



The role of commensal microbes on the effect of metformin in *Drosophila melanogaster*

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Abstract

Metformin has been commonly used in the treatment of type 2 diabetes because it has many beneficial effects such as good safety profile, efficacy, and comparatively reduced cost. Nevertheless, treatment with metformin is often associated with gastrointestinal side-effects and reduced quality of life of patients. Some researches demonstrated that metformin causes the imbalance of intestinal fluid homeostasis. Since it is well known that the alteration of intestinal microbes is related to gastrointestinal dysfunction, we hypothesized that the gastrointestinal side-effects of metformin may be related with the change of commensal microbial flora in the gut. To prove our hypothesis, we used the fruit fly, *Drosophila melanogaster*, which is one of the excellent model organisms in the host-microbe study. Similar to the results in human subjects, the incidence ratio of fly with an intestinal barrier dysfunction was increased under 25 mM of metformin. Since it is known that the fly with intestinal barrier dysfunction has a shorten lifespan, we measured the lifespan of fly fed the metformin and observed that supplementation of metformin decreased the lifespan of flies. To test whether the effect of metformin on the intestinal barrier and lifespan of fly was related with the commensal microbe, we investigated the lifespan of axenic fly and the microbial flora alteration in fly with supplementation of metformin. Interestingly, the reduced lifespan of fly by supplementation of metformin slightly recovered by removal of commensal microbes, indicating that the presence of commensal microbes is involved in the effect of metformin on fly lifespan. Also, supplementation of the 25 mM metformin increased the total microbial load in a fly, especially *Lactobacillus*, one of the main phyla in the fly's gut. In the case of *Acetobacter*, another main phylum in the fly's gut, the number of bacteria was rather decreased by supplementation of 25 mM metformin. To investigate whether that *Lactobacillus* performs a key role in the side-effects of metformin on lifespan, we measured the lifespan of axenic fly inoculated with *Acetobacter* or *Lactobacillus* under the supplementation of metformin. Interestingly, the median lifespan of fly inoculated with *Lactobacillus* showed a similar trend to that of conventionally reared fly, indicating that *Lactobacillus* is related with the side-effects by metformin, but not that of fly inoculated with *Acetobacter*. Our results will provide the fundamental knowledge to address the side-effects of taking metformin.

Key words: Metformin, commensal microbe, lifespan, intestinal barrier dysfunction, host-microbe interaction, *Drosophila melanogaster*

