* values). (*C*) Expression changes of genes encoding mitochondrial complex components (colors indicate $\log_2$ ratio of *dMef2 > ND75-i* versus *dMef2 > w-i*. Lines indicate potential protein interactions). (*D*) Expression changes of genes encoding regulators of β-oxidation process (heat map indicates $\log_2$ fold-change of gene expression). (*E*) Schematic of hormone/ligand-induced muscle-to-fat-body communication. Muscle secretes hormone(s) or ligand(s) to target fat body and affects signaling downstream gene expression. (*F*) Heat map illustrating the expression changes of target genes of the TGF-β signaling pathway in larval fat bodies. (*G*) Representative confocal images of p-MAD in third instar larval fat body cells (red, p-MAD; blue, DAPI). (*H*) Western blots of p-dSMAD2 in larval fat bodies.

fat body (Fig. 2*E*). Because ligands classically trigger intracellular signaling pathways that regulate transcriptional factor-dependent gene expression, monitoring target gene expression should help identify the signaling pathway(s) involved (Fig. 2*E*). Strikingly, most of the TGF-β signaling pathway target genes (11–13) were significantly up-regulated in the *dMef2 > ND-75-i* larval fat body (Fig. 2*F*), indicating that TGF-β signaling was potently increased. Consistent with this indication, immunostaining and immunoblotting revealed that levels of the two major readouts of TGF-β signaling, p-Mad and p-dSmad2, were significantly elevated in the *dMef2 > ND-75-i* larval fat body (Fig. 2 *G* and *H*). We previously found that midgut-induced activation of TGF-β signaling in the fat body leads to metabolic dysregulation (14). Based on this idea, we speculated that nonautonomous activation of TGF-β signaling also participates in muscle-to-fat body communication.

Muscle Mitochondrial Perturbation Remotely Affects Mitochondrial Function in the Fat Body Function via Production of Actβ. To test whether enhanced TGF-β signaling in the fat body is a response to TGF-β family ligand(s) produced by the muscle, we performed quantitative PCR (qPCR) analysis to examine the expression of *Drosophila* TGF-β ligands in complex I-perturbed muscle. There are seven TGF-β ligand-encoding genes in the *Drosophila* genome: four members of the TGF-β/Activin subgroup, namely *Act*β, *dawdle* (*daw*), *maverick* (*mav*), and *myoglianin* (*myo*), and three members of the TGF-β/BMP subgroup, namely *decapentaplegic* (*dpp*), *glass bottom boat* (*gbb*), and *screw* (*scw*) (15). The ligands encoded by these genes bind to receptor complexes composed of type I and type II receptors. We found that whereas levels for most ligand-encoding genes were unaffected, mRNA levels for *Act*β and *daw* were dramatically increased in complex I-perturbed muscles (Fig. 3*A* and Fig. S3*B*). Note that *scw* was not included in the analysis because it is not expressed in larvae or adults. We next tested the putative roles of the ligands in muscle-to-fat-body communication by performing RNAi knockdown of each ligand in *dMef2 > ND-75-i* muscle. Surprisingly, only *Act*β knockdown dramatically alleviated TG accumulation in whole animals (Fig. 3 *B* and *C*), an event that correlates with mitochondrial perturbation in the muscle.

We next asked whether removal of *Act*β in complex I-perturbed muscle could alleviate mitochondrial dysfunction in the muscle or fat body. Interestingly, the ADP-induced O_2 consumption rate in isolated mitochondria from *dMef2 > ND-75-i+ Act*β*-i* larval fat bodies, but not muscles, was restored by *Act*β knockdown, compared with *dMef2 > ND-75-i+w-i* (Fig. 3*D*, *Left*). *dMef2 > ND-75-i+Act*β*-i* larvae also exhibited higher ATP production in the fat body, but not in muscles (Fig. 3*D*, *Right*). We also examined lipid metabolism and found that fewer neutral lipid droplets accumulated

