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Gut bacteria mediated the lipid delivery of an invasive bark beetle via hypoxia-induced factor 1α

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Dear Editor,

Symbiotic microbes play crucial roles in animal biology, contributing significantly to the invasion success and outbreaks of the red turpentine beetle (RTB, *Dendroctonus valens*) in China.[1,2] While the previous work revealed that gut bacteria promote RTB larval growth via Hif-signaling-mediated glucose transport,[3] the molecular mechanism of gut microbial regulation of other essential nutrients, particularly lipids, remains to be fully elucidated. Similar to observations in mosquitoes,[4,5] we found that gut bacteria are indispensable for lipid accumulation in the RTB fat body. Specifically, axenic larvae exhibited significant reductions in triacylglycerol (TAG) content and weight gain, both of which were rescued by the re-introduction of live gut bacteria.

In this study, we found that two key genes in lipid uptake in insects, lipophorin (*Lpp*) and its receptor (*LppR*) are required for the delivery of lipids from the gut to fat body, and *Lpp* and *LppR* are the downstream genes of Hif-signaling in RTB. Moreover, Hif-1α binds directly to the hypoxia response elements (HREs) in *Lpp* and *LppR* promoters to drive their expressions, inducing lipid accumulation in the fat body of RTB larvae, which finally supports larval growth. Findings in this study extend the previous study[3] and present direct evidence supporting Hif-1α regulation on lipid delivery in RTB. Notably, this regulatory logic appears to extend beyond RTB. Our analysis in mosquitoes reveals that Hif-1α similarly activates *Lpp* expression through direct promoter binding. This finding provides the missing mechanistic link in previous mosquito studies[4,5] and suggests that the Hif-1α-mediated coordination of glucose and lipid metabolism may be a shared feature across diverse insect-microbe interactions. Taken together, all of these results elucidate the molecular mechanism by how gut bacteria regulates RTB larval development enriching our knowledge of insect-microbe interactions and providing novel targets for the management of pest species.

By using antibiotic treatment, we generated the axenic (AX) RTB larvae and observed that AX RTB larvae gained less weight (Figure 1A) and accumulated significantly less lipid in fat body than conventional (CN) RTB larvae (Figure 1B). The triacylglycerol content (TAG) in CN RTB larvae fat body was also significantly higher than AX RTB larvae (Figure 1C). Moreover, our bacteria re-introduction experiments showed that all live gut bacteria could rescue the block of lipid transportation from gut to fat body in AX RTB larvae (Figures 1D-E). These findings are consistent with earlier study showing that mosquito gut bacteria affect the lipid transport from gut to fat body.[5] Building on previous findings that gut bacteria influence glucose transport, these results suggest that gut bacteria not only regulate the glucose uptake but also facilitate lipid transport from gut to fat body thereby promoting the RTB larval growth.