Result

1. Metformin increases intestinal barrier dysfunction of *D. melanogaster*

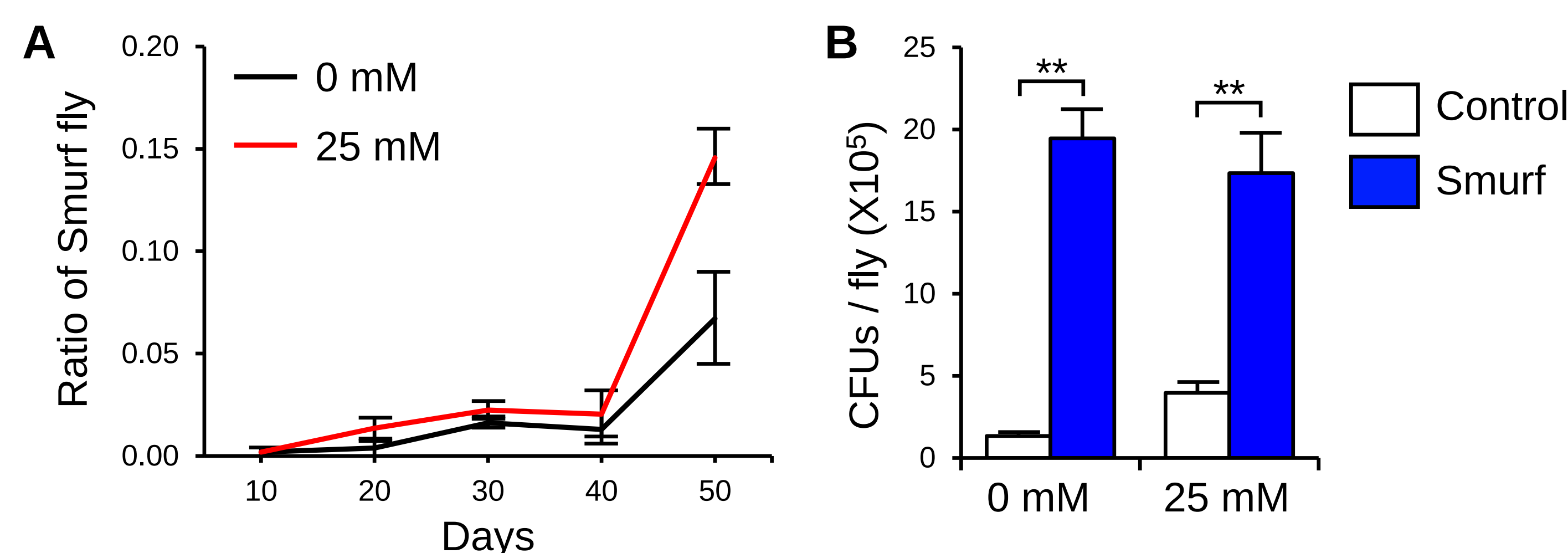


Fig. 1 The incidence ratio and microbial load of fly with an intestinal barrier dysfunction (A) The incidence ratio of the fly with intestinal barrier dysfunction on supplementation of 25 mM metformin. (B) The number of bacteria colony forming unit (CFU) of Smurf fly. Asterisks indicate significant differences between groups (*t*-test, ***p* < 0.001). Error bars represent the SEM.

2. The effect of metformin on the lifespan of fly is related with commensal microbes

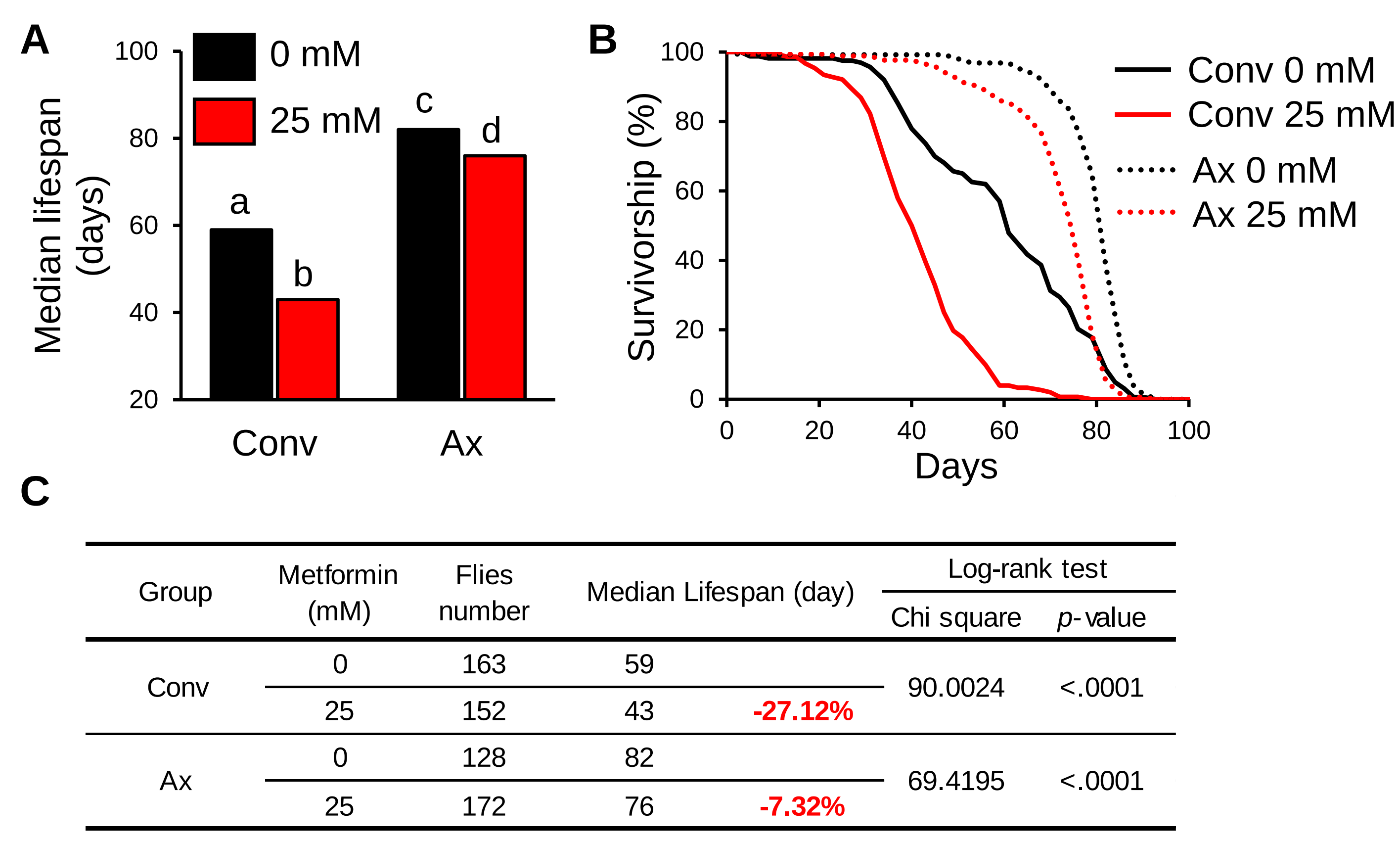


Fig. 2 The survival of axenic fly fed metformin (A) Median lifespan of conventionally reared fly (Conv) or axenic fly (Ax) fed 25 mM metformin. Different letters indicate significant differences between groups (ANOVA, Tukey's HSD post-hoc analysis). (B-C) Survival of Conv and Ax fly fed 25 mM metformin.

