High-sucrose-induced maternal obesity disrupts ovarian function and decreases fertility in *Drosophila melanogaster*

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Abstract

As the obesity epidemic worsens, the prevalence of maternal obesity is expected to rise. Both high-fat and high-sucrose diets are known to promote maternal obesity and several studies have elucidated the molecular influence of high-fat feeding on female reproduction. However, to date, the molecular impact of a high-sucrose diet on maternal obesity remains to be investigated. Using our previously reported *Drosophila* high-sucrose maternal obesity model, we sought to determine how excess dietary sucrose impacted the ovary. High-sucrose diet (HSD) fed adult females developed systemic insulin resistance and exhibited an ovarian phenotype characterized by excess accumulation of lipids and cholesterol in the ovary, decreased ovary size, and impaired egg maturation. We also observed decreased expression of antioxidant genes and increased protein carbonylation in the ovaries of HSD females. HSD females laid fewer eggs; however, the overall survival of offspring was unchanged relative to lean control females. Ovaries of HSD females had increased mitochondrial DNA copy number and decreased expression of key mitochondrial regulators, suggestive of an ineffective compensatory response to mitochondrial dysfunction. Mitochondrial alterations were also observed in male offspring of obese females. This study demonstrates that high-sucrose-induced maternal obesity promotes insulin resistance, while disrupting ovarian metabolism and function.

Keywords

Obesity; *Drosophila*; ovary; mitochondria; pregnancy; nutritional programming

1. Introduction

In recent years, maternal obesity has emerged as a major health concern not only for the mother but also for the offspring. In the United States alone, 19-38% of pregnant women are

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obese and thus at increased risk for gestational diabetes mellitus, pre-eclampsia, miscarriage, caesarian delivery, hypertension, and preterm birth [1–5]. The offspring of obese mothers exhibit a higher incidence of neural tube defects, fetal macrosomia, shoulder dystocia, orofacial clefts, high birth weight, and, in later childhood, type 2 diabetes mellitus, obesity, and cardiovascular disease [1,3,6–10]. There is also a strong association between maternal obesity and offspring congenital heart defects [11].

To understand the molecular mechanisms by which maternal obesity impacts female fertility and offspring health, several reports have employed the use of high-fat or high-fat-high-sucrose diet rodent models. From these studies it is clear that high-fat and high-fat-high-sucrose feedings contribute to a disrupted ovarian phenotype that includes endoplasmic reticulum stress, mitochondrial dysfunction, intracellular lipid accumulation, and apoptosis that correlate strongly with the decreased fertilization rates, delayed offspring development, and fetal brain abnormalities also observed in these same model systems [12–15]. Despite the strong associations between excess sugar consumption and obesity and its co-morbidities, only a few studies have investigated the specific contribution of sucrose to maternal obesity and offspring health [16–23]. Moreover, to date, the role of excess sucrose consumption on the molecular features of maternal obesity remains to be explored.

Drosophila melanogaster has emerged as an invaluable tool in metabolic disease research in part due to the high conservation of metabolic tissues and pathways in the fly, well developed metabolic methodology, ease of diet manipulation, and rapid life-cycle [24–28]. Additionally, in female flies, the ovary is the largest and one of the most well characterized organs in *Drosophila*, providing a highly accessible way to study egg maturation under various genetic and environmental conditions that mimic human diseases [29]. The fact that diet and insulin can control ovary size, ovariole stem-cell proliferation, and egg production is well documented and further underscores the benefits of using female *Drosophila* to investigate the impact of diet on reproduction [30– 34]. For these same reasons, we and others have used the fly to further understand the influence of nutrition on both parental and offspring health [35–41]. We previously described a *Drosophila* maternal obesity model in which adult females fed a high-sucrose diet (HSD) exhibited an obese-like phenotype characterized by increased levels of whole-body triglycerides and the disaccharide trehalose, the most abundant circulating sugar in the fly [39]. Offspring of high-sucrose fed females showed altered metabolism and disrupted expression of carbohydrate and lipid metabolic pathway genes, and went on to produce progeny with altered body composition — highlighting the transgenerational impact of maternal obesity [39].

We employed our *Drosophila* model to study the impact of sucrose-induced maternal obesity on fertility and offspring survival. Our data demonstrate that high-sucrose-induced maternal obesity promotes insulin resistance, while disrupting ovarian function and altering maternal and offspring mitochondria.

