

***Drosophila melanogaster* with TPI Deficiency Do Not Exhibit Infection Susceptibility**

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ABSTRACT

Humans with glycolytic deficiencies, such as those caused by mutations in Triose Phosphate Isomerase (TPI), exhibit numerous symptoms including progressive neurodegeneration and infection susceptibility. *Drosophila melanogaster* with a mutant allele of TPI, called *TPI^{sugarkill}*, have been used as model for the study of TPI deficiency. *TPI^{sugarkill}* flies exhibit the progressive neurodegeneration and early death that is observed in human patients. Flies utilize a similar innate immune system pathway to humans to defend against pathogens but lack an adaptive immune system. Therefore, this study examined *TPI^{sugarkill}* flies to determine if they exhibit susceptibility to infection. It was found that *TPI^{sugarkill}* flies do not appear to be infection susceptible suggesting that the infection susceptibility observed in human patients is due to a malfunction in the adaptive, but not innate immune system in TPI Deficiency patients.

Keywords: Glycolytic Enzymopathy; Innate Immune System; Triose Phosphate Isomerase

Triose phosphate isomerase (TPI) is a glycolytic enzyme responsible for the interconversion of dihydroxyacetone phosphate (DHAP) and glycerol-3-phosphate. This step is critical to efficient energy production from glucose metabolism. Several mutations in TPI have been shown to cause a disorder called TPI deficiency in humans. Patients with TPI deficiency exhibit progressive neurodegeneration, susceptibility to infection, haemolytic anemia and premature death (Orosz, Olah, & Ovadi, 2009). While the exact mechanism by which mutations in TPI result in susceptibility to infection is unclear, gaining an understanding of the pathology of this symptom may result in development of future therapies to treat the infection susceptibility observed in TPI deficient patients. Much of the work on TPI deficiency has been conducted in model organisms such as *Drosophila* and yeast and has focused primarily on the enzymatic function of the mutant TPI proteins and the

cellular pathways disrupted (Roland et al., 2015; Roland et al., 2013; Seigle, Celotto, & Palladino, 2008). To date, no studies have looked at infection susceptibility in TPI deficiency model systems.

Drosophila melanogaster with a M80T transition in the TPI protein have been used as a model for TPI deficiency. This mutant is known as *TPI^{sugarkill}* (Celotto, Frank, Seigle, & Palladino, 2006). Homozygous *TPI^{sugarkill}* flies exhibit similar phenotypes to humans with TPI deficiency such as reduced lifespan and neurodegeneration. However, it has not been determined if *TPI^{sugarkill}* flies exhibit the infection susceptibility observed in humans with the disorder. TPI is a highly conserved enzyme and the high degree of structural similarity observed in the crystal structure of TPI from various organisms indicates that data collected from flies will be applicable to humans (Celotto et al., 2006). In this study homozygous mutant flies carrying *TPI^{sugarkill}* and flies homozygous for the wild type TPI

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allele were examined to determine if *TPI^{sugarkill}* flies model this characteristic of the human disease.

Previously published studies in *Drosophila* have identified genes involved in the immune response to bacterial infection. While flies do not exhibit an adaptive immune system, they do utilize an innate immune system pathway to recognize and defend against pathogens. Using the innate immune system, flies are able to produce anti-microbial peptides that activate transcriptional responses to mediate an infection response. This signaling system is conserved in higher eukaryotes, including humans. However flies lack the white blood cells and antibody production from the adaptive immune system observed in humans and other mammals (Vidal et al., 2001). The infection pathway can be challenged by simple inoculation of the gut with a needle that has been dipped in a bacterial culture. In this experiment wild type and *TPI^{sugarkill}* flies were infected with gram positive bacteria (*Staphalococcus aureus*) or gram negative bacteria (*Escherichia coli* or *Pseudomonas aeruginosa*). *E. coli* is a rod shaped gram negative bacterium that should not be overly pathogenic to flies with properly functioning innate immune systems (Tinsley, Blanford, & Jiggins, 2006). *Staphylococcus* is a round cluster gram positive bacterium, it should have greater effect on the viability of flies with functioning immune systems than *E. coli* but should still not cause significant mortality to the population (Tinsley et al., 2006). However, when the innate immune system function is compromised both *E. coli* and *S. aureus* induce significant mortality in the population (Vidal et al., 2001). *Pseudomonas aeruginosa* is a coccobacillus gram negative bacterium that should cause significant mortality in flies (Christofi & Apidianakis, 2013). As humans with TPI deficiency exhibit infection susceptibility, it is hypothesized that *TPI^{sugarkill}* flies may also exhibit infection susceptibility (Orosz et al., 2009). It is expected that *TPI^{sugarkill}* flies should die at a higher rate when challenged with bacteria, but wild type flies should be able to fight off the bacteria with their innate immune system and exhibit a lower level of mortality.

