HHS Public Access

Author manuscript

Mol Cell Endocrinol. Author manuscript; available in PMC 2017 November 05.

Published in final edited form as:

Mol Cell Endocrinol. 2016 November 5; 435: 20–28. doi:10.1016/j.mce.2015.12.003.

Drosophila melanogaster: An emerging model of transgenerational effects of maternal obesity

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Abstract

The prevalence of obesity in the world is endemic with one rapidly growing health concern being maternal obesity. Obesity during pregnancy increases the risk of gestational diabetes, miscarriage, and preeclampsia, while rendering offspring susceptible to developmental anomalies and long-term metabolic complications including type 2 diabetes and cardiovascular disease. Several studies in humans and rodents demonstrate a correlation between the risks of maternal overnutrition and factors such as epigenetics, mitochondrial dysfunction, insulin resistance, ER stress, and immune system disruption. At present, the molecular mechanisms connecting these factors to maternal obesity are unknown. This review focuses on the use of *Drosophila melanogaster* to study human metabolic diseases, including obesity, and its emerging use to elucidate the mechanisms of maternal overnutrition and the impact on offspring.

Keywords

Obesity; drosophila; diabetes; mitochondria; pregnancy; nutritional programming

1. Introduction — Prevalence and clinical impact of maternal obesity

The prevalence of obesity among adults has become a global epidemic; in 2014 over 1.9 billion adults were overweight, with 600 million classified as obese (Organization, 2015). This trend is also impacting the young; among children under the age of 5, 42 million were classified as obese or overweight (Organization, 2015). Individuals are identified as overweight or obese based on their body mass index (BMI), with a BMI ≥ 25 denoting an overweight individual, and a BMI ≥ 30 considered obese. While the impact of obesity on human health has been widely studied, one rapidly growing health concern is the prevalence of obesity in pregnant women.

Between 25 to 30% of pregnant women in the United States are obese and are at greater risk for developing major health complications not only for themselves but also for their

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offspring (Vahratian, 2009; Yogev and Catalano, 2009)(Yogev and Catalano, 2009). In early pregnancy, maternal obesity increases the risk of single and recurrent miscarriages as well as fetal congenital anomalies that include neural tube defects, cardiovascular disorders, and orofacial clefts (Dokras et al., 2006; Metwally et al., 2008; Stothard et al., 2009). Later in pregnancy, obese mothers exhibit a higher incidence of preeclampsia, gestational diabetes mellitus, hypertension, preterm delivery, and have twice the risk for stillbirth compared to normal weight mothers (Leddy et al., 2008; O'Brien et al., 2003). Rates of caesarian delivery also double in obese mothers and the risk of operative morbidity increases (Leddy et al., 2008; Nohr et al., 2009). The offspring of obese females are at increased risk for fetal macrosomia, shoulder dystocia, and increased birth weight (Leddy et al., 2008). There is also evidence suggesting maternal overnutrition increases the offspring's risk of developing type 2 diabetes, obesity, cardiovascular disease, and asthma in later childhood (Patrick M Catalano et al., 2009; Patrick M. Catalano et al., 2009; Clausen et al., 2009; Diesel et al., 2015; Forno et al., 2014; Lowe et al., 2011; Nohr et al., 2009). For the mother, maternal obesity also correlates with an increase in cardiovascular disease and premature death later in life (Lee et al., 2015).

In an effort to mitigate these health risks, physicians have amended prenatal testing practices in obese females to allow for early detection of complications (Leddy et al., 2008). At present the molecular mechanisms by which maternal obesity imposes these detrimental effects on mother and offspring are not known.

2. Impact of Maternal Nutrition on Offspring

The concept that maternal diet impacts offspring health was introduced by David Barker in 1990, and proposed that the manifestation of adult cardiovascular disease was a result of intrauterine and neonatal environments; a novel concept since later childhood influences such as diet, housing conditions and familial income were originally attributed to adult disease (Barker, 1990). This concept later gave rise to Hales and Barker's "thrifty phenotype" hypothesis, which suggested that maternal under-nutrition forces an adaptive fetal response that impairs development of target organs (i.e., decreased pancreatic beta-cell mass and islet function) leading to disadvantaged fetal growth and permanently altered metabolism (Hales and Barker, 1992). Hales and Barker also suggested a contribution by infant malnutrition in their hypothesis. Poor fetal and infant nutrition would be advantageous in offspring who remained malnourished into adulthood; however, because these offspring are metabolically pre-adapted to famine-like conditions, overnutrition renders them susceptible to type 2 diabetes and insulin resistance (Hales and Barker, 2001).

