RNA-Sequencing of Drosophila melanogaster Head Tissue on High-Sugar and High-Fat Diets

Wayne Hemphill, Osvaldo Rivera, and Matthew Talbert
Department of Biology, University of Louisiana at Monroe, Louisiana 71209

ABSTRACT
Obesity has been shown to increase risk for cardiovascular disease and type-2 diabetes. In addition, it has been implicated in aggravation of neurological conditions such as Alzheimer’s. In the model organism Drosophila melanogaster, a physiological state mimicking diet-induced obesity can be induced by subjecting fruit flies to a solid medium disproportionately higher in sugar than protein, or that has been supplemented with a rich source of saturated fat. These flies can exhibit increased circulating glucose levels, increased triglyceride content, insulin-like peptide resistance, and behavior indicative of neurological decline. We subjected flies to variants of the high-sugar diet, high-fat diet, or normal (control) diet, followed by a total RNA extraction from fly heads of each diet group for the purpose of Poly-A selected RNA-Sequencing. Our objective was to identify the effects of obesogenic diets on transcriptome patterns, how they differed between obesogenic diets, and identify genes that may relate to pathogenesis accompanying an obesity-like state. Gene ontology analysis indicated an overrepresentation of affected genes associated with immunity, metabolism, and hemocyanin in the high-fat diet group, and CHK, cell cycle activity, and DNA binding and transcription in the high-sugar diet group. Our results also indicate differences in the effects of the high-fat diet and high-sugar diet on expression profiles in head tissue of flies, despite the reportedly similar phenotypic impacts of the diets. The impacted genes, and how they may relate to pathogenesis in the Drosophila obesity-like state, warrant further experimental investigation.

KEYWORDS
obesity fly transcriptome obesogenic

Obesity is a low-grade inflammatory condition characterized by a positive energy imbalance, resulting from overabundant energy intake, and/or inadequate energy expenditure. The most commonly used assessment of obesity is the Body Mass Index (BMI) score, which is calculated as weight in kilograms divided by the height in meters squared. Obesity is characterized by the World Health Organization as a BMI of 25–30 characterizes being overweight (Lehnert et al. 2013). By this metric, 60% of adults in the US population are estimated to be overweight, 30% of which are estimated to be obese (“Adult Obesity Facts| Overweight and Obesity|CDC” n.d.). Obesity’s detriment to health is largely attributed to its facilitation of comorbidities such as cardiovascular disease, type-2 diabetes, and neurological decline. Conditions such as these are not only increased in prevalence, but also exacerbated, in obese individuals (Must et al. 1999; Langdon et al. 2011). Obesity fosters a molecular environment conducive to comorbidities, and so that environment, and the mechanisms by which it does induce disease, are of great biomedical interest.

While some mechanisms of pathogenesis leading to comorbidity in an obesity or obesity-like state in mammals remain unknown, studies with mice and humans have revealed increased inflammation, aberrant cell signaling, decreased insulin sensitivity, and increased oxidative stress to be involved (Xu et al. 2003; Furukawa et al. 2004; Montague et al. 1997). The elevated release of pro-inflammatory cytokines is characteristic of the inflammatory state in obesity, which can be initiated by accumulation of lipids in adipocytes, retardation of autophagy, and the unfolded protein response, and is characterized by the infiltration of visceral adipose tissue by macrophages (Gregor and Hotamisligil 2011). Pro-inflammatory cytokines such as tumor necrosis factor α (TNFα), interleukin-6 (IL-6), and resistin can promote insulin resistance that can, in turn, be ameliorated by a decrease in visceral adipose tissue (Piya et al. 2013). Leptin resistance has been observed in mice following prolonged exposure to a high-fat diet, and this has been hypothesized to further exacerbate positive energy imbalance...
Our objective was to identify gene expression patterns of interest in the head tissues of fruit flies exposed to a HSD, HFD, or a normal diet (ND) via poly-A selected RNA-sequencing, and to establish differences between the physiological effects of two conventional obesogenic diet types (HSD and HFD) through expression profiling and gene ontology (DAVID). Furthermore, we hoped to determine the functional nature of genes affected by these diets, with the goal of ascertaining pathogenic mechanisms accompanying obesity in flies, and, by extension, possibly in mammals. Heads were used since they contain the brain, glia, neurosecretory cells (including insulin-like peptide-producing cells, and potentially the corpora cardiaca, which can accompany removal of the head), a pericerebral fat body, and, therefore, many of the components of central energy homeostasis. This approach aimed to reduce competing local transcription patterns produced by the rest of the fly tissues, while still accounting for many of the major tissues involved with metabolism and obesity.