2. Methods and Materials

2.1 Primers

Primers used for quantitative PCR (qPCR) were: *dILP2 F* 5′-AGGTGCTGAGTATGG TGT-3′, *R* 5′-TATCGAGTTATCCTCCTCCT-3′; *dILP3 F* 5′-AACTCTCTCCAAGCTCTGT-3′, *R* 5′-GAACCGAACTATCACTCAAC-3′; *dILP5 F* 5′-CTTGATGGACATGCTGAG-3′, *R* 5′-AAGTGGTCCTCATAATCGAA-3′; *ATP synthase F* 5′-TGCATCTAAAGCCGATAAGAATTTG-3′, *R* 5′-TCATCAAACTGGACATCGACG-3′ *Cytochrome c oxidase I F* 5′-AAAGTTGACGGTACACCTGG-3′, *R* 5′-AGGAACACTTTCAATTACAATCGG-3′; *mt-TFB1 F* 5′-TCCCTAGTTCGTAAGCGTTTTC-3′, *R* 5′-ACCAGCTCCCGAATTGTG-3′ *Spargel F* 5′-ATTCGGTGCTGGTGCTTCCTT-3′, *R* 5′-CGTGCCTTCTGTCGTTCATCTTAC-3′; *delg F* 5′-CGCGTCCTGCAGCCAAAAC-3′, *R* 5′-GCGGAGCCGGACGACATC-3′; *Parkin F* 5′-GGCGGAAGCAGTCGATACAGGTGA-3′, *R* 5′-CCGGCGGGGAGAAGGATTTTG-3′; *Rosy F* 5′-GGTGGTGAGCCTGTTCTTCAAG-3′, *R* 5′-ACTGGTGTGTGGAATGTCTCGG-3′; *Cytochrome c oxidase III F* 5′-CACGAGAAGGAACATACC-3′, *R* 5′-GCGGGTGATAAACTTCTG-3′; *SOD1 F* 5′-CAAGGGCACGGTTTTCTTC-3′, *R* 5′-CCTCACCGGAGACCTTCAC-3′; *SOD2 F* 5′-AATTTCGCAAACTGCAAGC-3′, *R* 5′-TGATGCAGCTCCATGATCTC-3′; *GstD1 F* 5′-TCGCGAGTTTCACAACAGAA-3′, *R* 5′-TGAGCAGCTTCTTGTTCAGC-3′; *α Tubulin 84B F* 5′-ACACTTCCAATAAAAACTCAATATGC-3′, *R* 5′-CCGTGCTCCAAGCAGTAGA-3′

2.2 Fly Stocks

w^{1118} Drosophila stocks were obtained from the Bloomington Drosophila Stock Center and maintained in vials containing molasses-based fly food (recipe available upon request). All flies were housed at 25°C in a humidified, temperature-controlled incubator with a 12 hour on/off light cycle. Virgin female flies were collected from stocks and placed within 24 hours of eclosion on either a low-sucrose (LSD) or HSD diet for seven days as described previously [39]. Both foods were isocaloric for fat and protein and differed only in the amounts of sucrose added. Low and high sucrose food were composed of 0.15 mol/l and 1.0 mol/l sucrose, respectively.

2.3 Ovarian imaging and electron microscopy

Ovaries were isolated from low- and high-sucrose fed females, immediately placed on glass slides with PBS, and viewed with a Zeiss fluorescent microscope equipped with an Axiocamera. Number of stage 14 eggs were individually counted and total ovary area, length, and width were determined using ImageJ. For electron microscopy, ovaries were isolated from low- and high-sucrose fed females and immediately fixed in Modified Karnovsky's Fixative solution and processed for electron microscopy by the Washington University Research Electron Microscopy Core. Samples were viewed on a JEOL 1200EX electron microscope and images acquired via a high resolution CCD based camera. Mitochondrial size was determined using ImageJ.

2.4 Egg laying capacity and offspring survival studies

After seven days of low- or high-sucrose feeding, females were mated with w^{1118} males for 18 hours on grape agar plates supplemented with the appropriate low- or high-sucrose food. Eggs were counted and transferred to vials containing molasses-based food. Number of eggs that developed into wL3 larvae, then pupae, and finally adults was determined by counting individual offspring at each developmental stage and dividing by number of eggs laid.