In previous studies, lipophorin (*Lpp*), which serves as the major neutral lipid carrier, and its receptor (*LppR*) are critical for lipid uptake by fat body in insects.[6] Quantitative PCR (qPCR) revealed significantly lower transcript levels of *Lpp* and *LppR* in AX RTB larvae compared to CN RTB larvae (Figure 1F). Our previous study has demonstrated that RTB gut bacteria induce a local hypoxic environment (O_2 dropping from 10% to 5%).[3] Further investigations revealed that gut bacteria induced hypoxic environment is essential for the gut mediated promotion on RTB larvae via Hif-signaling pathway.[3] Hypoxia-inducible factor-1α (Hif-1α) is the key hypoxia regulatory protein regulating this process.[3] Here inhibition of Hif-1α expression in CN larvae using PX-478, an Hif-1α inhibitor, significantly reduced the expression of *Lpp* and *LppR* (Figure 1G). Conversely, treatment of AX RTB larvae with FG-4592, which is a prolyl-4-hydroxylation domain inhibitor and upregulates Hif-1α expression, significantly increased *Lpp* and *LppR* expression (Figure 1G). Consistent with the qPCR results, PX-478 treatment decreased the lipid content in the fat bodies of CN larvae, whereas FG-4592 induced lipid accumulation of AX larvae (Figures 1H-I), pattern consistent with findings in mosquitoes.[5] Knockdown of *Lpp* and *LppR* via gene-specific dsRNA injections significantly reduced lipid content in the fat bodies of RTB larvae (Figures 1J-L). Additionally, gene silencing of *Lpp* and *LppR* resulted in a significant reduction in larval weight gain (Figure 1M). Thus, these results demonstrated that *Lpp* and *LppR* are required for the delivery of lipids from the gut to fat body, and *Lpp* and *LppR* are the downstream genes of Hif-signaling in RTB. Moreover, the fat body synthesizes and secretes *Lpp* into the hemolymph. While its receptor (*LppR*) presents at the plasma membrane of various organs and facilitates lipid absorption in the gut. *Lpp* delivers lipids to various organs via the hemolymph as a reusable shuttle. Collectively, these findings reveal that gut bacteria regulate lipid flow from intestinal absorption to fat body storage by modulating the Hif signaling pathway and its downstream effectors, *Lpp* and *LppR*. Beyond direct energy deficiency, elimination of gut bacteria may also impair the synthesis of essential hormones. Sterols represent a class of non-hydrolyzable lipids and are converted by insects into a wide array of metabolites, including ecdysteroids, which require cholesterol precursors and control insect development and reproduction. Therefore, the growth retardation caused by silencing *Lpp* and *LppR* may result not only from energy deficiency but also from the inhibition of ecdysteroid biosynthesis.

To investigate the regulation mechanism of Hif-1α on the expression of *Lpp* and *LppR*, we analyzed the promoter sequence of *Lpp* and *LppR* revealing the presence of putative hypoxia response elements (HREs) within the promoter region of *Lpp* and *LppR* (Figure 1N). Yeast one-hybrid assays demonstrated that Hif-1α binds directly to these promoters of *Lpp* and *LppR* (Figure 1O). This finding further verified by electrophoretic mobility shift assays (EMSA) showing Hif-1α interaction with the HREs of *Lpp* and *LppR* (Figure 1P). Dual luciferase assays (LUC) further indicated that Hif-1α activates the expression of *Lpp* and *LppR* (Figures 1Q-R). Taken together, these results establish that Hif-1α binds directly to HREs in *Lpp* and *LppR* promoters to drive their expressions, inducing lipid accumulation in the fat body of RTB larvae, which finally supports larval growth.

The role of Hif-signaling in lipid storage in the fat bodies is conserved in other insects, including mosquito larvae.[5] Treatment with PX-478, a Hif-1α inhibitor, reduced *Lpp* expression in gnotobiotic (GN) mosquito larvae colonized with wild-type *Escherichia coli*, that typically supports larval development. Conversely, FG-4592, which enhances Hif-1α activity, increased *Lpp* expression in GN larvae with Δ*cydB*-Δ*cydD*::*kan E. coli*, unable to rescue larval growth.[3,5] Promoter sequence analysis of mosquito *Lpp* also showed a presence of HRE (Figure 1S). Subsequently EMSA and LUC assays indicated that mosquito Hif-1α also activates the expression of *Lpp* by directly binding to HRE in *Lpp* promoter (Figures 1T-U). Collectively, our results suggested that gut bacteria regulate lipid storage within fat bodies of mosquito larvae by inducing Hif-1α, which directly activated *Lpp* expression. Additionally, the activation of Hif-1α integrates glycolytic and lipid anabolic pathways, contributing to altered metabolism in pathologic cardiac hypertrophy.[7] Findings of the regulatory role of Hif-1α on both glucose and lipid metabolism in RTB indicate that this function might be broadly conserved across animal

