Title: Sex-specific regulation of the insulin pathway.

Background. Biological sex is a key determinant of body size, adiposity and metabolism. In humans, sex also affects health: men and women differ in the development of diseases such as cardiovascular disease, obesity, and type 2 diabetes. While sex hormones contribute to these differences, recent studies in mammals show biological sex also affects body size independently of sex hormones. Yet the molecular mechanisms underlying sex hormone-independent effects of sex on body size are unclear, as sex is often overlooked as a variable in biomedical research.

Rationale & Hypothesis. The lab discovered that the conserved insulin pathway mediates sex differences in body size in fruit fly larvae. It was found that female larvae, which are larger, have higher levels of insulin signaling than males. Since the insulin pathway augments body size in all animals, including humans, our findings suggest a model of growth in which increased female body size is caused by high levels of insulin signaling. In support of this model, the team's unpublished data shows that females have higher mRNA levels of insulin pathway genes that activate insulin signaling, such as the insulin receptor (InR), and lower levels of negative regulators of the pathway, such as PTEN.

The **goal** is to determine how sex differences in the regulation of insulin pathway genes affects insulin signaling and body size using the *Drosophila* larva as a model. Larvae are an ideal model for these studies, as they lack sex hormones, are non-reproductive, and males and females have identical body composition and food intake. This offers a pared-down system to uncover the molecular mechanisms underlying the effects of biological sex on body size. **It is hypothesized that the sex-specific regulation of insulin pathway genes stimulates insulin signaling in females to increase body size.**

Experimental Approach. To test the hypothesis two approaches will be used: first, it will be determined whether forcing 'female' regulation of insulin pathway genes in males will augment insulin signaling and body size. For example, since females normally have higher levels of InR, InR will be overexpressed in males using GAL4/UAS system, which allows precise spatial and temporal control over gene expression. To determine whether overexpression of InR in males affects insulin signaling and body size, levels of P-Akt will be measured as a read-out for insulin signaling, and pupal volume to determine body size. It is predicted that if increased expression of InR in females promotes insulin signaling and a larger body size, then males overexpressing InR will have higher levels of P-Akt, and achieve a larger pupal volume than control males. Second, it will be determined whether forcing male regulation of insulin pathway genes in females will block their larger body size. For example, PTEN will be overexpressed in females and P-Akt and pupal volume will be measured. If increased expression of PTEN in males normally inhibits insulin signaling and growth, we expect that females overexpressing PTEN will have lower levels of insulin signaling and a smaller body size.

Significance. Studies in flies have provided vital mechanistic insight into how the insulin pathway controls cell and body size in response to nutrients, highlighting the strength of using flies to reveal the molecular mechanisms underlying the effects of biological sex on insulin signaling. Given that the regulation and action of the insulin pathway is conserved from flies to humans, knowledge provided will assist in developing therapies to combat the sex-biased risk of metabolic diseases.

Feasibility of the Project. Overexpression and RNAi transgenes for all insulin pathway genes are in the lab, thus it is anticipated that significant progress will be made during the four-month training period. Marcus has generated all the preliminary data for this project, and is proficient at culturing flies and measuring body size. The experiments will be completed concurrently, as samples to perform Western blotting and body size measurements will be collected from the same batch of animals.