**MATERIALS AND METHODS**

**Flies/rearing**

All flies used in the experiment were wild-type, Oregon R-C (Bloomington stock 5) genotype. Upon eclosing, female virgins were collected and housed on low calorie media at approximately equal densities of 30 flies/vial until in excess of 450 flies were obtained. Females were then synchronously mated with males of the same genotype in a 2:1 ratio for 2 d. After mating, males were removed, and the females were divided equally into three groups, and placed on one of the three corresponding media types (ND, HSD, or HFD). The fly groups were kept on their respective medium for 7 d, being transferred to fresh medium every 3–4 d. Flies were kept in an incubator at 25°C on a 12-hr light/dark cycle with consistent humidity, until decapitation, excluding any handling without anesthesia for media transfers.

**Media**

The ND acted as our control diet, and consisted of 2.6% w/v cornmeal, 4% w/v dry inactivated yeast, 0.8% w/v agar, 3% w/v sucrose, 1.5% v/v Tegosept (20% w/v in 70% ethanol), and 0.3% v/v propionic acid. The HFD and HSD were identical to the ND, except for an addition of 20% w/v coconut oil in the HFD, and the use of 20% w/v sucrose instead of 3% in the HSD. The low calorie (LC) diet briefly used for collections and mating contains 5.2% w/v cornmeal, 5% w/v dry inactivated yeast, 1% w/v agar, 3% w/v sugar, 1.5% v/v Tegosept (20% w/v in 70% ethanol), 0.3% v/v tetracycline (10 mg/ml), and 0.3% v/v propionic acid.

**RNA extraction**

Flies were transferred from their media briefly into empty vials and anaesthetized using FlyNap (Carolina). The flies were then submerged in a Petri dish with 1 ml TRIzol reagent, and their heads were pulled off with forceps in rapid succession. The heads (N = 20) were then immediately placed in 200 μl of TRI Reagent in a 1.5-ml microcentrifuge tube. There were six biological replicates (tubes) for each media group, with each replicate/tube containing 20 heads. The head tissue in each
Figure 1 (A) Venn diagram illustrating the number of transcripts with $p < 0.05$ for the HFD, HSD, both diet groups, or neither diet group. (B) Venn diagram illustrating the number of transcripts with $q < 0.05$ in the HFD, HSD, both diet groups, or neither diet group. (C) A heat map of 233 transcripts with $p < 0.05$ in both HSD and HFD groups. The map is divided (black horizontal lines) into six expression priority groups, and, within each group, the transcripts are further arranged by increasing z-score within the HFD column. Bright green indicates the most negative z-score, while bright red indicates the most positive z-score. (D) A heat map of 79 transcripts with $q < 0.05$ in both the HSD and HFD groups. The map is divided (black lines) into five expression priority groups, and, within each group, the transcripts are further arranged by decreasing z-score.
tube was then homogenized using an electric pestle. RNA extraction and purification was performed using a Direct-zol RNA MicroPrep kit (Zymo Research). The RNA was eluted with sterile water, after which purity and concentration were confirmed via 260/280 and 260/230 ratios measured using a Nano Drop 2000c (Thermo Scientific). N = 6 per experimental condition.

Sequencing
Twelve RNA samples (four replicates from each of three dietary treatment groups) of the highest yield (>100 ng/μl) and sufficient purity (260/280 and 260/230 ratios of 1.8–2.1 and >1.5, respectively), ascertained via NanoDrop2000c (Thermo Scientific) readings, were sent to Louisiana State University Health Science Center in Shreveport for initial Poly-A selected RNA-sequencing in the Genomics Core Facility. mRNA was isolated from the RNA via poly-adenylated RNA selection using oligomer beads, and sequenced using a NextSequation 500 (Illumina), targeting at least 50 million stranded, paired-end reads of 75 bp in size. Raw sequencing data were passed through a software filter (RTA v2.4.6.0), and sent to the laboratory of U. Cvek at Louisiana State University at Shreveport for further analysis to ascertain reads per million, perform differential expression analysis between the three conditions, generate the preliminary transcript statistics (discussed later), within the HFD column. Bright green indicates the most negative z-score, while bright red indicates the most positive z-score. (E) A bar graph quantifying the number of genes, among those with \( q < 0.05 \) or \( p < 0.05 \) in both obesogenic diet groups, having various RPKM value priorities in the three diet groups.