While these studies focused on undernourished fetal environments, later work demonstrated a similar pre-disposition to metabolic disruptions in fetuses exposed to over-nourished environments, and proposes that maternal obesity impacts offspring metabolism well into adulthood. Maternal overnutrition is positively associated with increased offspring BMI and an increased occurrence of insulin resistance from birth through adulthood (Hochner et al., 2012; Reynolds et al., 2010). Children and adult offspring of obese mothers showed a higher incidence of hypertension, increased serum glucose and triglycerides, and decreased levels of HDL cholesterol (Clausen et al., 2009; Godfrey et al., 1994; Hochner et al., 2012; Laor et

al., 1997). While the fetal impacts from maternal obesity are clearly apparent, the contributions of socioeconomic status, diet, genetics, and physical activity during childhood and adulthood in offspring of obese mothers may also contribute to these negative outcomes on offspring health (Davey Smith et al., 2009). However, studies in siblings from obese mothers suggested that the intrauterine environment does contribute to offspring metabolic health (Clausen et al., 2009; Kral et al., 2006).

There is also evidence demonstrating the transgenerational impact of maternal obesity, albeit from animal models. F2 and F3 offspring of obese female mice exhibit increased body weight, and in swine the F2 generation shows increased adiposity and altered lipid profiles (Dunn and Bale, 2011, 2009; Gonzalez-Bulnes et al., 2014). However, in the mouse model, insulin sensitivity is reduced in the F2 generation, but is normalized in the F3 offspring (Dunn and Bale, 2011, 2009).

Given the endemic increase in maternal obesity in Western society, several studies have focused on using animal models to define the modes by which a calorically excessive maternal diet impacts offspring development and health. Key factors that are thought to contribute to this phenomenon include epigenetics, mitochondrial dysfunction, insulin resistance, endoplasmic reticulum (ER) stress, and immune system disruption.

Epigenetics

Epigenetic control of gene expression is required for normal cellular functions and accounts for differences in gene expression between cell types. Epigenetic gene silencing can occur by the methylation of cytosine in CpG sites, histone modifications (either via acetylation or methylation) that can repress or activate gene expression, and RNA silencing that can promote the formation of heterochromatin, histone modifications, or DNA methylation (Egger et al., 2004).

In addition to regulating basic cellular functions, epigenetics also has a role in modifying gene expression in response to environmental factors such as diet. With regards to obesity, human studies have demonstrated a correlation between increased maternal BMI and offspring DNA methylation in neonates (cord blood) and in later childhood (Herbstman et al., 2013; Sharp et al., 2015). Interestingly, sites of DNA methylation in neonates from overnourished mothers were different from the modified sites of neonates from undernourished mothers, suggesting that differential gene regulation is dependent on specificities of the maternal environment (Sharp et al., 2015). A similar trend was also reported in rodent models of maternal obesity demonstrating an impact on methylation and expression of genes that regulate behavior (Vucetic et al., 2010), this is of particular interest because maternal obesity has been implicated in the occurrence of behavioral disorders (Buss et al., 2012).

Mitochondrial dysfunction

Mitochondria utilize oxidative phosphorylation to generate the majority of ATP in the cell, making them the main center of cellular energy metabolism. Unlike other organelles, mitochondria contain a functional genome (mtDNA) that encodes for a portion of the genes required for energy production. Because mitochondria are maternally inherited, maternal

mutations in mtDNA or disruptions in mitochondrial function are passed along to the offspring. This characteristic of mitochondria makes it a prime vessel by which obese mothers may predispose their offspring to metabolic dysfunction.

Recent reports have demonstrated that female overnutrition contributes to mitochondrial disruption in mother and offspring (Szendroedi et al., 2012). Oocytes of obese females exhibited numerous mitochondrial defects including abnormal mitochondrial distribution, altered mitochondrial membrane potential, and increased swelling and vacuole number relative to lean controls (Igosheva et al., 2010; Luzzo et al., 2012; Wu et al., 2015; Wu and Brown, 2006). In rodent offspring of obese females, levels of hepatic electron transport chain complexes (ETC; I, II, III, and IV) and *SIRT3* and *peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α)* mRNA, key regulators of fatty acid oxidation and mitochondrial biogenesis, are reduced (Borengasser et al., 2014; Bruce et al., 2009; Kong et al., 2010; Taylor et al., 2005). There are conflicting reports as to whether mtDNA levels are altered in oocytes from obese females and the exact impact of maternal obesity on mitochondrial membrane potential; however, these contrasting reports may be attributed to strain and diet differences (Igosheva et al., 2010; Luzzo et al., 2012; Wu et al., 2010). Though some data is conflicting, there is the consensus that maternal obesity negatively impacts oocyte mitochondria.