METHODS

Fly Strains and Media

A homozygous stock of *TPI^{sugarkill}* flies was used for the study. Canton S was utilized as the wild type control. Flies were raised using the Bloomington cooked fly food recipe prepped from the pre-mixed powder from Genesee Scientific. For all studies, flies were reared at 25°C.

Infection Protocol and Analysis

Flies were collected at Day 3-7 after eclosion and challenged with bacteria using inoculation from a specimen needle. Bacterial cultures were grown in luria broth to log phase as determined by spectrophotometer (absorbance at 595nm). A specimen needle was flame sterilized and dipped into the culture. This needle was inserted into the abdomen of the fly that was anesthetized with carbon dioxide. Flies were then placed in vials of fresh flood and incubated at 25°C. The percent death was counted every 12 hours for 3 days. A stainless steel insect pin (Indigo Instruments, 33416-2) dipped in sterile phosphate buffered saline (PBS, BioRad) was used as a negative control to assess death from the wounding process. This control was selected based on previously published infection studies in *Drosophila* (Tinsley et al., 2006). Furthermore, the pricking method has been shown to trigger anti-microbial peptide expression to a lesser extent than the injection method with negative controls (such as PBS). Thus it the needle pricking method was selected for this study (Lemaitre, Reichhart, & Hoffmann, 1997). All bacterial strains were tested on isolated sets of males or females as differences in resistance to infection have been observed between the sexes (Vincent & Sharp, 2014). The percent death at the 72 hour timepoint was assessed for significance compared to the wild type control by a student's T test. N = 100 for each condition tested.

RESULTS

TPI^{sugarkill} flies were examined for an infection susceptible phenotype compared to a wild type control. As flies were wounded in the abdomen to introduce a bacterial load, a

sterile PBS control was also included to determine the percentage of death due to wounding. The first bacterial strain tested was *P. aeruginosa*, a bacterial strain that should induce death even in a wild type fly. As expected, wild type flies challenged with *P. aeruginosa* exhibited 45% and 58% mortality in the population 72 hours following infection in males and females, respectively (Figure 1). The mortality of wild type flies when challenged with PBS (11% and 19% mortality 72 hours post-wounding in wild type males and females respectively) is significantly less than the mortality observed when challenged with *P. aeruginosa* ($p < 0.001$). This suggests the infection protocol is successful at introducing pathogens to the gut of the flies as has been previously reported (Christofi & Apidianakis, 2013). *TPI^{sugarkill}* flies tested with *P. aeruginosa* displayed percent death rates of 41% (males) and 68% (females) (Figure 1). This was not significantly different than the percent death in the wild type flies challenged with *P. aeruginosa* by t-Test ($p > 0.05$). Furthermore, this is significantly different than the PBS control that resulted in 11% and 17% death in male and female *TPI^{sugarkill}* flies respectively ($p < 0.005$). Again, this suggests that the death observed is due to exposure to active *P. aeruginosa* and not merely wounding.

Next, susceptibility to infection by *S. aureus* was examined. This strain was expected to be less lethal to the wild type control. However if the *TPI^{sugarkill}* flies exhibit infection susceptibility, significant death would be observed following bacterial challenge. As expected, the wild type flies were relatively adept at fighting off a *S. aureus* infection with 20% (males) and 14% (females) death observed 72 hours after bacterial challenge (Figure 2). Furthermore, *TPI^{sugarkill}* flies exhibited 33% and 25% mortality 72 hours following *S. aureus* infection in males and females respectively. T-test analysis indicated that there was no significance between wild type and *TPI^{sugarkill}* infection response to *S. aureus* ($p > 0.05$).

Finally, susceptibility to infection with a Gram-negative bacterium, *E. coli*, was assessed. No susceptibility of infection to *E. coli* was observed comparing the wild type control to the *TPI^{sugarkill}* flies ($p > 0.05$) (Figure

3). Similar levels of mortality at the 72 hour mark, 25% and 24%, were observed in male *TPI^{sugarkill}* and wild type flies respectively. Females resulted in similar levels of death with 23% and 16% mortality observed in *TPI^{sugarkill}* and wild type flies.