| Table 1 | Select genes of functional interest and their associated sequencing and statistical values |
|--------|-------------------------------------------------------------------------------------|
| Gene   | ND RPKM | HFD RPKM | HFD fold change | HFD p-value | ND RPKM | HSD RPKM | HSD fold change | HSD p-value |
| PepB1  | 1.83123 | 24.2993  | 3.73004         | 5.00E-05    | 1.83797 | 110.991  | 5.91618        | 5.00E-05    |
| Decay  | 0.715349 | 8.71145  | 3.60619         | 0.0014      | 0.721689 | 12.8802  | 4.15764        | 5.00E-05    |
| Damm   | 0.360169 | 6.06931  | 4.07478         | 5.00E-05    | 0.360761 | 4.9797  | 3.78695        | 5.00E-05    |
| Hsp26  | 39.7857  | 203.681  | 2.35599         | 5.00E-05    | 39.9639 | 426.535  | 3.41589        | 5.00E-05    |
| Hsp70Bc| 3.0237   | 7.43374  | 1.29777         | 0.00655     | 3.03621 | 29.0915  | 3.26025        | 5.00E-05    |
| Amy-p  | 30.1851  | 336.333  | 3.47797         | 5.00E-05    | 30.4986 | 282.113  | 3.20946        | 5.00E-05    |
| Dpt    | 3.95319  | 379.545  | 6.58511         | 5.00E-05    | 3.95992 | 35.3653  | 3.15879        | 5.00E-05    |
| Hsp27  | 10.1213  | 64.9198  | 2.68127         | 5.00E-05    | 10.1665 | 85.7934  | 3.07704        | 5.00E-05    |
| PGRP-SC2| 4.39994  | 41.5454  | 3.23915         | 5.00E-05    | 4.41733 | 28.6289  | 2.69622        | 5.00E-05    |
| Hsp23  | 14.4853  | 42.3095  | 1.54639         | 0.00015     | 14.556 | 67.6048  | 2.21551        | 5.00E-05    |
| Akh    | 222.111  | 208.278  | -0.0927698      | 0.67655     | 223.073 | 93.5279  | -1.25405       | 0.00005     |
| AkhR   | 46.5207  | 83.6116  | 0.845831        | 0.0002      | 46.7487 | 48.4237  | 0.0507891      | 0.79895     |
| CecAl1 | 11.2097  | 204.471  | 4.18908         | 0.00025     | 11.2106 | 15.7291  | 0.488577       | 0.4498      |
| AttA   | 10.2262  | 97.1934  | 3.24856         | 0.00005     | 10.2502 | 18.2709  | 0.8339         | 0.02065     |
| Dro    | 41.444   | 366.232  | 3.14352         | 0.00005     | 41.5591 | 71.8219  | 0.78926        | 0.0012      |
| Drs    | 93.0297  | 636.395  | 2.77416         | 0.00005     | 93.3272 | 136.457  | 0.548075       | 0.03445     |
| IM23   | 273.451  | 715.195  | 1.38705         | 0.00005     | 274.247 | 411.722  | 0.586195       | 0.00485     |
| CG10562| 24.4223  | 33.0321  | 0.43567         | 0.12025     | 24.502  | 41.71    | 0.767493       | 0.0014      |
| CG13659| 3.90307  | 4.412    | 0.176823        | 0.70795     | 3.91773 | 7.94422  | 1.01989        | 0.01075     |
| CG31288| 8.12872  | 6.97689  | -0.220444       | 0.59165     | 8.15775 | 17.2851  | 1.08328        | 0.0006      |
| CG33510| 0.185615 | 0.340132 | 0.873777        | 1           | 0.186477| 0.776494| 2.05798        | 0.00535     |
| CG6830 | 15.4968  | 25.4499  | 0.715695        | 0.0512      | 15.5596 | 40.5246  | 1.38099        | 0.00095     |
| CG10621| 179.404  | 92.1356  | -0.961381       | 0.00005     | 180.208 | 104.82  | -0.781741      | 0.00015     |
| CG9150 | 154.356  | 85.8748  | -0.845953       | 0.0003      | 154.951 | 66.232   | -1.22621       | 0.00005     |
| CG4500 | 19.5954  | 8.43517  | -1.21602        | 0.00045     | 19.6746 | 5.88     | -1.74245       | 0.00005     |
| CG3999 | 35.1408  | 17.3141  | -1.0212         | 0.00005     | 35.2905 | 17.783   | -0.988785      | 0.00005     |

Represented are 10 genes with \( q < 0.05 \) in both obesogenic diet groups, six genes with \( q < 0.05 \) in the HFD group only, and six genes with \( q < 0.05 \) in the HSD group only. Those provided represent the genes included in the most enriched annotation (gene ontology keyword) of relevance (described in Materials and Methods) among the genes with \( q < 0.05 \) in each of three mentioned groups (both diet groups, HFD only, HSD only). Also included are the four genes whose expression was decreased, and had \( q < 0.05 \), in both obesogenic diet groups; these were absent from the functional annotation results.
and identify the sequenced transcripts via mapping against the *Drosophila* mRNA records using Cufflinks (Trapnell et al. 2010).