2.5 Insulin sensitivity assay

Insulin sensitivity of adult females (3 flies pooled per biological replicate) was determined as previously described for wL3 larvae [42]. Briefly, live adults were rinsed in PBS, bisected, and pinned open prior to incubation with 0.5μM human insulin (Sigma-Aldrich; catalog# I0259) or control buffer (10mM HEPES) at room temperature for 15 minutes. Animals were transferred to lysis buffer and homogenized. Protein concentrations were quantified from lysates by bicinchoninic acid assay (ThermoFisher Scientific) and phosphorylated S505 AKT, total AKT, and actin were detected by Western blot using antibodies against phospho-*Drosophila* AKT (Ser505) #4054 and total AKT (pan) #4691 from Cell Signaling and β-actin #A2228 from Sigma-Aldrich. Blots were visualized and bands quantified using the LI-COR Odyssey imaging system.

2.6 Ovarian composition measurements

Measurements for triacylglycerides (TAG) and glucose were performed on ovaries (28 ovaries pooled per biological replicate) isolated from adult females, following methods adapted from a previous study that used whole-body adults and larvae [39]. Briefly, animals were rinsed in PBS, ovaries were dissected and homogenized in PBS + 0.1% Tween-20 and for glucose measurements 2μl of homogenate was added to 98μl of Infinity Glucose Hexokinase Liquid table Reagent (Fischer Scientific), incubated for 15 minutes at 37°C and absorbance measured at 340nm on a Tecan Infinite 200 PRO microplate reader. For ovarian TAG measurements, homogenized ovaries were incubated for 5 minutes at 65°C to inactivate lipases and 2μl added to 198μl of Thermo Infinity Triglyceride Reagent (ThermoFisher Scientific) and absorbance measured at 560nm on a microplate reader. Ovary cholesterol concentration (30 ovaries pooled per biological replicate) was measured as previously described using the Amplex Red Cholesterol Assay Kit (Invitrogen) and microplate reader [43]. Ovarian composition measurements were normalized to total protein.

2.7 Quantitative PCR

Adult female heads (5 heads pooled per biological replicate) and ovaries (6-14 ovaries pooled per biological replicate) or wL3 male larvae (5 larvae pooled per biological replicate) were collected, rinsed in PBS, and homogenized in TRIzol reagent (Invitrogen) followed by subsequent RNA isolation as per manufacturer's instructions. RNA was quantified and used to generated cDNA via the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Synthesized cDNA was used for quantitative PCR (qPCR) using SYBR Green (Applied Biosystems), appropriate primers, and performed in triplicate on a Stratagene Mx3005P qPCR machine. Relative quantification after normalization was calculated using

the $2^{-\Delta CT}$ formula. For ovarian gene expression 6-14 ovaries were pooled for each biological replicate.

For mitochondrial DNA quantification, larvae (5 larvae pooled per biological replicate) or ovaries (6-14 ovaries pooled per biological replicate) were homogenized in RNA-Bee (Tel Test) as per manufacturer's instructions to obtain total DNA, which was used in qPCR with the primers cytochrome c oxidase III (CG34074; mitochondrial gene) and Rosy (CG7642; nuclear gene). Relative quantification after normalization was calculated using the $2^{-\Delta CT}$ formula and values for cytochrome c oxidase III were subtracted from Rosy to determine amount of mtDNA per sample.

2.8 Protein Carbonylation Assay

Whole-cell lysates were prepared from homogenized ovaries (10-14 ovaries pooled per biological replicate) in RIPA buffer (25mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS) with EDTA-Free protease complete tablet (Roche), PMSF and 2% 2-mercaptoethanol. Homogenates were run through a 27 gauge needle 20-times followed by protein quantification. A total of 20µg of protein per sample was loaded onto 10% SDS PAGE gels. Carbonylated proteins were detected using the Oxyblot Protein Oxidation Detection Kit (S7150; EMD Millipore) as per the manufacturer's instructions. Blots were visualized and bands quantified using the LI-COR Odyssey imaging system.

2.9 Statistics

Data are expressed as mean ±standard error of the mean (SEM). Comparisons between groups were performed by unpaired, two-tailed Student's *t*-test.

3. Results

3.1 Insulin sensitivity and production are impaired in high-sucrose fed females

Our previous work demonstrated that female adult flies fed a high-sucrose diet exhibited an obese-like phenotype, characterized by elevated whole-body TAG, glycogen, and trehalose [39]. Because insulin resistance is a comorbidity of obesity and the insulin signaling pathway is highly conserved in *Drosophila,* we sought to determine whether this high-sucrose feeding also impacted insulin sensitivity. Following 7 days on an LSD or HSD diet, bisected adult females were exposed to human insulin to stimulate the insulin signaling pathway. Pathway activation was assessed by measuring levels of AKT phosphorylation on Serine 505, which is homologous to the mammalian AKT Serine 473 position (Figure 1A). Both HSD and LSD adult females responded to insulin treatment; however, LSD females showed a 9-fold increase in phosphorylated AKT levels compared to HSD females, demonstrating that stimulation of the insulin signaling pathway is impaired in HSD obese-like female flies. These studies are in agreement with previous work by Na, *et al.* which reported that mixed-sex adult populations fed a high-sucrose diet for 3 weeks are insulin resistant [26]. In the fly, *Drosophila* insulin-like peptides (dILPs) are homologs of mammalian insulin, and, like insulin, respond to changes in nutrient availability [44]. We next investigated whether insulin production was altered in HSD females by measuring *dILP* gene expression using qPCR. Compared to LSD flies, HSD females exhibited a decrease in