It should be noted that there was no significant difference in the mortality ($p > 0.05$) between PBS treatment and *S. aureus* or *E. coli* in the experiment for both wild type and *TPI^{sugarkill}* of both sexes. This is not completely unexpected as a minimal level of mortality is observed in flies that have functioning immune systems (Tinsley et al., 2006). Furthermore, sex does not appear to influence infection susceptibility in the wild type and *TPI^{sugarkill}* strains, $p > 0.05$ when the mortality rates of both sexes challenged by *P. aeruginosa*, *E. coli* or *S. aureus* were compared at the 72 hour time point in each genotype. Overall, the studies suggest that *TPI^{sugarkill}* flies are able to fight off infection by both gram positive and negative bacteria at levels comparable to wild type flies.

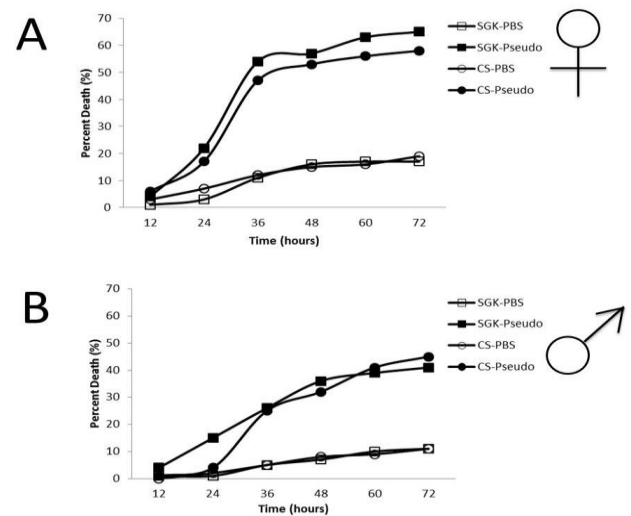


Figure 1. *TPI^{sugarkill}* homozygotes are not more susceptible to infection by *P. aeruginosa* than wild type flies. During each trial Canton S wild type (CS) or *TPI^{sugarkill}* (SGK) flies were poked with a needle treated with sterile PBS or *P. aeruginosa* (Pseudo). The treatment and genotype are indicated in the key above. For example, SGK-PBS indicates the data set represent *TPI^{sugarkill}* flies treated with PBS. The graphs above represent the percent death for the population recorded for each 12 hour interval for a 72 hour period for female flies tested with *P. aeruginosa* (A), and male flies tested with *P. aeruginosa* (B). Each line of the graphs represent an N=100. There was no

difference between CS and SGK flies infection response as determined by t-test analysis ($P>0.05$). However, there was a significant difference between flies treated with PBS and flies treated with *P. aeruginosa* for both CS and SGK flies as determined by t-test analysis ($P<0.05$). No difference in infection susceptibility of the different sexes of the same genotype was observed ($P>0.05$) for both CS and SGK.

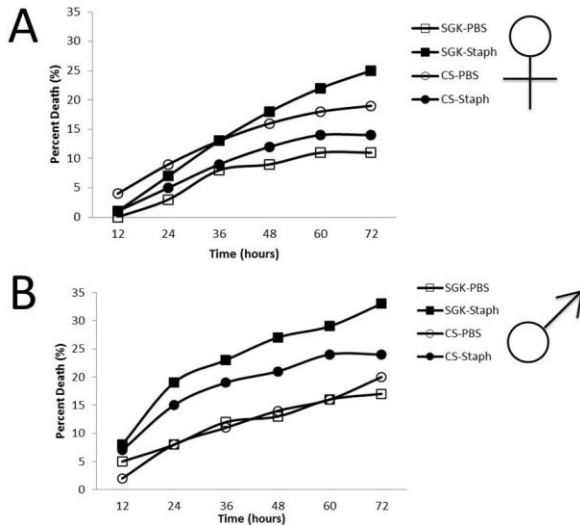


Figure 2. $TPI^{sugarkill}$ homozygotes are not more susceptible to infection by *S. aureus* than WT flies. During each trial Canton S wild type (CS) or $TPI^{sugarkill}$ (SGK) flies were poked with a needle treated with sterile PBS (control) or *S. aureus* (Staph). The treatment and genotype are indicated in the key above. For example, SGK-PBS indicates the data set represent $TPI^{sugarkill}$ flies treated with PBS. The graphs above represent the percent death for the population recorded for each 12 hour interval for a 72 hour period for female flies tested with *S. aureus* (A), and male flies tested with *S. aureus* (B). Each line of the graphs represent an N=100. There was no significant difference between CS and SGK flies infection response as determined by t-test analysis ($P>0.05$). No significant difference in infection susceptibility of the different sexes of the same genotype was observed ($P>0.05$) for both CS and SGK.