### Statistical analysis

With RNA-Sequencing, the degree of gene expression is gauged (in one case) by a signal value in Reads Per Kilobase per Million reads (RPKM). This is calculated as the number of reads (usually of fragments of comparable size) identifying the transcript, divided by the length of the known whole transcript in kilobases, and divided again by the total number of reads (in millions) for all sequenced transcripts in the sample. Another metric used to illustrate the relative change in RPKM of a transcript between two groups is fold change (FC), which is calculated as the negative log base two of the experimental to control RPKM ratio.

P- and q-values were also generated, and they were applied to each transcript, in each obesogenic diet group. After obtaining a transcript’s RPKM values for all replicate samples in each diet group, the SD of each of these sample data sets was calculated, and that value applied to a normal distribution of that transcript’s RPKM values for each diet group. These distributions were then compared between the ND and HSD groups, as well as between the ND and HFD groups, and a p-value calculated for each obesogenic diet group, in all cases via Cuffdiff (Trapnell et al. 2010). Q-values were calculated using the p-values of each transcript, in each obesogenic diet group (relative to the ND group), via the Benjamini-Hochberg False Discovery Rate (B-H FDR) adjustment method (Benjamini and Hochberg 1995). Prism includes an iteration of this q-value when performing a B-H FDR adjustment on p-values; it indicates the fraction of Type I errors present among all p-values less than or equal to its associated p-value. Prism 7.0c (GraphPad) was used for all necessary calculations involved with the B-H FDR adjustment.

Figure 2 For the cluster graphs (left), bars of the same grouping represent annotations of related functions, belonging to the same cluster (aka annotation clusters), and those bars at the far right of a group represent the enrichment score of the entire cluster (geometric mean of all composing annotations). (A) Graph displaying the three most enriched annotation clusters among genes whose expression was significantly (q < 0.05) increased in the HSD group. (B) Graph displaying the most enriched individual annotations among genes whose expression was significantly (q < 0.05) increased in the HSD group.
Heat maps

Heat maps were created using Heatmap Generator software (Khomtchouk et al. 2014). Transcript data were arranged in Microsoft Excel, and the data were then subdivided into six groups, corresponding to the six possible comparative iterations of the RPKM values for each media type (i.e., RPKM values of ND, HSD, HFD, or any other arrangement, for each implicated transcript). A standard normal distribution is generated for each transcript using the RPKM values of that transcript from each diet group, and then a z-score for each diet groups’ RPKM value on that distribution for the transcript is calculated. This z-score is then used to assign a color to that transcript for each diet group, with the most positive z-scores (those with RPKM values above the transcript’s average RPKM value across all diet groups) being bright red, the most negative (those with RPKM values below the transcript’s average RPKM value across all diet groups) being bright green, and anything between being a varied-ratio mix of the two colors.

Functional annotation

Functional annotation was performed using DAVID Bioinformatics Resources Version 6.8 (Dennis et al. 2003). Six gene lists were constructed to be provided, consisting of transcripts with \( q < 0.05 \) and FC > 0 HFD group values, \( q < 0.05 \) and FC < 0 HFD group values, \( q < 0.05 \) and FC > 0 HSD group values, \( q < 0.05 \) and FC < 0 HSD group values, \( q < 0.05 \) and FC > 0 HFD and HSD group values, and \( q < 0.05 \) and FC < 0 HFD and HSD group values, respectively. Each gene list was then separately provided to the software and annotated.

For functional annotation of this data, the “Official Gene Symbol” identification was used, and the species background used was “D. melanogaster.” The “Functional Annotation Clusters” feature was used to generate groupings/clusters of related annotations, and their total and individual enrichment values within each gene list. The “Functional Annotation Chart” feature was also used to generate lists of the top individual annotations for each gene list, and their p-values. For both features, DAVID Bioinformatics Resources suggested defaults were used for all options, including the database sources, background, and the nature of annotations used, as well as all statistical parameters for determining annotation clusters.

Functional elaboration of select genes

Genes with \( q < 0.05 \) in at least one obesogenic diet group were first identified. Those genes were then divided into three lists: those with

Figure 3

For the cluster graphs (left), bars of the same grouping represent annotations of related functions, belonging to the same cluster (aka annotation clusters), and those bars at the far right of a group represent the enrichment score of the entire cluster (geometric mean of all composing annotations). (A) Graph displaying the three most enriched annotation clusters among genes whose expression was significantly \( (q < 0.05) \) decreased in the HSD group. (B) Graph displaying the most enriched individual annotations among genes whose expression was significantly \( (q < 0.05) \) decreased in the HSD group.