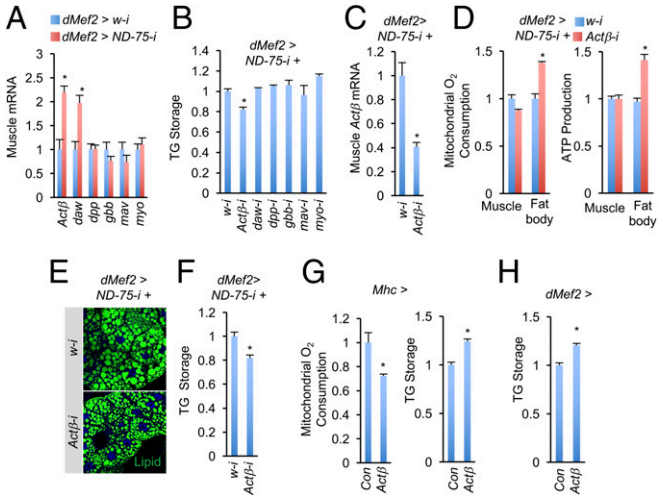

Fig. 3. Actβ mediates nonautonomous regulation of mitochondrial dysfunction between muscle and fat body. (*A*) Relative mRNA levels of TGF-β family ligands in third instar larval muscle ($n = 3$, 10 pooled tissues per replicate). Note that *scw* is not expressed in larva or adult. (*B*) Whole body TG level in third instar larvae. *UAS-RNAi* lines were crossed to *UAS-ND-75-i, tub-Gal80; dMef2-Gal4*, and the progenies were grown at 29 °C until third instar stage ($n = 3$, 5 pooled larvae per replicate). (*C–F*) Muscle *Act*β mRNA levels (*C*, $n = 3$, 10 pooled tissues per replicate), ADP-induced mitochondrial O_2 consumption rates (*D, Left*, $n = 3$, 20 pooled tissues per replicate) and ATP level (*D, Right*, $n = 3$, 5 pooled tissues per replicate), and staining of neutral lipids (*E*) and TG level in the fat body (*F*, $n = 3$, 5 pooled tissues per replicate) of *dMef2 > ND-75-i+ Actβ-i* third instar larvae. Genotypes are as in *B*. (*G*) Mitochondrial O_2 consumption rates (*Left*, $n = 3$, 20 pooled fat bodies per replicate) and TG levels (*Right*, $n = 3$, 5 pooled fat bodies per replicate) in the fat bodies of *Mhc > Con* (*Mhc-Gal4/+*) and *Mhc > Actβ* (*UAS-Actβ/+; Mhc-Gal4/+*) third instar larvae. (*H*) Fat body TG levels (*I*, $n = 3$, 5 pooled fat bodies per replicate) in *dMef2 > Con* (*dMef2-Gal4/+*) and *dMef2 > Actβ* (*UAS-Actβ/+; dMef2-Gal4/+*) third instar larvae. Data are presented as means ± SEM, $^*P < 0.05$.

and less TG is stored in the *dMef2 > ND-75-i+Actβ-i* fat body compared with *dMef2 > ND-75-i+w-i* (Fig. 3 *E* and *F*). Altogether, our results indicate that production of TGF-β ligand Actβ is required for complex I-perturbed muscle to remotely cause mitochondrial dysfunction and lipid imbalance in the fat body.

We further investigated whether increased Actβ expression in wild-type muscle is sufficient to impair mitochondrial function and lipid homeostasis in the fat body. We overexpressed Actβ in wild-type larval muscle by using *Mhc-Gal4*, a driver that induces exogenous expression in the late larval stage. Consistent with our expectation, forced expression of Actβ in the larval muscle significantly lowered ADP-induced O_2 consumption rate from isolated mitochondria and increased lipid storage in the fat body (Fig. 3*G*). Similar results were also obtained by using *dMef2-Gal4* (Fig. 3*H*). Taken together, our gain- and loss-of-function results demonstrate that Actβ is essential for muscle-to-fat-body communication in the context of mitochondrial complex I perturbation.