Studies using human umbilical cord blood have correlated maternal nutrition with offspring mitochondrial dysfunction. Cord blood of newborns from obese mothers exhibited significantly decreased levels of mtDNA content compared to offspring from healthy mothers; however, mtDNA content of offspring from undernourished mothers was also decreased, suggesting maternal malnutrition in general impacts mitochondrial function (Gemma et al., 2006). A subsequent study demonstrated a positive correlation between increasing maternal BMI and methylation of the offspring PGC-1α promoter, suggestive of an epigenetic mechanism (Gemma et al., 2009). There have also been reports demonstrating increased oxidative stress in offspring cord blood from obese mothers (Gallardo et al., 2015; Malti et al., 2014). Although oxidative stress is not a direct measure of mitochondrial function, because mitochondria are a major source of cellular reactive oxygen species generation, it is suggestive of an impact on the organelle. While many animal studies highlight an association between maternal obesity and offspring mitochondrial dysfunction, more research is needed to strengthen this correlation in humans.

Insulin resistance

Insulin serves as a major regulator of lipid and glucose homeostasis, controlling several downstream signaling pathways that allow cells to respond to changes in nutrient availability (Cheng et al., 2010; Saltiel and Kahn, 2001). Failure of cells to respond to insulin is a key feature of obesity and type 2 diabetes, resulting in hyperlipidemia, hyperglycemia, and hyperinsulinemia (Saltiel and Kahn, 2001).

Both rodent and human studies have suggested a link between maternal obesity and offspring insulin resistance. Human offspring of obese mothers are insulin resistant *in utero* and as adults (Patrick M. Catalano et al., 2009; Mingrone et al., 2008; Tan et al., 2015). In rodents, adult offspring of obese females exhibit increased adiposity and elevated fasting

plasma insulin levels and develop β-cell exhaustion and hyperglycemia (Fernandez-Twinn et al., 2014; Samuelsson et al., 2008; Srinivasan et al., 2006). Exactly how maternal overnutrition contributes to offspring insulin resistance is not known. Interestingly, in obese rodents and humans, there is a strong correlation between insulin resistance and the occurrence of mitochondrial dysfunction; however, whether insulin resistance leads to mitochondrial disruption or vice versa remains up for debate (Cheng et al., 2009; Goo et al., 2012; Kelley et al., 2002; Morino et al., 2005; Petersen et al., 2004; Sleigh et al., 2011; Stump et al., 2003).

ER stress

ER stress is a key molecular characteristic of obesity and treatment with ER chaperones not only alleviates hepatic ER stress in rodents, but also restores systemic insulin sensitivity and glucose homeostasis (Ozcan et al., 2006, 2004). Interestingly, ER stress is also present in the oocytes and offspring of obese female rodents (Melo et al., 2014; Wu et al., 2015). Pharmacological inhibition of ER stress with either salubrinal or BGP-15, an inducer of sarcoplasmic reticulum Ca^{2+} ATPase activity, improved oocyte function, resulting in an increased rate of ovulation and improved oocyte viability and blastocyst development, oocyte mitochondrial membrane potential and mtDNA copy number were also normalized (Wu et al., 2015). Additionally, alleviating ER stress also normalized mtDNA copy number in the offspring kidney and liver, highlighting a potential link between ER stress and offspring mitochondrial dysfunction (Wu et al., 2015).

Immune system disruption

Inflammation is a characteristic of obesity and is implicated in obesity-related insulin resistance and other metabolic dysfunctions (Gregor and Hotamisligil, 2011). Maternal obesity causes increased circulating cytokines and chemokines in the mother and is associated with disruptions to the offspring immune system (Challier et al., 2008; Frias et al., 2011; Schmatz et al., 2010). Pregnant obese humans exhibit placental macrophage accumulation and increased levels of TNFα, IL-1, and IL-6 (Challier et al., 2008; Frias et al., 2011; Schmatz et al., 2010). The infant cord blood from obese mothers also has increased cytokines and chemokines as well as reduced immune cell populations (Wilson et al., 2015). Children of overnourished mothers exhibit increased levels of circulating pro-inflammatory factor C-reactive protein and have a higher risk of developing asthma (Forno et al., 2014; Leibowitz et al., 2012). Murine models of maternal obesity report offspring with increased susceptibility to and decreased survival from bacterial infections such as *E. coli* and *Staphylococcus aureus* (Myles et al., 2013).