A

Top Annotation Clusters of Genes with \( q<0.05 \) and FC>0 in HSD Group

| Cluster A               | Cluster B                                | Cluster C                                 |
|------------------------|------------------------------------------|-------------------------------------------|
| 1                      | Cell cycle                               | Homeobox, conserved site                   |
| 2                      | Mitosis                                  | IBMX                                      |
| 3                      | Cell division                            | Homodomain                                |
| 4                      | regulation of chromatin binding          | S. TKEc                                   |
| 5                      | mitotic sister chromatid segregation     | DNA-binding region, Homodomain             |
| 6                      | SMO132                                   | DNA-binding region, Homodomain             |
| 7                      | Cyclin, C-terminal domain                | DNA-binding region, Homodomain             |
| 8                      | Cyclin, N-terminal domain                | DNA-binding region, Homodomain             |
| 9                      | Cyclin                                   | DNA-binding region, Homodomain             |
| 10                     | CYCLIN                                   | DNA-binding region, Homodomain             |
| 11                     | synaptotriad mutant-like cell cycle       | DNA-binding region, Homodomain             |
| 12                     | Cyclophosphamide-like transcript         | DNA-binding region, Homodomain             |
| 13                     | Cluster Geometric Mean                   | DNA-binding region, Homodomain             |
| 14                     | Sema/thromboside-protein kinase          | DNA-binding region, Homodomain             |
| 15                     | protein kinase                           | DNA-binding region, Homodomain             |
| 16                     | domain Protein kinase                    | DNA-binding region, Homodomain             |
| 17                     | nucleoside phosphate-binding region, ATB | DNA-binding region, Homodomain             |

| 1                      | mitotic cytokinesis                      | DNA-binding region, Homodomain             |
| 2                      | cell cycle                               | DNA-binding region, Homodomain             |
| 3                      | midbody                                  | DNA-binding region, Homodomain             |
| 4                      | mitosis                                  | DNA-binding region, Homodomain             |
| 5                      | cell division                            | DNA-binding region, Homodomain             |
| 6                      | spindle                                  | DNA-binding region, Homodomain             |
| 7                      | spindle midzone                          | DNA-binding region, Homodomain             |
| 8                      | regulation of chromatin binding          | DNA-binding region, Homodomain             |
| 9                      | protein kinase-like domain               | DNA-binding region, Homodomain             |
| 10                     | protein phosphorylation                  | DNA-binding region, Homodomain             |

B

Top Annotations of Genes with \( q<0.05 \) and FC<0 in HSD Group

1
2
3
4
5
6
7
8
9
10

\textbf{Figure 3} For the cluster graphs (left), bars of the same grouping represent annotations of related functions, belonging to the same cluster (aka annotation clusters), and those bars at the far right of a group represent the enrichment score of the entire cluster (geometric mean of all composing annotations). (A) Graph displaying the three most enriched annotation clusters among genes whose expression was significantly \( (q < 0.05) \) decreased in the HSD group. (B) Graph displaying the most enriched individual annotations among genes whose expression was significantly \( (q < 0.05) \) decreased in the HSD group.
q < 0.05 in both obesogenic diet groups, those with q < 0.05 in the HSD group only, and those with q < 0.05 in the HFD group only. Each of those gene lists was then separately provided to the DAVID bioinformatics software (Dennis et al. 2003), and the genes contained within the most enriched, relevant (potential implications in obesity-related pathogenesis; relating to immunity, metabolism, neural function, etc.) annotations were investigated in FlyBase (Gramates et al. 2017) for literature supporting functions with experimental evidence. All of these genes were initially considered for elaboration, but those that did not have relevant functions (i.e., functions potentially related to such pathogenesis) were removed from the final list of genes to be discussed; 10 genes were presented with q < 0.05 in both obesogenic diet groups, and six genes presented from each of the other two lists.

**Data availability**

All data that was generated or analyzed during this research is present in this publication, or its corresponding supplemental files. Raw sequencing data can be accessed on the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus via accession number GSE104336. Supplemental files include comparisons of the expression levels for each gene between the ND and HFDs (Supplemental Material, File S1) and between the ND and HSDs (File S2). RNA-sequencing quality control information is available in File S3.

**RESULTS**

**Alterations in gene expression**

Counts of genes with significant expression changes by two standards (q- and p-values) indicate there are over twice as many genes whose expression was significantly affected by a single obesogenic diet, than by both (Figure 1, A and B). Additionally, 11 of the 79 genes whose expression was most significantly affected (q < 0.05) were upregulated by the HFD, but downregulated by the HSD, while the opposite was true for no genes (Figure 1, C–E). Curiously, the HFD also upregulated more genes, while the HSD was responsible for the downregulation of more genes, among those significantly affected (Figure 1E and data not shown).