The ROS/NF-κB Cascade Autonomously Regulates Actβ Expression in Mitochondria-Perturbed Muscle. We next analyzed how Actβ is induced in mitochondria-perturbed muscle. Perturbation of the mitochondrial complex I has been shown to robustly increase intracellular production of ROS (reactive oxygen species) signaling molecules and to activate various downstream kinase/transcriptional factor signaling pathways (16–18). We have also shown that complex I perturbation in the larval muscle triggers ROS production and activates transcription factors like FoxO, JNK, and NF-κB/Rel (8). Thus, we hypothesized that mitochondrial perturbation might induce *Act*β expression in larval muscles via ROS production and tested the effect of decreasing ROS levels in complex I-perturbed muscles (8). Strikingly, overexpression of a ROS-eliminating enzyme PHGPx (8) significantly down-regulated Actβ levels in larval muscles and TG storage in the fat body (Fig. 4*A*), indicating that mitochondrial-stressed muscle induces Actβ expression via ROS generation.

Several signaling pathways are known to act downstream of ROS, including FoxO, JNK, and NF-κB/Rel (8). To explore which of these factors contribute to Actβ induction, we overexpressed the relevant kinases or transcriptional factors in wild-type larval muscles. Interestingly, whereas overexpression of *foxO* or *hep-CA*, an active form of Hep, failed to affect Actβ expression (Fig. 4*B*), overexpression of *Rel-68*, an active form of Rel, in wild-type muscle significantly elevated Actβ expression and remotely increased the level of TG storage in the fat body (Fig. 4*D*), suggesting that NF-κB/Rel signaling promotes Actβ production. We also confirmed activation of NF-κB/Rel signaling in complex I-perturbed muscle by using a Diptericin-GFP (Dpt-GFP) reporter (14, 19) (Fig. 4*C*). Further, we suppressed NF-κB/Rel signaling by knocking down *Rel* expression in complex I-perturbed muscles and found that both muscle *Act*β induction and fat body TG accumulation were at least partially alleviated (Fig. 4*A*). Collectively, these results strongly suggest that complex I perturbation autonomously activates a ROS-induced NF-κB signaling cascade that induces Actβ expression in the larval muscle.

TGF-β/Activin signaling is evolutionarily conserved. For example, activin A is the homolog of *Drosophila* Actβ and is an important regulator of lipid metabolism and mitochondrial activity (14, 20). To ask whether functional conservation extends to ROS-induced Activin production in mammals, we treated C2C12 myoblasts with rotenone, a well-established complex I inhibitor (21), which resulted in rapid and robust ROS elevation as indicated by CellROX, a dye that specifically detects ROS (Fig. S4*A*). In addition, rotenone also induced mRNA expression of *Inhba*, a gene that encodes a subunit of activin A, but not *Inhbb*, in myoblasts by about two folds (Fig. S4*B*, *Left*). Similar results were also obtained in differentiated C2C12 myotubes (Fig. S4*B*, *Right*). Because intracellular ROS has been extensively shown to activate NF-κB signaling in muscle cells (16), we wondered whether NF-κB signaling is required for rotenone-induced up-regulation of *Inhba*. To address this question, we blocked NF-κB signaling by adding Withaferin A, a natural NF-κB inhibitor (22), before rotenone treatment of C2C12 myoblasts and observed that the induction of *Inhba* in myoblasts was abolished (Fig. S4*B*, *Left*). Thus, our data indicate that complex I perturbation autonomously induces activin mRNA expression in mammalian muscle cells in a conserved manner.