3. Metformin increases the microbial load in fly, especially *Lactobacillus*

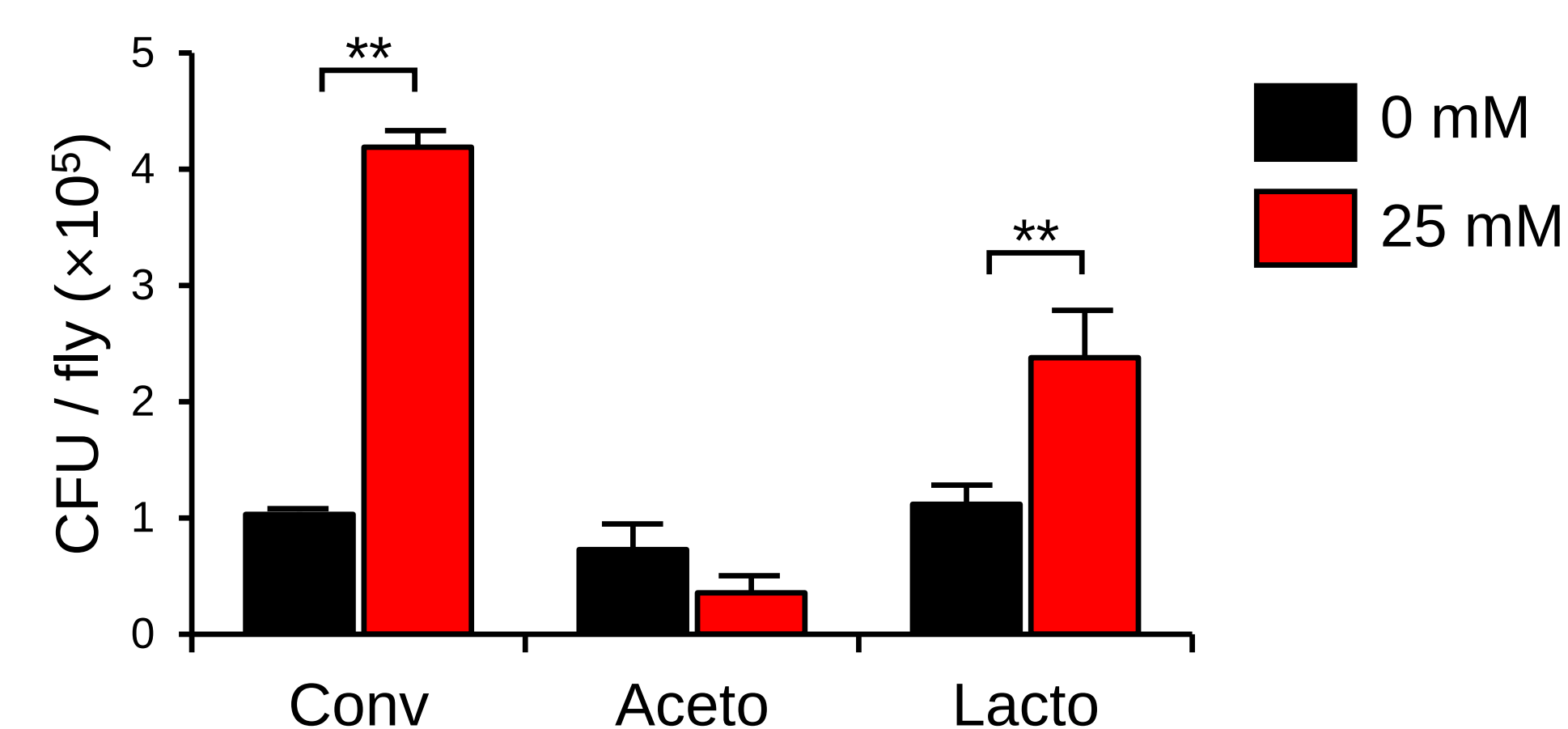


Fig. 3 Changes of bacterial load by supplementation of metformin The number of bacteria colony forming unit (CFU) of Conv fly or the fly inoculated with *Acetobacter* (Aceto) or *Lactobacillus* (Lacto). Asterisks indicate significant differences between groups (*t*-test, ***p* < 0.001). Error bars represent the SEM.