**Functional annotation**

The aforementioned genes with significant (q < 0.05) expression alterations for at least one obesogenic diet group were further subdivided
to produce the gene lists on which to perform functional annotation (described in Materials and Methods), and the DAVID Bioinformatics Resources were utilized with suggested defaults to determine the top individual gene annotations and top annotation clusters for each.

No functional annotation enrichment data could be gathered from genes whose expression was decreased by both obesogenic diets given the small sample number ($n = 4$). These four genes will be discussed individually later, and are listed in Table 1. The HSD increased expression of genes associated with enzymatic activation, peptide bond cleavage, checkpoint kinases, ascorbate/aldarate and retinol metabolism, cytochrome P450 activity, mitochondrial activity, sugar molecule processing, and heme (Figure 2), and decreased expression of those involved with the cell cycle, kinase activities, cytochrome P450 function, and DNA-binding and transcription (Figure 3). The HFD increased expression of genes with ontology related to immunity and infection, chitin metabolic processing, and protein activities associated with metabolism (Figure 4), and decreased expression of those related to pyridoxal phosphate function, nutrient storage, and signaling, as well as those implicating hemocyanin and immunoglobulins (Figure 5). Genes significantly ($q < 0.05$) upregulated by both obesogenic diets had ontology related to proteolysis, peptide processing, metabolic enzymes, signaling, and carbohydrate metabolism (Figure 6). This indicates gene ontologies that appear characteristic of a HFD (immunity/infection, hemocyanin, and immunoglobulins) or HSD (cytochrome P450 action, CHK, and mitochondrial activity).

### DISCUSSION

It is important to re-establish that our findings are within a single genetic background in *D. melanogaster*, using mated females, and utilizing the media recipe variants that we indicated. It is unclear yet how each of

![Figure 5](image_url)

**Figure 5** For the cluster graphs (left), bars of the same grouping represent annotations of related functions, belonging to the same cluster (aka annotation clusters), and those bars at the far right of a group represent the enrichment score of the entire cluster (geometric mean of all composing annotations). (A) Graph displaying the three most enriched annotation clusters among genes whose expression was significantly ($q < 0.05$) decreased in the HFD group. (B) Graph displaying the most enriched individual annotations among genes whose expression was significantly ($q < 0.05$) decreased in the HFD group.

### Notable genes

A selection of differentially expressed genes (with $q < 0.05$ in at least one obesogenic diet) with obvious functional relevance were further investigated (Table 1). The remainder of genes not discussed here is publicly available, as noted in Data availability.

There was a notable increase in expression of several of the heat shock proteins (in both obesogenic diet groups, except for Hsp70Bc in the HSD group only), which are known to be involved in responses to stress and promotion of longevity in the fly model. There was also increased expression of genes associated with response to bacterial infection, production of antimicrobials, and apoptotic processes, as well as those relating to metabolic regulation. The genes presented for the HSD only list were mostly proteins of unknown function, which were annotated in FlyBase (Gramates et al. 2017) as having CHK kinase-like activity, but whose alleged functions were supported by no concrete experimental evidence. Finally, the four genes whose expression was decreased, and had $q < 0.05$, in both obesogenic diet groups contained one gene relating to triglyceride storage and sleep homeostasis, and three genes of unknown biological function.
these major variables could impact gene expression in these dietary contexts, and we anticipate that further study will elucidate this. It is also important to note that any extrapolation from expression change to a definitive functional impact or physiological mechanism is by nature speculative without direct experimental validation, but a consideration of the implications of our results is necessary. For all genes discussed hereafter, it should be assumed they were significantly affected ($q < 0.05$) in the manner stated, unless otherwise indicated.

Transcriptome changes

An overview of the transcriptomes of the flies in each dietary group indicates a notable difference in the effects of the two obesogenic diets on gene expression in the fly model. Comparisons between these two obesogenic diet groups’ effects also suggest that the HSD might be more prone to instituting a decrease in gene expression than the HFD, while the HFD might preferentially upregulate gene expression (Figure 1).

Annotation patterns

Genes with functions related to peptide and carbohydrate processing appear to be the most enriched among genes upregulated by both diets (Figure 6). This is expected, given the increased caloric intake. No functional classes could be ascertained from the four genes noted to be significantly downregulated in both obesogenic diet groups, and these four genes are included in the table of individual genes (Table 1), to be discussed later.

