**Genetic characterization of a Drosophila model of type 2 diabetes**

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**Introduction**

Diabetes mellitus is the 5th leading cause of death in the world. In 2007, healthcare costs of the disease totaled $174 billion. Both type 1 and 2 diabetes include retinopathy, nephropathy, peripheral neuropathy, cardiovascular disease, and early mortality. In addition, insulin resistant patients are often obese. All of these pathologies can be modeled in the fruit fly, Drosophila melanogaster. Drosophila have eyes, kidneys, hearts, and a combined adipose/lever equivalent called the fat body. Flies also have insulins and almost all components of metabolic pathways found in humans.

We have developed a simple dietary method of eliciting type 2 diabetes-like phenotypes in Drosophila by increasing the carbohydrate content of the food. High sugar (1 M sucrose) feeding induces hyperglycemia, insulin resistance, and obesity-all hallmarks of type 2 diabetes. Genomic and genetic analyses of high sugar-fed Drosophila demonstrate that lipogenesis plays a protective role. A transcriptional network analysis in control, high sugar-fed, and lean high sugar-fed animals identified factors that trended with disease-like phenotypes. This network was used as the basis for a functional study of several transcription factors in the fat body. We identified genes that were previously unknown to regulate growth and fat storage on a high sugar diet. Our model represents a platform on which to rapidly test and identify genes that function in developing type 2 diabetes.

**Aim**

Our goal for this project was to test the transcriptional network generated from expression profiling of sugar-fed control and D-Chirp mutant diabetic Drosophila fat bodies. This network predicted that several gene products would function in high sugar-induced diabetes. Therefore, we knocked down these genes in larvae and characterized their phenotypes.

**Background**

We fed developing wild type larvae a diet of Bloomington semi-defined Drosophila media containing sucrose as the only source of sugar and counted the number of days to pupariation, the onset of meiosis, and the larval weight. (A) Animals reared on control feed (0.15 M sucrose) normally reach the onset of meiosis within 3-4 days of egg laying. Those on a high sugar diet (1 M sucrose) are delayed, taking an additional 2-3 days to reach this transition. The same delay is seen on control feed in larvae homozygous for the Drosophila insulin receptor (Shingleton et al, PLoS Biol Sep 2005; 3(9):e289). (B) High sugar-fed larvae take in 84% more calories (data not shown), but are 94% larger than controls. Larvae reared on both diets were also weighed at wandering.

Because high sugar feeding makes wild type larvae act like insulin resistant larvae, we analyzed blood sugar, fat accumulation, and insulin resistance:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Blood Sugar (mM)</th>
<th>Fat Accumulation</th>
<th>Insulin Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.55</td>
<td>2.5</td>
<td>Normal</td>
</tr>
<tr>
<td>High Sugar</td>
<td>4.5</td>
<td>15</td>
<td>Slight delay</td>
</tr>
</tbody>
</table>

**Methods**

1. A transcriptional network of sugar-dependent gene expression in the Drosophila fat body. A CLR (context likelihood of relatedness) algorithm was used to analyze genes that trended together.

2. Fat body-specific loss of function using cgGAL4 and UAS-broad.

3. F1 progeny of this cross were characterized using described methods. Briefly, animals were observed for developmental delays on all diets, compared to genetically-matched controls. Larvae and adults were weighed to identify genes that affected growth. Finally, Ispase-based enzymatic triglyceride (TAG) assays were used to quantify stored fat in control and mutant animals.

**Table 1**

<table>
<thead>
<tr>
<th>Gene Targeted</th>
<th>C/EBP gamma</th>
<th>C/EBP alpha</th>
<th>Hr39</th>
<th>Broad</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 M sucrose</td>
<td>Delayed</td>
<td>Normal</td>
<td>Normal</td>
<td>Delayed</td>
</tr>
<tr>
<td>1 M sucrose</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>0.5 M sucrose</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
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<td>15 M sucrose</td>
<td>Normal</td>
<td>Normal</td>
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<td>Normal</td>
</tr>
<tr>
<td>1 M sucrose</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>0.5 M sucrose</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

**Results**

**Figure 1. Female mutant larvae weights and TAG**

Mutant larvae reared on 1 M sucrose were assessed for TAG content and weight. cgGAL4, UAS-broad, and UAS-srp larvae are more obese than controls. cgGAL4, UAS-broad larvae are larger than controls, while UAS-srp, cgGAL4 mutants are smaller. Other mutants were not significantly different from controls.

**Figure 2. Male mutant larvae weights and TAG**

Mutant larvae reared on 1 M sucrose were assessed for TAG content and weight. Similar results were observed in males as in females.

**Figure 3. Female mutant larvae weights and TAG**

**Figure 4. Male mutant larvae weights and TAG**

While results are not as striking as the corresponding females, r4GAL4, UAS-broad larval pupae do not eclose, similar to broader mutants. This is the first time that this phenotype has been observed in a tissue-specific manner, to our knowledge.

**Summary**

Our results show that the transcriptional network successfully identified novel mediators of high sugar diet-induced diabetes-like phenotypes in Drosophila. The use of an algorithm-based analysis to identify physiologically relevant gene products increases the efficiency with which interesting genes can be isolated. This method could be applied to other research projects that model the genetic bases of human disease. By identifying critical factors in the response of the diabetic fly to a high sucrose diet, we can better understand the pathophysiology of diet-induced type 2 diabetes in humans.

**Conclusions and next steps**

- Aplerous, Bifid, Kruppel, and Srp appear to act as fat body-specific mediators of high sugar diet-induced phenotypes.
- Knockdown of gene expression will be confirmed with qRT-PCR of dissected control and mutant fat bodies.
- The physiological responses of mutant animals will be further characterized, including hemolymph glucose measurements and assays for insulin sensitively.
- Fat body-specific profiling of some mutants will be performed to refine network analyses and make a better transcriptional network.

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