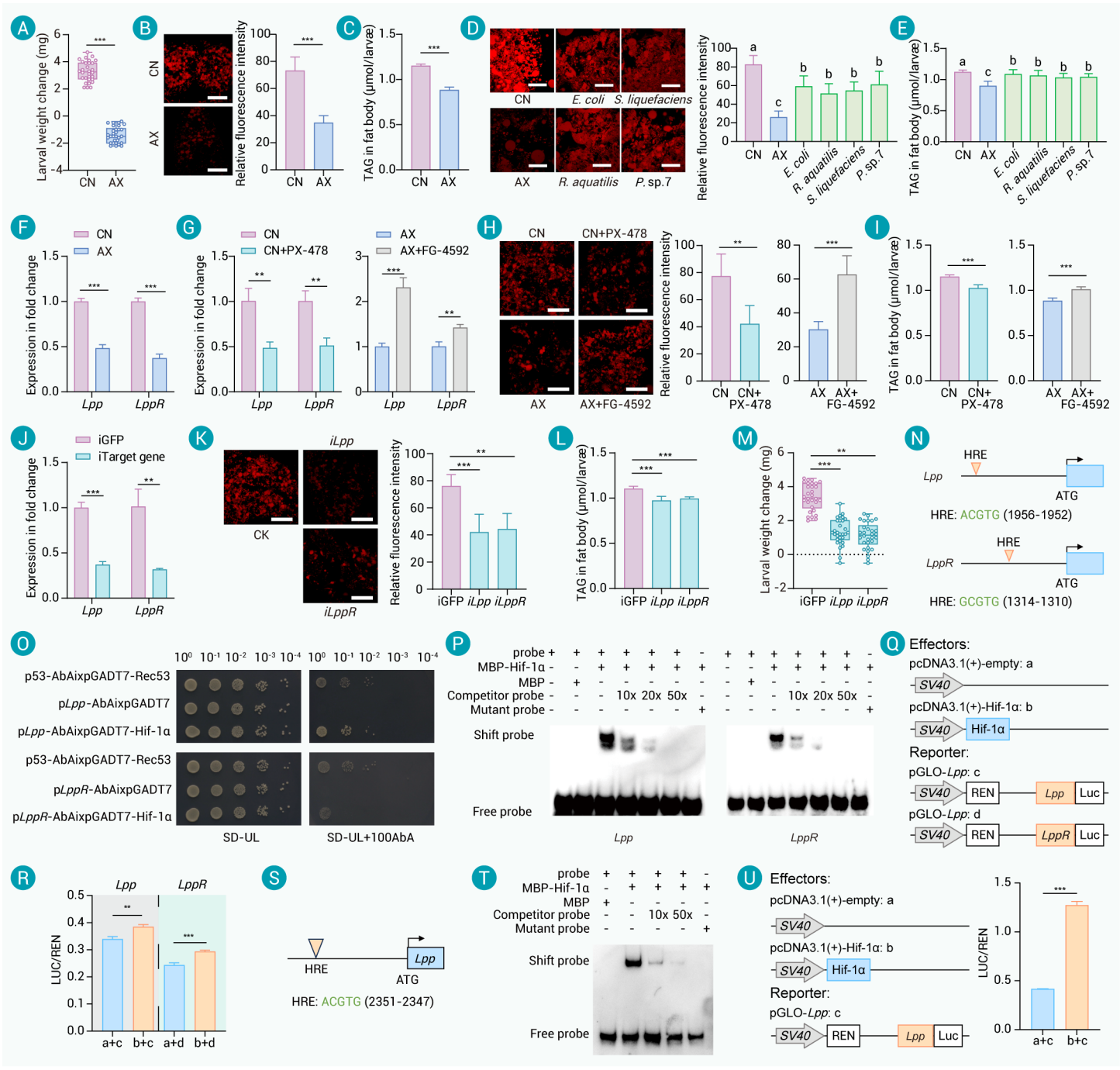

Figure 1. Gut bacteria–induced Hif-1α directly activates *Lpp*/*LppR* to promote lipid transport and storage in the fat body of RTB larvae (A) Impact of gut bacteria elimination on the weight change of RTB larvae ($n \geq 30$ individuals, Student's t test). (B) Lipid drop change in the fat body of RTB larvae using Nile red staining ($n = 5$, Student's t test). (C) Triacylglycerol (TAG) content in the fat body of RTB larvae ($n = 5$, Student's t test). Influence of bacteria re-introduction on the lipid drop change (D) and TAG content (E) in the fat body of RTB larvae. *E. coli*: *Escherichia coli*, *R. aquatilis*: *Rahnella aquatilis*, *S. liquefaciens*: *Serratia liquefaciens*, *P.* sp.7: *Pseudomonas* sp.7. Different letters in (D) and (E) mean significant difference ($n = 5$, ANOVA, $P < 0.05$). (F) Expression of *Lpp* and *LppR* in CN and AX RTB larvae ($n = 3$, Student's t test). (G) Expression of *Lpp* and *LppR* in fat bodies from CN RTB larvae, CN RTB larvae with PX-478, AX RTB larvae and AX RTB larvae with FG-4592 ($n = 3$, Student's t test). Lipid drop change (H) and TAG content (I) in the fat body of CN RTB larvae, CN RTB larvae with PX-478, AX RTB larvae and AX RTB larvae with FG-4592 ($n = 5$, Student's t test). (J) RNA interference efficiency of *Lpp* and *LppR* after injection of specific dsRNA ($n = 3$, Student's t test). Influence of silencing *Lpp* and *LppR* on the lipid drop (K) and TAG content (L) in fat body ($n = 5$, Student's t test), and larval weight change (M) of CN RTB larvae (Student's t test, $n = 30$). Mean ± SE, ** $P < 0.01$ and *** $P < 0.001$. (N) Presence of HREs in the promoter region of *Lpp* and *LppR*. (O) Y1H of Hif-1α and the promoter of *Lpp* and *LppR*. (P) EMSA of Hif-1α and HREs from *Lpp* and *LppR* promoter regions. (Q) Schematic diagram of constructs used in dual luciferase assay. (R) Dual luciferase reporter assays in HEK294T cells showing the activation of Hif-1α on the expression of *Lpp* and *LppR* ($n = 3$, Student's t test, Mean ± SE, ** $P < 0.01$ and *** $P < 0.001$). (S) Presence of HRE in the promoter region of *Lpp* (AAEL009955). (T) EMSA of mosquito Hif-1α and HREs from *Lpp* promoter region. (U) Dual luciferase reporter assays showing the activation of mosquito Hif-1α on the expression of *Lpp* ($n = 3$, Student's t test, Mean ± SE, *** $P < 0.001$).