Both diets also upregulated genes functioning in protein cleavage, enzyme activation, and macromolecule processing (Figure 2 and Figure 4). This could be a result of the obesogenic diet-associated macromolecules within the fly, as discussed above. However, the independent enrichment of metabolism related annotations in the HFD- (Figure 4) and HSD- (Figure 2) specific gene ontology lists that are distinct from those enriched in the combined HFD–HSD gene ontology list also implies some distinction in the specific metabolic genes affected. This is unsurprising, given that each diet is enriched with a different macromolecule. These annotations could reflect that, or be an artifact of the DAVID software protocols. However, among those genes upregulated by the HSD, the enriched metabolic processing annotations do appear to relate mostly to sugar/carbohydrate processing (“pentose and glucuronate interconversions,” “transferase activity, transferring hexosyl groups,” and “glycosyltransferase”) (Figure 2).

An enrichment of genes functioning in immunity and response to infection was apparent among genes upregulated by the HFD group (Figure 4). This might imply that the fly is utilizing response mechanisms for the obesogenic diets that appear similar to those used during infection or other immune reactions. This alludes to an overlap in nutrient processing pathways and inflammatory pathways in *Drosophila*, which is supported by the existence of an inflammation-like response during immune reactions in the fly (Shaukat et al. 2015).

Among the genes upregulated by the HSD (Figure 2), there was an enrichment of genes relating to checkpoint kinases. Genes coding for...
CK kinase-like proteins exist in Drosophila (Patil et al. 2013), and, in several models, CHK has been established as an arrester of the cell cycle in response to DNA damage. Additionally, it has the suspected function of indirectly inducing apoptotic mechanisms in response to such damage (Dai and Grant 2010). The increased expression of genes associated with these CHK-related functions in response to the HSD, paired with the decreased expression of genes with functions related to the cell cycle, could imply the ability of diets high in sugar to increase levels of DNA damage and subsequent cell cycle activity impairment. This hypothesis is supported by experimental evidence in humans, where obese individuals experience a prolonged increase of free ROS, which are known contributors to DNA damage, following the consumption of a meal high in sugar content (Patel et al. 2007).

Among genes downregulated by the HFD, there was enrichment of genes related to hemocyanin (Figure 5), the functional equivalent of hemoglobin in the fly. Recent study has convincingly illustrated the existence of a response to hypoxia in the adipose tissue of obese mammals, and this response may represent a new mechanism for the development of chronic inflammation, macrophage infiltration, insulin resistance, and other detrimental characteristics of an obesity state (Ye 2009). This decrease in expression of genes related to hemocyanin in the HFD group could also imply a relationship between exposure to a HFD and initiation of adipose tissue hypoxia (ATH) in the fly, via the rationale of decreased hemocyanin available for oxygen delivery to adipose tissue. Among genes upregulated by the HSD, those with functions related to heme (major biochemical component of hemoglobin and hemocyanin) processing were enriched, which could suggest a similar ATH state occurring due to the HSD. However, the involvement of heme with a variety of processes in the fly limits definitive conclusion regarding this.

**Genes of interest**

Increased expression, in both obesogenic diet groups, of several heat shock proteins suggests that the obesogenic diets may be placing a significant amount of physiological stress on the fly that the Hsps are trying to combat, given the Hsp’s roles in mitigating various types of physiological stress via protein folding and refolding, among other mechanisms (Zhao et al. 2010; Vos et al. 2016; Azad et al. 2009; Wang et al. 2004). The Hsps are also identified as having a significant impact on the promotion of longevity in flies, with as much as a 30% increase in lifespan when overexpressed, in contrast to the decreased longevity associated with obesogenic diets (Wang et al. 2004; Vos et al. 2016). This could imply existence of another mechanism whose effects on lifespan outweigh that of the Hsps. Curiously, activity of Hsp27 has also been associated with an increased resistance to starvation (Hao et al. 2007). This seems counterintuitive given the increased caloric intake; however, it is unclear from these data alone whether this function of Hsp27 is active in these flies.

Akh is exclusively downregulated by the HSD, and AkhR upregulated by the HFD group. Knockdown of Akh in flies has produced an increase in triglyceride stores, a decrease in glycan stores coupled with hypoglycemia in the fly hemolymph, and a decrease in oxidative stress tolerance (Galičkova et al. 2015). Downregulation of Akh by the HSD is expected for increased metabolite anabolism, and not catabolism, but also might further indicate a potential source of oxidative stress to the fly. In the HFD group, the upregulation of AkhR might be promoting the effects of Akh, which is contrary to the expected physiological needs of the fly, and might suggest an avenue of aberrant signaling resulting in excess circulating metabolites. Given these data, the corpora cardiaca could be a fruitful avenue of future directed study into these hypotheses.

There was also upregulation, in both obesogenic diet groups, of two genes known to be involved in apoptotic processes: Decay and Damm (Harvey et al. 2001; Dorstyn et al. 1999). Increased expression of Decay and Damm in our obesogenic diet groups may imply that cell apoptosis is increased in response to obesogenic diets and obesity-like states in Drosophila. Overall, more in-depth studies are warranted to investigate these traits in flies on obesogenic diets.