DISCUSSION

Patients with TPI deficiency exhibit several symptoms that contribute to the early death of the patient including progressive neurodegeneration and infection susceptibility. The mechanism by which a mutation in TPI, a glycolytic enzyme, results in infection susceptibility in humans is not understood. As

these patients are rare, clinical studies are difficult (Orosz et al., 2009). However, a *Drosophila melanogaster* model for TPI deficiency has been established. This fly line, called $TPI^{sugarkill}$, has a missense mutation that changes a methionine to a threonine at position 80 in the TPI amino acid sequence. Homozygous $TPI^{sugarkill}$ flies exhibit the progressive neurodegeneration and shortened lifespan observed in human patients with TPI deficiency (Celotto et al., 2006). This study examined if the $TPI^{sugarkill}$ flies also exhibit the infection susceptibility observed in human patients with TPI deficiency. When challenged with three different bacterial pathogens, *P. aeruginosa*, *S. aureus*, and *E. coli*, $TPI^{sugarkill}$ flies did not exhibit significantly different mortality when compared to a wild type control. Furthermore, flies with marked infection susceptibility would be expected to exhibit a large percentage of death when challenged by bacterial infection with *E. coli* and *S. aureus* (>60%) (Buchon, Silverman, & Cherry, 2014; Christofi & Apidianakis, 2013; Tinsley et al., 2006; Unckless, Rottschaefer, & Lazzaro, 2015). $TPI^{sugarkill}$ flies did not exhibit greater mortality than wild type flies indicating that $TPI^{sugarkill}$ flies are not susceptible to infection.

In human patients both the innate and adaptive immune responses are utilized to fight an infection. However, *Drosophila*, the model organism used in this study, relies only on the innate immune system to fight off pathogens (Buchon et al., 2014). Therefore if infection susceptibility was observed in $TPI^{sugarkill}$ flies then the innate immune system could be examined for deficiencies. As no significant difference in response to bacterial challenge was observed in $TPI^{sugarkill}$ flies it can be inferred that the infection susceptibility could be the result of deficiencies in the adaptive immune response in humans, not from a poor innate immune response. Therefore *Drosophila* is not an ideal model organism for the study of this aspect of the symptoms observed in patients with TPI deficiency.

Future work should focus on a model with an immune system more similar to humans. TPI null mice that lack a functional TPI enzyme were non-viable and thus are not a course of study. However, a TPI mutant

mouse with a substitution (A149G) in mice resulted in a viable animal that exhibit haemolytic anemia, another symptom observed in humans (Pretsch, 2009). The blood from these mice exhibited ~57% the TPI activity observed in the control mice. It would be interesting to determine if these mice also exhibit susceptibility to infection. Then future work could probe what aspect of the adaptive immune system is deficient in patients to better develop therapies for these individuals.

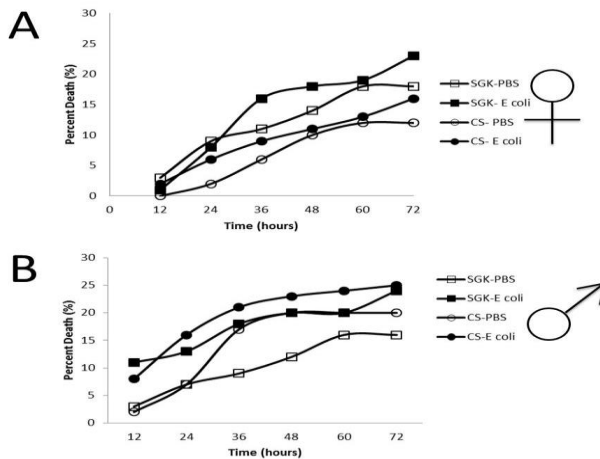


Figure 3. *TPI^{sugarkill}* homozygotes are not more susceptible to infection by *E. coli* than WT flies. During each trial Canton S wild type (CS) or *TPI^{sugarkill}* (SGK) flies were poked with a needle treated with sterile PBS (control) or *E. coli*. The treatment and genotype are indicated in the key above. For example, SGK-PBS indicates the data set represent *TPI^{sugarkill}* flies treated with PBS. The graphs above represent the percent death for the population recorded for each 12 hour interval for a 72 hour period for female flies tested with *E. coli* (A), and male flies tested with *E. coli* (B). Each line of the graphs represent an N=100. There was no significant difference between CS and SGK flies infection response as determined by t-test analysis ($P>0.05$). No significant difference in infection susceptibility of the different sexes of the same genotype was observed ($P>0.05$) for both CS and SGK.

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