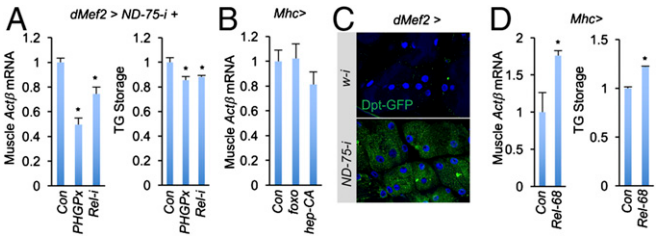

Fig. 4. ROS/NF-κB signaling regulates *Act*β expression in complex I-perturbed muscle. (*A*) Muscle *Act*β mRNA levels (*Left*, $n = 3$, 10 pooled tissues per replicate) and fat body TG levels (*Right*, $n = 3$, 5 pooled tissues per replicate) in indicated third instar larvae. Control or *UAS* lines were crossed to *UAS-ND-75-i, tub-Gal80; dMef2-Gal4*, and the progenies were grown at 29 °C until third instar stage. (*B*) Muscle *Act*β mRNA levels in *Mhc > foxo* (*UAS-foxo/+; Mhc-Gal4/+*) and *Mhc > hep* (*Mhc-Gal4/UAS-hep-CA*) larvae ($n = 3$, 10 pooled tissues per replicate). (*C*) Representative images of Relish reporter expression (*Dpt-GFP*) in larval muscle cells. (*D*) Muscle *Act*β mRNA levels (*Left*, $n = 3$, 10 pooled tissues per replicate) and fat body TG levels (*Right*, $n = 3$, 5 pooled tissues per replicate) in *Mhc > Rel-68* (*UAS-Rel-68/+; Mhc-Gal4/+*) third instar larvae. Data are presented as means ± SEM, $^*P < 0.05$.

The TGF-β Type I Receptor Baboon Regulates Mitochondrial Function and Lipid Homeostasis in the Fat Body. In *Drosophila,* TGF-β ligands bind to either of two type II receptors, Punt (Put) and Wishful thinking (Wit), and to one of three different type I receptors, to activate distinct downstream signaling events (15). For example, the TGF-β/Activin ligands Actβ and Daw target the type I receptor Baboon (Babo) and activate the downstream transcriptional factor dSmad2, whereas the TGF-β/BMP ligands Dpp, Gbb, and Scw target the type I receptors Saxophone (Sax) and Thickveins (Tkv) and activate Mad (15). To test whether Actβ-associated Babo signaling autonomously regulates lipid metabolism in the fat body, we knocked down fat body *babo* mRNA levels by using the *CG-Gal4* driver. Knockdown of *babo* robustly enhanced the ADP-induced O_2 consumption rate of isolated mitochondria and ATP production in the *CG > babo-i* fat bodies (Fig. 5 *A* and *B*). Conversely, activation of Babo signaling via overexpression in the fat body of *babo-CA*, an active form of Babo, or of *dSmad2*, significantly decreased ATP production (Fig. 5*B*). These data suggest that, consistent with muscle Actβ production, Babo signaling in the fat body autonomously regulates mitochondrial function. Finally, we examined lipid metabolism in the fat body. Decreasing Babo signaling by knocking down *babo* or *put* significantly reduced both lipid droplet mass and TG storage in the fat body, whereas activation of Babo signaling by overexpressing *babo-CA* or *dSmad2* increased both lipid droplet mass and TG storage in the fat body (Fig. 5 *C* and *D*). Taken together, our results indicate that Babo signaling in the fat body autonomously regulates mitochondrial function and lipid metabolism.

Discussion

Mitochondria, which are semiautonomous organelles essential for the cellular energy supply, have been shown to integrate metabolic signals and regulate systemic physiology, including aging and energy homeostasis, in both an autonomous and a nonautonomous manner (5, 6, 8). Using *Drosophila* as a model, we demonstrate that impairment of mitochondrial function via complex I perturbation specifically in the muscle remotely impairs mitochondrial function in another metabolic tissue, the fat body, and causes an obesity phenotype. Using RNA-seq and genetic validation, we further found that complex I-perturbed muscles produce the TGF-β ligand Actβ, which then targets the fat body, affecting mitochondrial function and lipid mobilization in that tissue. The results of our study suggest the possibility of synchronized regulation of mitochondrial activity in distinct organs or tissues.