3. Paternal obesity and offspring health

The impact of paternal traits such as diet, age, and smoking to offspring health has been demonstrated in several human studies (El-Helaly et al., 2011; Veenendaal et al., 2013). An appreciation for the contribution of paternal overnutrition to offspring health is emerging. A relationship between male BMI and sperm impairment has been shown, specifically higher BMIs correlated with increased sperm DNA damage and reduced mitochondrial activity (Fariello et al., 2012). Studies investigating the contribution of paternal obesity to offspring

health demonstrated a correlation between increased paternal BMI and increased offspring weight (Chen et al., 2012; Danielzik et al., 2002; Patel et al., 2011). There is also evidence of an association between paternal obesity and methylation of metabolic genes in cord blood, suggestive of an epigenetic mechanism (Soubry et al., 2013). In rodents, several models of paternal obesity demonstrated a negative impact on offspring metabolic gene expression in adipose tissue and the pancreas, insulin disrupted embryonic development, mitochondrial activity, and reproductive function (Binder et al., 2012; Ng et al., 2014).

4. Drosophila as a model system for obesity

Much work has been achieved using rodent models to understand maternal obesity and its impact on offspring health; however, the fact remains that many studies, while informative, remain correlative and have not yet identified the molecular pathways at play. Recently, *Drosophila melanogaster* has emerged as a powerful model organism to identify and characterize the molecular mechanisms involved in obesity and other human metabolic disorders (Baker and Thummel, 2007; Birse et al., 2010; Fernández-Moreno et al., 2007; Musselman et al., 2011; Na et al., 2013; Sanchez-Martinez et al., 2006).

Many mammalian tissues important in lipid and carbohydrate metabolism can also be found in the fly (Liu and Huang, 2013; Akhila Rajan and Perrimon, 2013). The *Drosophila* midgut is the site of dietary nutrient absorption and digestion, mirroring functions of the mammalian stomach and small intestine. Carbohydrate and fat storage and lipid mobilization occur in the fat body – making this tissue analogous to mammalian liver and white adipose tissue. Secretion of adipokinetic hormone from the fly corpora cardiaca cells in the ring gland is equivalent to the secretion of glucagon from pancreatic α-cells (Liu and Huang, 2013; A Rajan and Perrimon, 2013). Production and secretion of insulin is also present in the fly; however, because *Drosophila* lack a pancreas insulin-like-peptides (dILPs 1–8) are secreted from insulin producing cells (IPCs) in the median neurosecretory region of the brain and from the fat body (Liu and Huang, 2013; A Rajan and Perrimon, 2013).

The presence of these analogous tissues in the fly underscores the fact that mammalian carbohydrate and lipid metabolic systems are highly conserved in *Drosophila*. Insulin signaling in the fly is stimulated during nutrient abundance by the leptin homolog Unpaired 2 (Upd2) and ablation of IPCs or Upd2 results in increased circulating sugars (Rajan and Perrimon, 2012; Rulifson et al., 2002). dILPs bind and activate the insulin receptor causing downstream activation of the PI3K-AKT/PKB pathway as in the mammalian system (Garofalo, 2002). The fat body stores triacylglycerides (TAG) in lipid droplets and serves as the site of *de novo* TAG biosynthesis utilizing enzymes homologous to mammalian glycerol-3-phosphate acyltransferase, 1-acylglycerol-3-phosphate-O-acyltransferase, phosphatidate phosphatase, and diacylglycerol acyltransferase (Liu and Huang, 2013). Lipid metabolism in the fly is also controlled by the highly conserved sterol regulatory element-binding protein (SREBP) pathway (Dobrosotskaya et al., 2002; Seegmiller et al., 2002). However, while mammalian SREBP responds to changes in cellular sterol levels, in the cholesterol auxotrophic fly, SREBP activity is instead regulated by phosphatidylethanolamine (Dobrosotskaya et al., 2002). The high conservation of both

metabolic tissue and biosynthetic and signaling pathways in the fly, make *Drosophila* an ideal organism for modeling human metabolic diseases.

