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LETTER TO THE EDITOR

Succinate: A microbial product that modulates *Drosophila* nutritional physiology

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Dear Editor,

Gut microorganisms process food in animal guts and release many metabolic by-products, which are predicted to influence host physiological processes such as energy and lipid metabolism. Here, we investigate how succinate, a TCA cycle intermediate that is a major predicted release product of gut bacteria in *Drosophila*, influences the nutritional physiology of its *Drosophila* host. We administered succinate as a dietary supplement to microbe-free *Drosophila*, and quantified key nutritional indices. Dietary succinate significantly reduced fly lipid levels by up to ∼50%. This response was not replicated in parallel experiments conducted with dietary fumarate supplement, indicating that it could not be attributed to a general effect of TCA intermediates. We hypothesize that microbe-derived succinate may contribute to the reduced lipid content of *Drosophila* bearing gut bacteria, relative to axenic *Drosophila*. More generally, this study highlights the importance of microbial-derived metabolites as regulators of host metabolism.

The focus of this study, succinate, is predicted to be released at substantial rates by several of the key bacterial members of the *Drosophila* gut microbiota (Ankrah *et al*., 2021). Succinate is a TCA cycle intermediate and it has been implicated as a modulator of host metabolism in mammalian systems. Specifically, microbial-derived metabolites from the TCA cycle play important roles in regulating host glucose homeostasis (De Vadder *et al*., 2016; Connors *et al*., 2019; Fernández-Veledo & Vendrell, 2019) and are correlated with the onset and progression of metabolic diseases such as obesity (Serena *et al*., 2018; Wang *et al*., 2019). To investigate the possible role of succinate in the nutritional physiology of *Drosophila*, we supplemented axenic (microbe-free) *Drosophila* diet with different concentrations of succinate and monitored changes to fly weight, triacylglyceride (TAG), glucose, and protein levels. (The basis for the succinate concentrations selected for this study is provided in Supplementary methods).

Drosophila fed on diet supplemented with succinate over 4 d displayed significantly reduced TAG levels in both female and male flies, with the TAG content halved on average in flies fed on diet containing 0.3 mol/L succinate relative to the succinate-free diet (Fig. 1A, B). Further analyses revealed that the effect of succinate was specific to TAG, and not mirrored by any significant effect on the glucose or protein contents of the flies, or on fly weight (Fig. S1). In addition, we observed a minimal negative effect of the dietary succinate supplement on survival of male flies and no effect on female flies over the 4-d experiment after Bonferroni correction for multiple comparisons (Fig. S1, Table S1).

To check whether the effect of succinate on fly lipid content was general to dicarboxylic acids we then analyzed flies fed on diets supplemented with fumarate, the product of succinate oxidation via the TCA enzyme succinate dehydrogenase and a metabolite that is not predicted to be released from *Drosophila* gut microorganisms (Ankrah *et al*., 2021). In contrast to flies fed on succinate, flies fed on fumarate displayed no statistically significant reductions in TAG levels compared to flies on the fumarate-free controls (Fig. 1C, D). The sole significant effect of fumarate-supplemented diets on the flies was significantly reduced glucose levels in male flies, specifically ∼41% decrease in average glucose levels in flies fed on 0.3 mol/L fumarate compared to male flies fed on the control diet (Fig. S1). Similar to succinate-supplemented diets we observed no significant changes in glucose levels for female flies (Fig. S1). The

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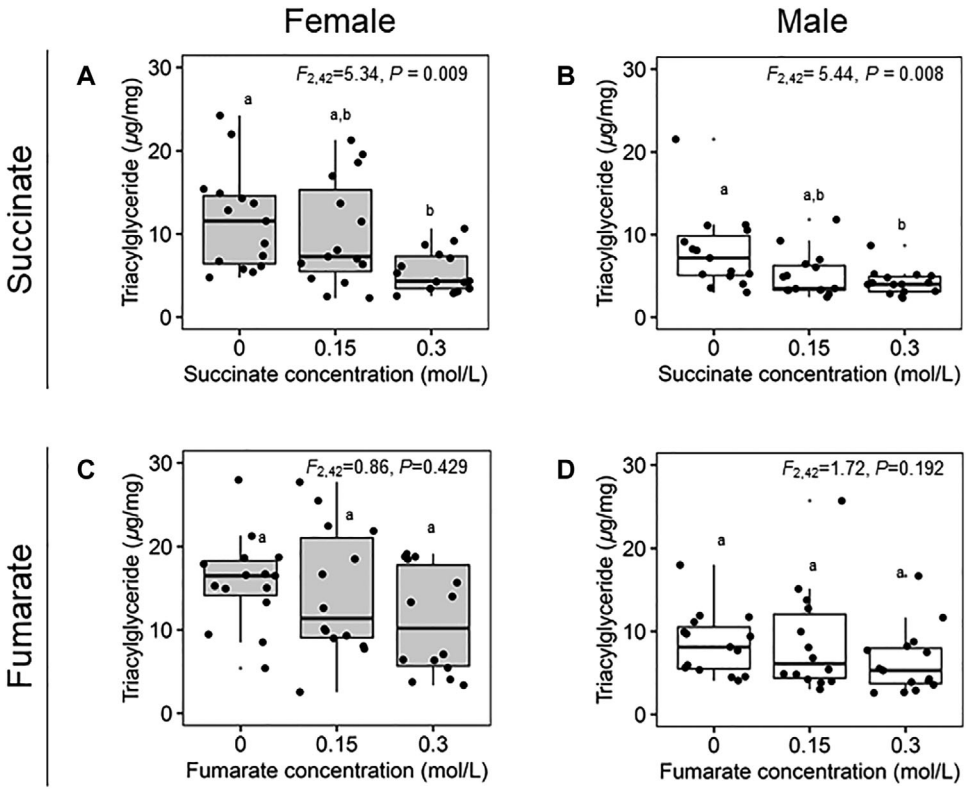