Evidence that supports this idea of "mitochondrial synchrony" has been reported in mammals. For example, a few myokines are secreted by muscles following exercise to enhance mitochondrial activity in adipose tissue (5, 23). Whether impaired mitochondrial activity in muscles is associated with simultaneous decreased mitochondrial function in other tissues is largely unknown. Here, we characterized Actβ as a myokine involved in mitochondrial synchrony in a mitochondrial dysfunction model.

In addition to acting as a neuropeptide expressed in central or peripheral nerves (24, 25), Actβ also acts as an endocrine peptide derived from enteroendocrine cells to target the fat body via the Babo receptor (14). Actβ is expressed at low levels in wild-type muscle (14); however, we observed a significant induction of Actβ expression when complex I was perturbed in muscle cells, suggesting that mitochondrial injury can turn on Actβ expression in the muscle to subsequently regulate fat body function. Actβ/Babo signaling has been shown to enhance the response to Akh in the larval fat body (14). Consistent with this finding, our RNA-seq data revealed that *AkhR* expression levels are increased by approximately two folds in the fat body of *dMef2 > ND-75* larvae (Dataset S2), an event associated with elevated Actβ production in larval muscles. Akh has been shown to regulate lipid mobilization in the fat body (26). Thus, our observation that Actβ/Babo signaling causes lipid accumulation suggests that Actβ/Babo signaling might impair mitochondria-associated lipid mobilization to overcome the lipolytic effect of Akh. In support of this idea, there is evidence from mammals that activin/TGF-β impairs mitochondrial function and lipid homeostasis via multiple mechanisms (27).

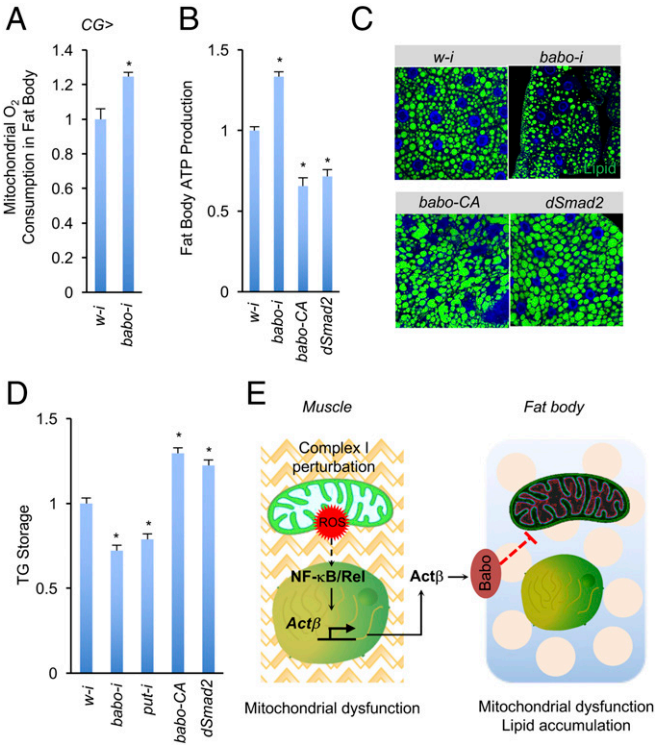

Fig. 5. Babo signaling regulates mitochondrial activity in the fat body. Mitochondrial O_2 consumption rates (n = 3, 20 pooled fat bodies per replicate) (*A*), ATP levels (n = 3, 5 pooled fat bodies per replicate) (*B*), staining of neutral lipids (*C*), and TG levels (*D*) (n = 3, 5 pooled fat bodies per replicate) in the fat body *CG > w-i* (*UAS-w-i/+*, *CG-Gal4/+*), *CG > babo-i* (*UAS-babo-i/+*, *CG-Gal4/+*), *CG > put-i* (*UAS-put-i/+*, *CG-Gal4/+*), *CG > babo-CA* (*CG-Gal4/UAS-babo-CA*), or *CG > dSmad2* (*CG-Gal4/UAS-dSmad2*) third instar larvae. (*E*) Muscle-to-fat-body communication in regulation of mitochondrial synchrony. Complex I-perturbed larval muscle increases Actβ production via ROS/NF-κB cascade. Muscle-derived Actβ further triggers Babo signaling in the fat body to decrease its mitochondrial activity and increase TG storage. Data are presented as means ± SEM, $*P < 0.05$.