Several genes upregulated in both obesogenic diet groups (Dpt, PGRP-SC2, and Pebp1) have functions relating to immunity or response to infection (Bischoff et al. 2006; Lemaitre et al. 1997; Wicker et al. 1990; Cronin et al. 2009; Reumer et al. 2009). Pebp1 binds phosphatidylethanolamine, and overexpression of this gene is associated with protection against both gram-positive and gram-negative bacterial infection in the fly via release of various immunity-related proteins into their hemolymph (Reumer et al. 2009). Dpt is noted as being released in varying degrees in response to infections by various types of bacteria, and its overexpression is also established as rescuing flies from the detrimental effects of these infections. Dpt is also released to a lesser extent in response to injury in the fly (Lemaitre et al. 1997), a response which often involves release of pro-inflammatory cytokines. Additionally, Dpt has been shown to be significantly upregulated in flies that have a tolerance to lifelong hyperoxia, though the significance of this function in the context obesity’s associated detriments is unclear (Wicker et al. 1990). PGRP-SC2 enzymatically interacts with peptidoglycan, a major component of bacterial membranes, and provides a specific means by which to protect against bacterial infection (Bischoff et al. 2006). It also plays a role in the regulation of the natural microbiota of Drosophila, acting to cleave the pro-inflammatory peptidoglycan of their membranes into nonstimulatory muropeptides, and subsequently prevent activation of the fly’s immune system in response to its own endogenous bacteria (Royer et al. 2011). There is precedent indicating the ability of diets high in sugar to alter the microbiota of flies in order to compensate for the needed change in metabolic processing (Whon et al. 2017), though no direct evidence of this change contributing to an inflammatory state or implicating PGRP-SC2 in the fly exists. Upregulation of these genes in both obesogenic diet groups supports the implication by functional annotation results that flies appear to respond similarly to infections and obesogenic diets (HFD in particular).

Several genes were exclusively upregulated by the HFD, and are established to be involved with immune response in Drosophila. These were CecA1, AttA, Dro, Drs, IM23, whose expressions are noted to be increased in response to humoral immune system challenge via microbial infection (Lemaitre et al. 1997; Uittenweiler-Joseph et al. 1998; Verleyen et al. 2006; Wagner et al. 2009; Levy et al. 2004). This diet-specific upregulation of immune related genes reinforces the potential physiologically distinct effects of different obesogenic diets. Ultimately, the large number of immune-associated genes affected by the obesogenic diets implicates hemocytes. This could be the source of these changes, however, infiltration of the fat body by macrophages during obesity-like states has also been established previously (Gregor and Hotamisligil 2011). Both possibilities warrant further study to elucidate this.

Among genes exclusively upregulated by the HSD, five of those related (gene ontology) to CHK kinase-like activity (most enriched annotation) currently have no experimentally supported biological function (Gramates et al. 2017). Also, of the four genes noted to be downregulated in both obesogenic diet groups, three had no reported biological function (Gramates et al. 2017). However, for one of the four genes, CG4500, there exists experimental data involving the knockdown of this gene in Drosophila. For these flies, a decrease in stored triglyceride was noted, as well as a negative disruption of sleep homeostasis.
in the flies studied (Thimgan et al. 2015). The suggested effect of obesogenic diets on triglyceride storage (i.e., decreased triglyceride storage via CG4500 decreased expression) is at odds with current direct experimental evidence concerning triglyceride storage in response to HSD and HFD, which could be more evidence of aberrant signaling during an obesity-like state in the fly. The decreased expression of CG4500 could also indicate a disruption of healthy sleep patterns in response to obesogenic diets, in the fly. There exists experimental evidence pointing to the ability of a HSD to selectively affect sleep behavior in Drosophila (Catterson et al. 2010), as well as, separately, for impaired sleep homeostasis to result in decreased longevity of the fly (Yamazaki et al. 2012). Overall, these data emphasize the lack of experimental data relating to genes affected by a HSD, as well as genes downregulated by obesogenic diets, and provide compelling evidence of their potential relevance.

Conclusions

Data from this research has illustrated a notable effect of obesogenic diets on the transcriptomic profile of D. melanogaster head tissue, as well as distinctions in the effects that diets high in sugar vs. fat have on gene expression. This data has additionally suggested a variety of physiological functions being activated or suppressed in response to these diets that may provide insight into the development of certain disease states associated with obesity, and indicated specific genes that may play a role in those functions. Overall, these results have provided insight into potential avenues of research to elucidate many of the unknown mechanisms associated with an obesity-like state in Drosophila, and provided data with which future investigators may guide their research.

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