Fig. 1 Effect of succinate (A, B) and fumarate (C, D) on *Drosophila* triacylglyceride levels. Significantly different ($P < 0.025$) samples by Tukey's Honestly Significant Difference (HSD) *post hoc* test are indicated by different letters. For each boxplot, the center line displays the median, and the lower and upper hinges correspond to the 25th and 75th percentiles; all data points are shown. Female and male flies are represented by gray and white boxes respectively.

protein contents, fly weight and survival (Fig. S1, Table S1) remained unchanged in both female and male flies feeding on fumarate or the fumarate-free controls.

As part of these experiments, we quantified food intake by the flies. Food consumption did not vary significantly with diet composition for either female or male flies (Fig. S2), indicating that the lower TAG content of flies on diets containing succinate could not be explained by an antifeedant effect of the dietary supplement. Additional statistical analysis performed to quantify variation between experimental replicates indicated that, apart from glucose content, the variation across experimental replicates was not statistically significant for both metabolites (Fig. S3).

Previous studies have demonstrated that the lipid content of *Drosophila* is significantly elevated by elimination of the gut microbiota (Shin *et al*., 2011; Huang & Douglas, 2015; Sommer & Newell, 2019). The capacity of certain individual bacterial taxa and bacterial communities, particularly of *Acetobacter* and *Lactobacillus* species, to lower fly lipid content has been attributed to two processes (which are not mutually exclusive): bacterial competition for dietary sugars, which are the principal source of stored lipid in *Drosophila* (Chaston *et al*., 2014; Huang & Douglas, 2015), and bacterial production of acetic acid, which modulates insulin signaling and reduces nutrient allocation patterns to lipid (Shin *et al*., 2011; McMullen *et al*., 2020). In this study, we demonstrate that succinate, predicted from metabolic models to be released at high rates from *Drosophila* gut bacteria, also functions to reduce *Drosophila* TAG content. The effect of succinate on fly lipid is specific in two ways: (i) other nutritional indices were not significantly affected by dietary succinate; and (ii) fumarate, a metabolically related TCA cycle intermediate, did not reduce fly lipid content. The effect of succinate on *Drosophila* lipid content is reminiscent of its effect on brown adipose tissue in mammals, where oxidation of accumulated succinate by the enzyme succinate dehydrogenase initiates production of reactive oxygen species which induces adipocyte damage and promotes lipolysis resulting in reduced lipid levels (Ayala *et al*., 2014; Mills *et al*., 2018).

Nevertheless, the global effect of succinate supplement on *Drosophila* is different from mammals, where increases in succinate levels have been associated with weight gain and obesity (Serena *et al*., 2018; Keiran *et al*., 2019; Wan *et al*., 2020). This effect has been attributed to the metabolic response of white adipose tissue to succinate, mediated by succinate binding to its cognate receptor succinate receptor 1 (SUCNR1/GPR91) to activate downstream signaling pathways that inhibit lipolysis (McCreath *et al*., 2015). Although the fat body of insects is, in some respects, functionally similar to mammalian white adipose tissue (Canavoso *et al*., 2001; Trinh & Boulianne, 2013), our data raise the possibility that the *Drosophila* fat body and mammalian adipose respond differently to succinate.

A priority for future research is to investigate the molecular basis of succinate transport across the gut epithelium and the effect of succinate metabolism on lipid synthesis and mobilization in the fat body. Identifying the molecular players such as transporters and receptor proteins involved in succinate assimilation and signaling will aid in understanding the mechanism by which succinate affects lipid homeostasis in insects. Altogether, our data reinforce the growing evidence that specific metabolites produced by gut microbiota play important roles in modulating host metabolism, and they specifically point to a physiological role for succinate in regulating insect lipid metabolism.

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Disclosure

The authors declare no competing interests.

Data availability

The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Author Contributions

FQZ, AED and NYDA designed the study. FQZ and JGM conducted experiments. FQZ and NYDA performed data analysis. FQZ wrote the first draft of the manuscript and revisions were made by all authors.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Effect of succinate (A) and fumarate (B) on *Drosophila* nutritional indices and survival.

Fig. S2. *Drosophila* food intake on succinate (A,B) and fumarate (C,D) supplemented diets.

Fig. S3. Among-trial variation in nutritional indices for (A) female and (B) male flies fed on control diet without metabolite added.

Table S1. Discrete survival analysis summary statistics for each metabolite administered to each sex.

Supplementary methods