In addition to Actβ, another activin ligand Daw also targets Babo/dSmad2 and regulates systemic carbohydrate metabolism and the aging process, probably via modulation of dILP2 secretion (28, 29). Although Daw is expressed in the muscle, we do not believe that Daw is involved in mitochondrial synchrony between muscle and fat body because Daw knockdown in complex I-perturbed muscles fails to restore normal levels of TG storage. However, Daw is expressed in other tissues beyond muscle, and we cannot rule out the possibility that Daw acts as a secondary hormone to activate Babo signaling in the fat body.

The results of our studies also uncovered that in both fly and mouse muscle cells, complex I disruption increases ROS generation, activating NF-κB/Rel and inducing Activin/Actβ expression. These findings are further supported by the previous observation that NF-κB activity is required to up-regulate *Inhba*/*Activin* in human myoblasts (30). *Inhba* encodes a β-subunit (β_A) of disulfide-linked dimeric inhibin/activin, including inhibin A ($\alpha\beta_A$), activin A ($\beta_A\beta_A$), and activin AB ($\beta_A\beta_B$). Inhibin moderately inhibits activin signaling via receptor-binding competition (31). Compared with relatively less-understood activin AB, activin A has been well established as a metabolic regulator of lipid homeostasis and mitochondrial activity. Importantly, levels of activin

A, which promote proliferation of human adipocyte progenitors, are significantly elevated in obese patients compared with lean subjects (32, 33). Genetic disruption of *Inhba*, achieved by replacing the mature domain of *Inhba* with that of *Inhbb*, results in significantly diminished activin A production and enhanced mitochondrial function in mouse adipose tissues, leading to lipid loss (20). Because Actβ/Activin exhibits conserved features in metabolic regulation in both flies and mammals (14, 34), our characterization of a mitochondria-ROS-Rel-Actβ axis in *Drosophila* should help further investigation of nonautonomous mitochondrial regulation between muscle and adipose tissues in mammalian animal models.

Experimental Procedures

***Drosophila* Strains and Cell Culture.** RNAi and overexpression fly stocks were obtained from the Transgenic RNAi Project (TRiP), National Institute of Genetics at Japan (NIG), and Bloomington Stock Center. See *SI Experimental Procedures* for more details.

Mitochondrial O_2 Consumption and ATP Measurement. Mitochondria Isolation Kit for Tissue (Abcam; ab110168), Extracellular Oxygen Consumption Reagent (Abcam, ab197242), and ATP Determination Kit (Thermo Fisher, A22066) were used. See *SI Experimental Procedures* for more details.

Quantitative Lipidomic Analysis and Triglyceride Measurement. Lipids from third instar larval fat bodies were extracted and submitted for spectrophotometric analysis at the Beth Israel Deaconess Medical Center (BIDMC) Mass Spectrometry Facility (www.bidmcmassspec.org). TG measurement was performed as described (35, 36). See *SI Experimental Procedures* for more details.

RNA-seq Transcriptome Analysis, Bioinformatics Analysis, and qPCR. Total RNA from larval fat body was extracted by using TriZol reagent and used for RNA-seq analysis at the Columbia Genome Center as described (37). RNA-seq data were deposited in the Gene Expression Omnibus (GEO, GSE100214). Gene expression indicated by qPCR was normalized to internal control *RpL32* (*Drosophila*) or *Actin-β* (*Actb,* mouse). See *SI Experimental Procedures* for more details.

Statistical Analyses. Data are presented as the mean ± SEM. Student's *t* test was used to compare two groups. EASE Score (38) was used for evaluating the gene enrichment. $P < 0.05$ was considered statistically significant.

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