# Muscle-specific expression of *tafazzin* or *spargel* (PGC-1α) restore exercise tolerance in a *Drosophila* model of Barth syndrome

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## Abstract

Barth syndrome is a mitochondrial disorder caused by mutations in the gene *tafazzin*. Mutations in *tafazzin (TAZ)* result in dysregulation of cardiolipin remodeling and ultimately lead to mitochondrial dysfunction. Mitochondrial dysfunction in Barth patients often causes severe exercise intolerance that substantially impacts quality of life. In fact, exercise intolerance is cited as one of the most prominent challenges Barth patients face when trying to live a fulfilling life. These challenges may be further exacerbated by the effects of a sedentary lifestyle. This study seeks to use knowledge gained from exercise training studies in animal models to identify pharmacological and genetic targets that could increase the exercise tolerance of Barth patients.

We used a lab-generated *Drosophila* endurance training machine to establish that *Drosophila* (*TAZ*) mutants have exercise intolerance reminiscent of Barth patients. We report the efficacy of overexpressing full-length *tafazzin* or *spargel* (PGC-1α homolog) to restore the exercise capacity of *TAZ* mutants. We first show that expression of wild-type *TAZ* in muscle alone is enough to rescue exercise capacity of *TAZ* mutants, indicating that muscle is the key therapeutic target to restore exercise ability. We further show that overexpressing *spargel* (PGC-1α homolog) specifically in muscle tissue is sufficient to rescue the exercise capacity of *TAZ* mutants. Our results indicate that *spargel* is an important modifier of exercise intolerance in *TAZ* mutants and suggest that PGC-1α could be targeted for future therapies.

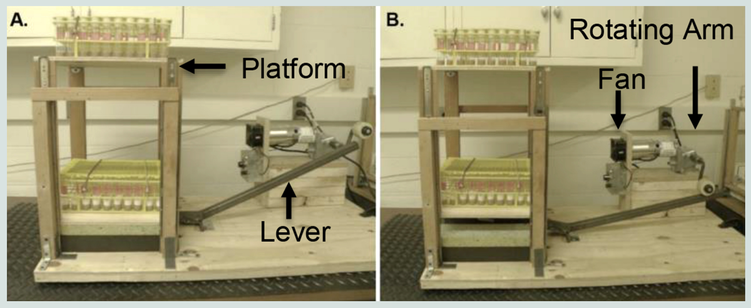
## Methods

CRISPR-CAS9 was used to delete 889 base pairs of the longest *tafazzin* transcript (*TAZ*$^{889}$), with W$^{1118}$ being the control background. All knockdown or overexpression experiments were achieved through a drug-inducible RNAi system, using a ubiquitous driver (*TUB5-GS*) and a muscle specific driver (*MHC-GS*). Flies were fed the drug for three days prior to any testing and were fed drug food throughout exercise training. Flies were reared on a 10% sugar/yeast diet and kept at 25 degrees Celsius.

## References

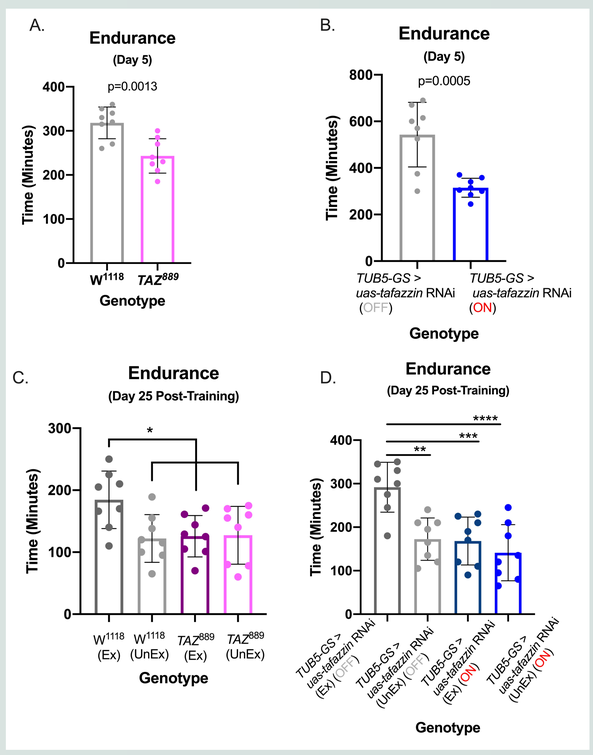
1.Damschroder, D., Cobb, T., Sujkowski, A., & Wessells, R. (2018). Drosophila Endurance Training and Assessment of Its Effects on Systemic Adaptations. *Bio-Protocol,8*(9). doi:10.21769/BioProtoc.3037