Drosophila offer well-developed genetics that allow for rapid whole-body, tissue-specific, and developmental stage-specific gene manipulation. Massive transgenic libraries exist for the fly including over 26,000 RNAi lines targeting 91% of the *Drosophila* protein-coding genome (Center, 2015; Dietzl et al., 2007). Overexpression lines covering a vast portion of the genome are also available, allowing for the expression of a plethora of various versions of a single gene. Additionally, generating new mutant fly lines with specific gene mutations is straightforward and rapid and can employ the use of transposons and CRISPR (Bassett et al., 2013). One prime example of employing the sophistication of fly genetics to better understand mammalian metabolism is a *Drosophila* RNAi obesity screen in which 11,594 transgenic RNAi lines targeting 10,489 open reading frames were used to identify 500 candidate genes of triglyceride regulation (Pospisilik et al., 2010). Interestingly, this approach uncovered a novel function for hedgehog signaling in fly fat body that extended to white and brown adipose determination in mammals (Pospisilik et al., 2010). Because many components of maternal obesity (i.e., ER stress, insulin signaling, immunity, and mitochondrial dysfunction) are intricately linked, being able to genetically manipulate one factor to address the impact on another in a disease setting, which the fly system affords, is invaluable in determining how each component contributes to a specific disorder (Gregor and Hotamisligil, 2011; Hotamisligil, 2010; Ozcan et al., 2004; Wu et al., 2015).

Pre-pregnancy obesity is associated with offspring cellular and organismal disruption; however, because mammalian embryogenesis occurs within the mother, determining the impact of pre-gestational versus gestational maternal obesity on offspring health would be highly invasive in humans, requiring *in vitro* fertilization (IVF). In mice, IVF has been used to specifically assess pre-gestational influences of maternal obesity, and has shown that embryos from obese mothers transplanted into non-obese females still exhibited increased fetal weight, decreased hepatic, cardiac, and renal mtDNA content, and increased hepatic mtDNA variants compared to embryos transplanted from lean littermates (Wu et al., 2015). Because fly embryogenesis occurs outside of the female physiologic milieu, the contribution of pre-gestational maternal obesity to offspring health can be specifically investigated without the use of invasive and time-consuming procedures. Additionally, the brief life cycle of the fly, 10 days from embryo to adult, is extremely beneficial for elucidating the mechanisms behind the transgenerational impact of maternal obesity since it allows studies on F3 and subsequent generations to be conducted in a short period of time.

The ease and speed of dietary manipulation in the fly also make it an ideal model for obesity research, primarily because fly diets can be produced within the lab. Concentrations of various fats and carbohydrates, as well as other dietary components, can be easily adjusted or omitted in fly diets and several methods exist to quantify the ingestion of food by the fly (Deshpande et al., 2014). Moreover, addition of specific compounds or pharmaceuticals can also be added to assess their therapeutic potential (Agrawal et al., 2005; Kang et al., 2002). With regards to immunity, measured amounts of bacteria can also be incorporated into diets to elicit an immune response (Neyen et al., 2014).

Obesity can be induced in the fly by either high-fat-diet (HFD) or high-sucrose-diet (HSD) feeding and mimics the pathophysiology of obesity observed in humans and rodents – insulin resistance, hyperglycemia, lipid accumulation, and increased expression of lipogenic and gluconeogenic genes (Birse et al., 2010; Musselman et al., 2011; Na et al., 2013; Pasco and Leopold, 2012). HSD feeding in larvae is known to cause increased hyperglycemia, systemic insulin resistance, developmental delays, and increased dILP2 secretion (Musselman et al., 2011). HFD feeding disrupted *Drosophila* cardiac function, resulting in cardiac lipid accumulation, reduced contractility, and structural damage, as has been reported in human and rodent obesity studies (Birse et al., 2010). Using the HFD model, it was discovered that disruption of the insulin-TOR pathway inhibits obesity-induced cardiac dysfunction (Birse et al., 2010). HSD feeding also disrupted cardiac function and was shown to be dependent on the hexosamine biosynthetic pathway (Na et al., 2013). New roles for other metabolic regulators such as SREBP, PGRC-1, and Retinol-Binding Protein 4 have also been identified using the *Drosophila* obesity model (Diop et al., 2015; Pasco and Leopold, 2012). These and other studies highlight the benefits of using *Drosophila* to identify novel regulators of obesity that have the potential to serve as therapeutic targets in humans.

