# Investigating differences in phenotype among 9G8, RSF1, and TRN KD flies and suspected antagonism for TRN SR transport shuttle in lipid storage

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The method of gene regulation underlying lipid storage related to obesity is poorly understood, yet alternative splicing (AS) appears to be an important mechanism for proper lipid storage. CPT1 (carnitine palmitoyltransferase 1) is a beta-oxidation enzyme involved in the breakdown of fatty acids. The gene coding for CPT1 is alternatively spliced by the SR protein 9G8 to produce two different products that vary in their activity. My project was to investigate if there was a difference in the AS phenotype among flies with decreased expression of 9G8, the SR protein antagonist RSF1, and the SR protein transporter TRN. First, a quantitative PCR (qPCR) protocol needed to be developed to detect the isoforms of CPT1. cDNA was generated from wild type flies that were fed (0 hrs), starved (65 hrs) or refed to optimize the likelihood that both isoforms would be produced. Following a published protocol, a qPCR procedure was carried out that skipped the elongation step (73℃) and instead cycled from the standard annealing temperature (60℃) to the denaturation step (95 ℃). Additionally, the PCR plasticware resulted in technical difficulties and required troubleshooting. Even though there were difficulties, the qPCR reactions saw good results. The preps were good quality confirmed by qPCR by having rp49 gene come in at 15-17 cycles. The individual isoforms 6A and 6B were both detected at distinct cycle numbers showing that the isoform ratios change depending on which gene KD is occurring and would directly affect lipid storage and contribute to fat reserves. Future directions include running triglyceride (TG) assays to determine if there is a change in TG levels among the 9G8, RSF1, and TRN knockdown flies and then investigate if their splicing patterns changed as well.

## Biography

Ben Borokhovsky is a current MD candidate student at Cooper Medical School of Rowan University. He completed his undergraduate education at Widener University and graduated Summa Cum Laude with a degree in Biochemistry. He did research throughout his entire undergraduate education and has consistently been asked to speak at Widener's Research Symposium and has even given a keynote presentation on his senior thesis, for which he won a first place award. During his time at Widener University, Ben's research involved looking at gene regulation of specific SR proteins and the roles they play in lipid metabolism using an alternative splicing model in D. *melanogaster* to better understand molecular and genetic links to obesity in humans.

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