dILP 2 and *5*, while *dILP 3* levels remained unchanged (Figure 1B). These data demonstrate that, in addition to insulin resistance, insulin production is also altered in HSD females.

3.2 High-sucrose diet disrupts mature egg production and ovarian lipid and sterol homeostasis

Using our *Drosophila* model of maternal obesity, we noted that HSD females are quantitatively smaller than LSD controls (Figure 2A-B) and, as our group demonstrated previously, weigh less [39]. This decreased size is also evident in the ovaries, whereby, HSD females had ovaries that were 28% smaller than their LSD counterparts (Figure 2C). Moreover, this decrease in overall ovarian size positively correlated with a decrease in the number of mature (stage 14+) eggs (Figure 2D).

We previously reported that HSD females exhibited elevated whole-body TAG levels and increased carbohydrates that were indicative of an obese-like phenotype [39]. Recent studies in high-fat fed mice have shown that the ovaries of obese females possess an abundance of lipids [12]. To further characterize the high-sucrose ovarian phenotype, we measured levels of TAG, cholesterol, and glucose specifically in the ovary (Figure 2E). TAG and cholesterol were significantly increased in the ovaries of HSD females relative to the ovaries of LSD controls. There was no observable change in ovarian glucose levels under high-sucrose conditions. These data demonstrate that, as in mice, maternal obesity in the fly causes an abundance of lipids in the ovary; moreover, our data are the first to demonstrate that a high-sucrose obesogenic diet causes excess accumulation of cholesterol in the ovary.

Accumulation of excess lipids in non-adipose tissues is associated with oxidative stress, which can disrupt normal organelle and cellular functions [45]. Recent studies in humans have shown a positive correlation between increasing maternal BMI and oxidative stress biomarkers [46,47]. Moreover, reactive oxygen species levels are elevated in the oocytes of mice fed a high-fat diet [14]. Based on these reports and our observations that high-sucrose feeding increased ovarian TAG levels, we next investigated whether there was evidence of oxidative stress in the ovaries of HSD flies. Protein carbonylation was higher in the ovaries of HSD females compared to LSD controls by 66% (Figure 2G). There was also a modest, yet significant decrease in gene expression of the antioxidant genes SOD1 and SOD2 (Figure 2F). Together these data suggest the presence of oxidative stress in the ovaries of HSD females.

3.3 High-sucrose diet increases ovarian mitochondrial copy number and alters expression of key mitochondrial regulators

Excess cellular lipid and sterol levels are known to disrupt mitochondrial function *in vitro* [45,48]. Additionally, in high-fat murine models of maternal obesity, there is a strong correlation between oocyte lipid accumulation and mitochondrial abnormalities [12]. Because both TAG and cholesterol accumulation were elevated in the ovaries of HSD females, we investigated whether high-sucrose ovaries exhibited a disrupted mitochondrial phenotype by assessing mitochondrial ultrastructure, number, and gene expression. Unlike in high-fat fed murine maternal obesity models, visualization of mature egg mitochondria by electron microscopy failed to show structural defects or alterations in organelle size in HSD

flies (Figure 3A) [13]. However, quantification of mitochondrial DNA copy number demonstrated a 54% higher copy number in the ovaries of HSD females compared to LSD controls (Figure 3B). We next investigated whether sucrose feeding affected expression of genes involved in mitochondrial function and observed that, compared to LSD females, the ovaries of HSD females showed increased expression of *ATP synthase* and decreased expression of several genes including the mitochondrial gene *cytochrome c oxidase I; mt-TFB1,* which controls mitochondrial translation; the *Drosophila* PGC-1α homolog *Spargel, a* key regulator of mitochondrial biogenesis and mass; the NRF-2α homolog *delg, a* regulator of mitochondrial mass; and *Parkin,* a ubiquitin-protein ligase important in mitochondrial remodeling [49–53] (Figure 3C). These data indicate that excess sucrose consumption promotes a disrupted mitochondrial gene profile in the ovary.