5. Contributions of paternal obesity to offspring health in the fly

Given the conservation of metabolic pathways and ease of dietary manipulation, the fly has emerged as a valuable tool to uncover the molecular mechanisms whereby paternal diet influences offspring health (Ost et al., 2014; Valtonen et al., 2012). Male flies exposed to a protein-poor diet from the embryonic state to adulthood produced offspring with shortened developmental times and larger male offspring compared to males raised on standard diet (Valtonen et al., 2012).

Valtonen *et al.* also went on to investigate the impact of exposing both parents to a protein-poor diet and demonstrated that offspring of these flies exhibited extreme developmental delays relative to offspring from control parents and offspring with only one parent on a protein-poor diet (Valtonen et al., 2012).

On the other spectrum, offspring from parents exposed to a high-protein, albeit low-sucrose, diet from the first instar larval stage to adulthood had a shorter developmental time compared to offspring from low-protein, high-sucrose parents; however, there was no difference in survival between the two groups of offspring (Matzkin et al., 2013). The high-protein parental diet also produced female offspring with increased egg production. These studies analyzed parental diet and, thus the contribution of altering protein and sucrose levels solely of the paternal diet was not investigated. However, in light of findings by others who demonstrated that manipulation of the paternal fly diet impacts the offspring, these data suggest a paternal effect may have contributed to the results reported by Matzkin *et al.*

Alterations to the offspring genome are also dependent on paternal diet (Aldrich and Maggert, 2015; Ost et al., 2014). Male flies exposed to a protein-rich diet during adulthood sired females with decreased rDNA copy number, which was rescued by supplementing the paternal diet with the mTOR inhibitor rapamycin. Interestingly, the impact on offspring

rDNA copy number was also observed in the F2 generation demonstrating the transgenerational impact of paternal diet in *Drosophila* (Aldrich and Maggert, 2015). The contribution of paternal-diet induced rDNA instability to offspring gene expression and therefore health is unknown; however rDNA integrity has been associated with global chromatin state changes as well as organismal longevity (Kobayashi, 2011; Kwan et al., 2013; Paredes and Maggert, 2009). These data highlight rDNA instability as a potential mechanism by which paternal-diet may impact offspring health.

An altered offspring chromatin state has been associated with changes in paternal-diet (Ost et al., 2014). Using adult male flies, Ost *et al.* demonstrated paternal TAG levels increased in a concentration dependent manner relative to dietary sucrose levels. This concentration dependent trend was transferred to the offspring since the body weight of adult progeny increased relative to paternal-diet sucrose concentrations. Moreover, when these offspring were challenged with a high-sucrose diet, body weight, TAG levels and lipid droplet size increased, suggesting these offspring are pre-sensitized to an obesogenic diet as a result of the paternal diet. Interestingly, only two days of high-sucrose paternal feeding was needed to induce this offspring obese phenotype. No changes were observed in offspring developmental timing or size relative to the paternal diet. Additionally, there was no indication of a transgenerational effect of the high-sucrose paternal diet. Analysis of adult offspring heterochromatin revealed progeny of high-sucrose fed males exhibited desilencing of peri-centric heterochromatin on the X chromosome, which correlated with an overall increase in gene expression (including several involved in energy metabolism and of unknown function) during the embryonic state of offspring from high-sucrose males. These observations suggest altered chromatin state as a potential mechanism of paternal-linked transgenerational inheritance. Interestingly, this trend of derepression was also evident in the sperm of high-sucrose fed fathers, implicating alterations in sperm gene expression as a vehicle for changes observed in offspring.

6. Maternal obesity in the fly

We and others have utilized the numerous genetic and developmental benefits of the fly to further understand the contribution of altered maternal diet on the health of the offspring and subsequent generations (Buescher et al., 2013; Matzkin et al., 2013; N.G. Prasad, Mallikarjun Shakarad, 2003; Valtonen et al., 2012; Vijendravarma et al., 2010). Most maternal diet studies have focused on undernourished conditions and highlight the fact that females reared on a protein- and sucrose-poor diet produced heavier eggs (Vijendravarma et al., 2010). When only dietary protein levels are altered, females reared on protein-poor conditions produced larger F1 offspring with longer developmental times relative to progeny of protein-rich females (Valtonen et al., 2012). With regards to offspring-survivorship, offspring of protein-poor females exhibited increased survival at the larval stage relative to offspring of protein-rich females, however, this advantage disappeared by the pupal stage (N.G. Prasad, Mallikarjun Shakarad, 2003). Interestingly, while offspring of protein-poor females are larger, actual egg production and ovary size are negatively correlated with dietary protein levels (Drummond-Barbosa and Spradling, 2001). The molecular mechanisms controlling female reproductive capacity and offspring health as a function of fly diet are not fully understood; however, several studies have demonstrated a requirement

for Hedgehog and insulin signaling pathways in diet-induced ovarian stem cell proliferation (Drummond-Barbosa and Spradling, 2001; Hartman et al., 2013; Hsu and Drummond-Barbosa, 2009; Hsu et al., 2008).