**Figure 1: The Power Tower is a treadmill for *Drosophila*.**



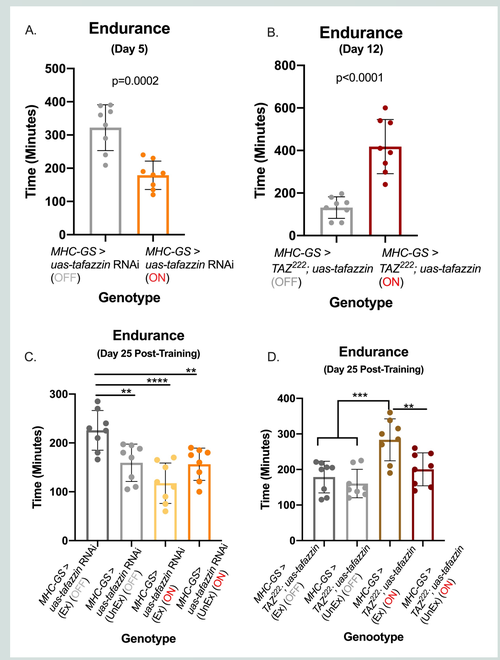

*Drosophila* have a natural instinct to climb upwards when knocked down to the bottom of their vial. The Power Tower stimulates flies to climb repeatedly by periodically dropping the platform of vials [1]. Using the Power Tower we can either measure endurance or chronically exercise train large cohorts of flies[1]. Endurance is measured by placing 8 vials of flies (n=20 per vial) on the Power Tower and having flies run until fatigue. A vial is defined as fatigued when 80% of the flies within that vial stopped climbing 1/2 cm upwards in the vial[1] Once fatigued, the time is recorded and the vial is removed. For training purposes, flies were trained once a day (Monday-Friday) for three weeks, with the time on the machine increasing by a half hour per week[1]. After training, wild-type flies adapt with increased endurance[1].

**Figure 2: Reduced *tafazzin* function causes exercise intolerance and an inability to adapt to chronic exercise training.**



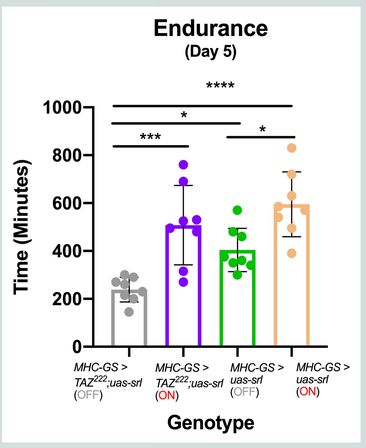

(A&B) Either *TAZ*$^{889}$ or a full-body knockdown of *TAZ* (*TUB5-GS*> *uas-tafazzin* RNAi ON) significantly decreases endurance (unpaired t-test, p=0.0013 and p=0.0005 respectively) to a similar extent. (C-D) Both *TAZ*$^{889}$ and full-body knockdown of *TAZ* flies failed to adapt to chronic exercise training, while W$^{1118}$ flies and control flies adapted with increased endurance (Post hoc Tukey test, p=0.02 and p=0.0013). These results indicate that full-body knockdown of *TAZ* mimics the exercise phenotypes of a genomic *TAZ* mutant.

**Figure 3: *tafazzin* in muscle tissue is required for normal exercise capacity and the ability to adapt to chronic exercise training.**




(A) Loss of *TAZ* in the muscle (*MHC-GS> uas-tafazzin* RNAi ON) significantly reduces endurance (t-test, p= 0.0002). This reduction in exercise capacity is similar to what was observed in the full-body knockdown of *TAZ.* (C) Similar to the full-body knockdown of *TAZ*, exercised muscle-specific knockdown flies failed to adapt to chronic exercise with increased endurance, while control exercised flies ran significantly longer than the other groups (Post hoc Tukey test, p= 0.0092, p<0.0001, p=0.0062). Therefore, muscle-specific *TAZ* is required for normal exercise capacity. (B) Muscle-specific *TAZ* rescue can restore exercise capacity of *TAZ* mutants by age day 12 (t-test, p<0.0001). (D) *TAZ* mutants (*MHC-GS> TAZ*$^{222}$*; uas-tafazzin* OFF) do not adapt to chronic exercise training with increased endurance, while muscle-specific *TAZ* rescue flies adapted with increased endurance (Post hoc Tukey test, p=0.0091). These results indicate that muscle tissue is a key therapeutic target for restoring exercise capacity in *TAZ* mutants.

**Figure 4: : Muscle specific rescue with *spargel* (PGC-1α homolog) restores exercise capacity of *tafazzin* mutants.**




After three days of drug feeding, muscle specific expression of spargel (*srl)* (MHC-GS> *TAZ*$^{222}$*; uas-srl* ON) rescued the exercise capacity of *tafazzin* mutants (Post hoc Tukey test, p=0.0005). Endurance was increased to the point there was no significant differences between control flies (*MHC-GS*> *uas-srl* OFF) and *srl* overexpressing flies (*MHC-GS*> *uas-srl* ON). These results confirm that muscle is a key therapeutic target for restoration of exercise capacity in *TAZ* mutants and that pharmaceutical activators of spargel could be a possible therapy for Barth syndrome.