systems.[3] Hif-1α represents a potential target not only for therapeutic drug development but also for novel pesticides. Moreover, multiple signaling pathways have been found to regulate Hif-1α activity.[8] PI3K-mTOR signaling upregulates the Hif-1α expression in mammalian cells.[9] Similarly, an activation of PI3K/Akt is mediated by gut bacteria induced Hif-signaling in mosquito.[5] Thus, we speculated that PI3K-mTOR signaling may regulate Hif-1α expression to coordinate glucose and lipid metabolism in insects and other animals. Future study about how PI3K-mTOR could also interact with gut microbes induced hypoxia and eventually co-regulate the expression of Hif-1α is required.

The gut microbiota performs many beneficial functions for the host.[10] In this study, we found that gut bacteria-induced Hif-signaling is critical for

larval growth in RTB and mosquitoes. Hif-1α directly regulates glucose and lipid transport genes expression by binding to HREs within their promoters.[3] All of these results elucidate the molecular mechanism by how gut bacteria regulates RTB larval development enriching our knowledge of insect-microbe interactions and providing novel targets for the management of pest species.

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AUTHOR CONTRIBUTIONS

Study conception and design, F.L. and J.S.; supervision, F.L. and J.S.; investigation and methodology, F.L., F.Y., Z.K., G.L., H.K., and D.L.; data collection and curation, F.Y., G.L., D.L., and F.L.; formal analysis, F.L., Z.K., and Y.W.; data interpretation and visualization, F.L. and F.Y.; drafting the manuscript, F.L., Z.K., and F.Y.; critical revision of the manuscript, F.L., F.Y., Z.K., Y.W., L.K., and J.S.; funding acquisition, F.L. All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

ETHICAL STATEMENT AND PATIENT CONSENT

Not Applicable.

DATA AND CODE AVAILABILITY

Data are available from the corresponding author upon reasonable request.