Studies of overnutrition in the fly have primarily focused on overall parental contributions to offspring health rather than specifically on the maternal dietary effect. To elucidate the molecular mechanisms that impact offspring health specifically as a result of maternal obesity, we developed a *Drosophila* model in which female adults were exposed to either high-sucrose or low-sucrose diets (HSD and LSD, respectively) prior to mating with standard diet-fed males (Buescher et al., 2013) (Figure 1). HSD females exhibited an obese-like phenotype characterized by increased levels of whole-body TAG, as seen in the paternal fly obesity model (Ost et al., 2014), glycogen, and the disaccharide trehalose as well as a failure to respond to insulin and elevated insulin-like peptide levels (Buescher et al., 2013, Duncan unpublished results).

Male offspring of HSD females possessed metabolic disruptions at both larval and adult developmental stages, while larval female offspring displayed decreased cholesterol levels relative to offspring from LSD females (Buescher et al., 2013). In contrast to the paternal fly obesity model, no difference in whole-body TAG was observed in the offspring (Ost et al., 2014). Whole animal gene expression profiling using RNA sequencing of male larval offspring from HSD and LSD females revealed many differentially regulated genes between these two progeny groupings, which were later confirmed by qPCR. Of these genes, several were regulators of lipid and carbohydrate metabolic pathways including Cpt1, PDK, DHR38, and the SREBP target genes FAS and dACC (Buescher et al., 2013).

Adult offspring of HSD females challenged with an obesogenic diet exhibited increased levels of whole-body trehalose, glycogen, and TAG along with differential expression of carbohydrate and lipid metabolic genes relative to challenged control offspring, suggesting that maternal obesity in the fly predisposes offspring to adiposity and metabolic dysfunction, similar to reports in rodents (Buescher et al., 2013). Moreover, a transgenerational effect was observed wherein both male and female F2 larvae of HSD females had altered body composition, this is in contrast to studies of paternal fly obesity (Buescher et al., 2013; Ost et al., 2014). Similar to reports from maternal obesity rodent studies, HSD female ovaries exhibited impaired mitochondrial function and altered number (Duncan unpublished results), thus current studies in the lab are focused on addressing the contribution of mitochondrial inheritance to these phenomena of maternal overnutrition.

6. Conclusion

Maternal obesity is not only detrimental to maternal and offspring health, but it also threatens the health of subsequent generations. While current rodent models of maternal overnutrition have identified factors that contribute to maternal programming, the molecular mechanisms underlying these factors remain unknown. We review a *Drosophila melanogaster* model of maternal obesity that imparts tools unique to the fly – highly developed genetics, easy diet manipulation, and a rapid life cycle – that will aid in elucidating molecular pathways of maternal programming. Many of the known factors that

contribute to maternal obesity are interconnected, thus being able to study these components individually will provide insight into their contributions to maternal overnutrition, a feat employable in the fly. For these reasons and the high conservation of metabolic pathways between humans and flies, *Drosophila* is an invaluable tool for understanding the complexity of maternal programming.

Acknowledgments

This work was supported by grants from the American Heart Association, 11IRG5450013 and 12GRNT12080056 (J.G.D), DRTC P30DK020579 (J.G.D) and the National Institutes of Health, K12HD001459 (R.T.B).