4. Monoassociation of *Lactobacillus* increases metformin susceptibility of axenic fly

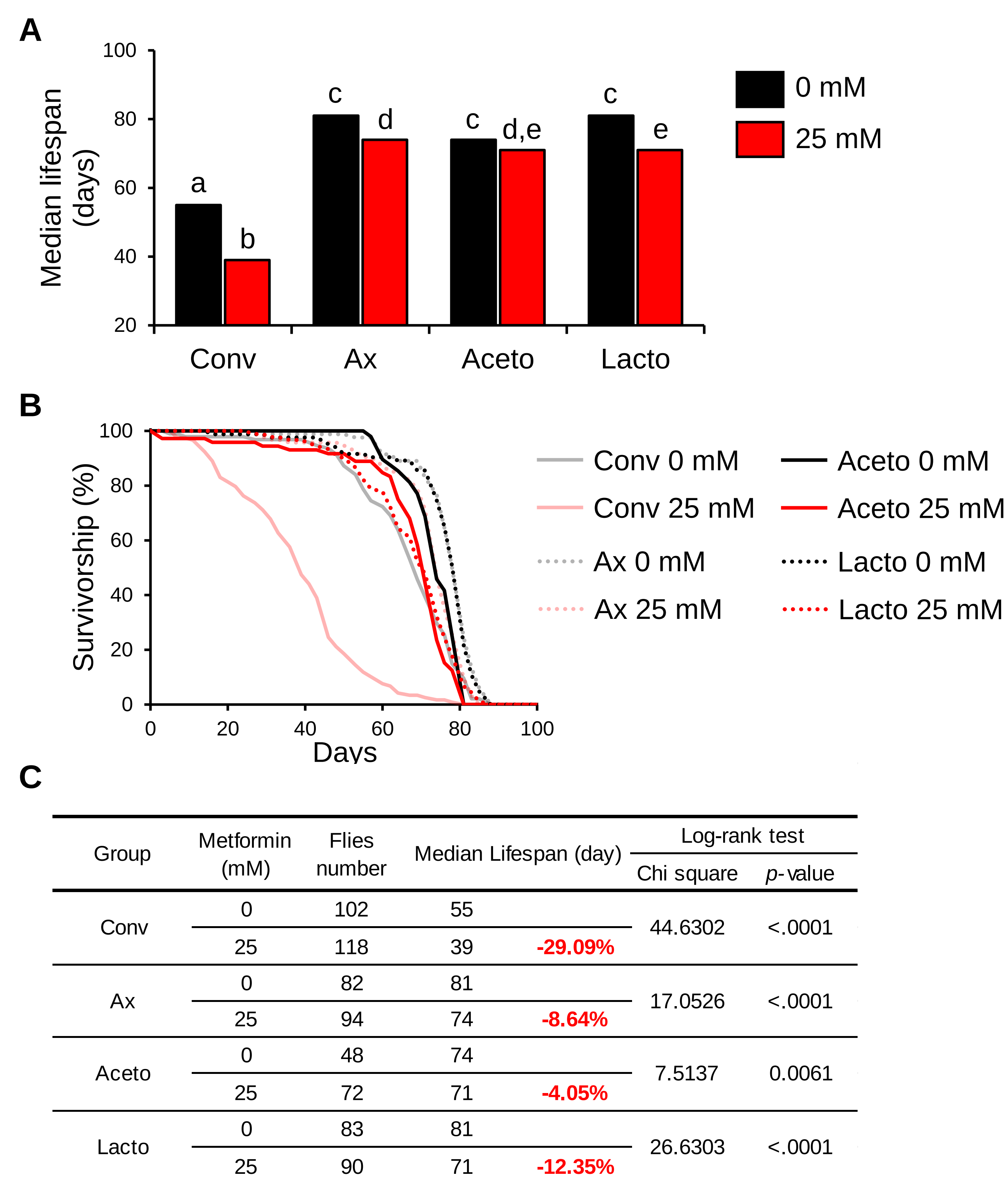


Fig. 4 The lifespan of fly inoculated with specific microbes (A) Median lifespan of axenic fly inoculated with *Acetobacter* (Aceto) or *Lactobacillus* (Lacto) under the supplementation of metformin. Different letters indicate significant differences between groups (ANOVA, Tukey's HSD post-hoc analysis). (B-C) Survival of gnotobiotic fly fed 25 mM metformin.

Conclusion

1. Metformin induces intestinal barrier dysfunction and lifespan reduction of fly
2. Lifespan reduction by supplementation of metformin is related with commensal microbes
3. *Lactobacillus* was increased by metformin supplementation and related with effect of metformin on fly lifespan
4. *Lactobacillus* may performs a key role in the side-effects by metformin.