Abbreviations

BMI	body mass index
ER	endoplasmic reticulum
ETC	electron transport chain
PGC-1α	peroxisome proliferator-activated receptor-γ coactivator 1α
dILPs	*Drosophila* insulin-like-peptides
IPCs	insulin producing cells
Upd2	Unpaired 2
TAG	triacylglycerides
SREBP	sterol regulatory element-binding protein
IVF	*in vitro* fertilization
HFD	high-fat-diet
HSD	high-sucrose-diet
LSD	low-sucrose-diet

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Highlights

- Maternal obesity is a rapidly growing health concern for both mother and offspring
- Epigenetics, the ER, and mitochondria are avenues of potential impact on progeny
- Molecular mechanisms that connect maternal overnutrition to these avenues are unknown
- *Drosophila* has provided insight into obesity and other human metabolic diseases
- A maternal obesity fly model mirrors findings in mammals and may aid in mechanistic insight

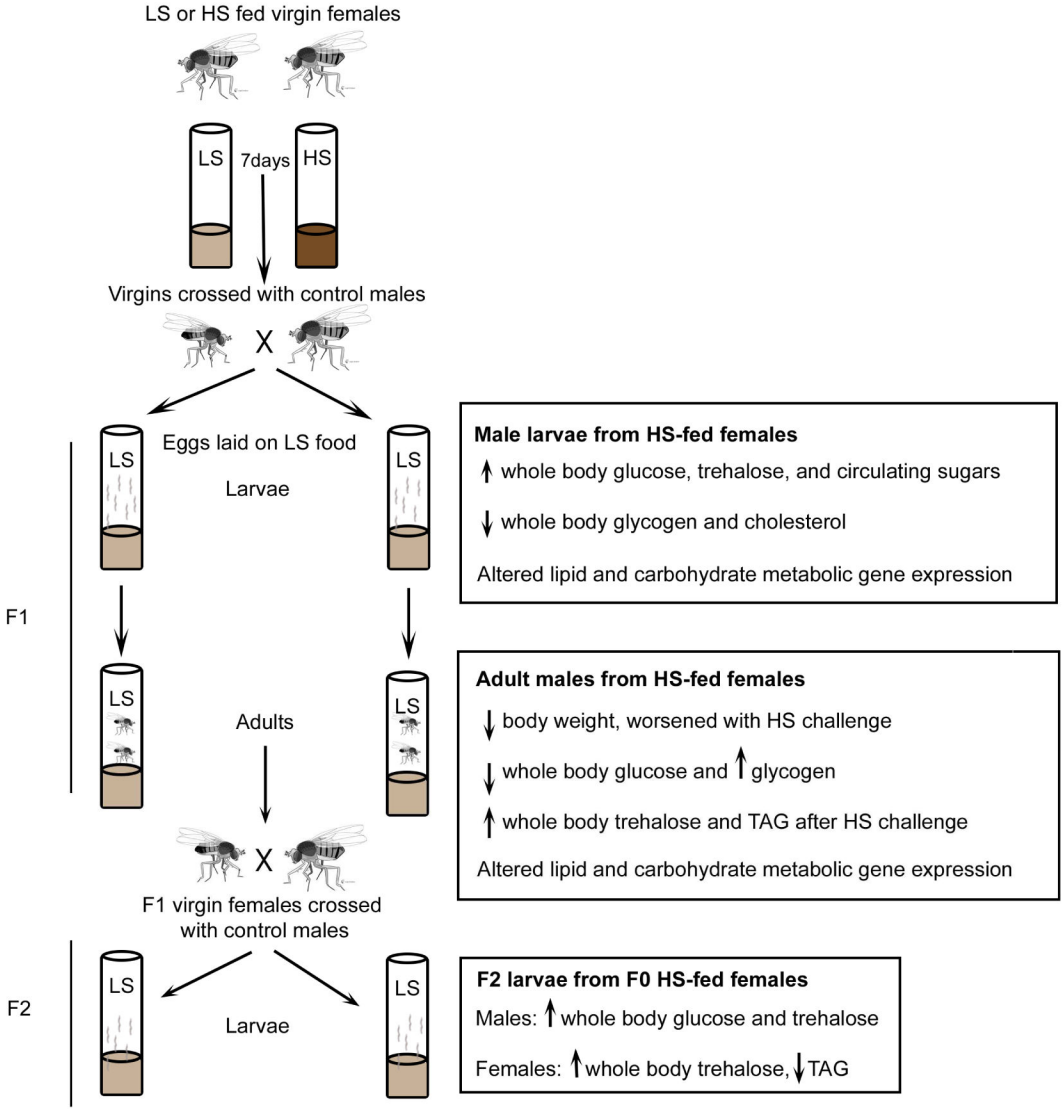

Fig. 1. *Drosophila* model of maternal obesity

Virgin w^{1118} female flies are fed LS or HS diet for 7 days and crossed with w^{1118} males fed stock food. All eggs for F1 and F2 generations are laid on LS food. Body composition changes and gene expression alterations are shown for each generation at the indicated developmental stage.