3.4 High-sucrose maternal feeding decreases fertility and alters offspring mitochondria

Based on our observations that high-sucrose diet increased ovarian mitochondrial DNA copy number and altered gene expression and the fact that maternal mitochondria are inherited by the offspring, we next investigated whether excess dietary sucrose impacted fertility and offspring mitochondria. To measure fertility, LSD and HSD adult females were mated with molasses-diet fed males on grape agar (Figure 4). Grape agar plates were supplemented with the appropriate LSD or HSD food, in lieu of yeast paste, in an effort to maintain females on their respective diets. This was done to avoid any dampening of our HSD phenotype by the addition of excess yeast, while still providing necessary nutrients for egg laying. Eggs laid were counted after 18 hours of mating and transferred to molasses food where the number of embryos developing into wL3, pupae, and adults was quantified. Compared to LSD females, high-sucrose feeding reduced the number of eggs laid by 62% (Figure 4A). This decrease in offspring number persisted at the larval, pupal, and adult stages; however, overall survival of offspring from LSD and HSD females remained the same regardless of maternal diet and the developmental stage at which survival was measured (Figure 4B). These data demonstrate that high-sucrose maternal feeding decreases fertility; however, offspring survival is not impaired. We next measured expression of key mitochondrial regulators in the male larval offspring of LSD and HSD females and observed a significant increase in the expression of regulators of mitochondrial translation, biogenesis, and mass (Figure 5A). Larval offspring of HSD females exhibited a decreased mitochondrial DNA copy number compared to controls (Figure 5B). While these data suggest an altered mitochondrial phenotype in the offspring of obese mothers, they also demonstrate that the impact to mitochondria varies from that of HSD female ovaries.

4. Discussion

There is strong evidence supporting a key role for excess sugar consumption in the pathogenesis of obesity and associated co-morbidities [21–23]. As the obesity epidemic worsens, the prevalence of maternal obesity is expected to increase, posing severe health threats not only to the mother and offspring, but to subsequent generations as well [38,39,54]. While the impact of high sucrose consumption on maternal and offspring health has been demonstrated, the molecular mechanisms by which excess sucrose contributes to maternal obesity remain unknown [16–20]. Using a *Drosophila melanogaster* model of

maternal obesity, this study demonstrated that high sucrose consumption in adult females resulted in insulin resistance and a reproductive phenotype characterized by accumulation of TAG and cholesterol in the ovary, decreased egg production and fertility, and increased mitochondrial copy number and decreased expression of key mitochondrial regulators. We also show that this sucrose-driven mitochondrial phenotype extends beyond the mother since mitochondrial copy number and gene expression are also altered in the male offspring of HSD females.

Drosophila has recently emerged as a valuable tool for modeling the metabolic aspects of obesity and diabetes [25,55]. We and others have demonstrated that high-sucrose and high-fat feeding results in metabolically obese larvae and flies, characterized by metabolic abnormalities that include insulin resistance, hyperglycemia, increased whole-body TAG levels, accumulation of lipids in non-adipose tissues, cardiomyopathy, decreased lifespan, and altered gluconeogenic and lipogenic gene expression [26–28,39,42,56–58]. Unlike in mammals, the largest organ in female *Drosophila* is the ovary, thus under high-sucrose conditions, this organ's size is diminished compared to low-sucrose fed females, which may account for the decrease in overall organismal size we observed (Figure 2A-C) [29]. We report here and previously that HSD-females share many of the same biological and reproductive impairments as rodent maternal obesity models, including excess circulating sugars, TAG accumulation both in whole-body and in the ovaries, decreased fertility, a mitochondrial phenotype, and a metabolic impact on the offspring [12,39,59]. *Drosophila* obesity models have also uncovered new roles for developmental and metabolic genes in obesity-associated diseases, in addition to exploring the impact of obesity on epigenetic inheritance [26–28,38,57,60–62].

This study demonstrates that HSD adult females have a blunted response to insulin stimulation compared to LSD females. We observed differences in the levels of whole-body phosphorylated AKT between insulin-stimulated LSD versus HSD females that were higher than a recently published report using *Drosophila* mixed-sex populations (Figure 1A) [26]. Although both studies showed that HSD feeding is associated with insulin resistance, the quantified degree of AKT phosphorylation observed between the two studies may have been affected by several differences including the amounts of exogenous insulin used (our 0.5μM versus Na *et. al*.'s 1.0μM concentration) [26]. The use of females versus mixed-sex populations in these experiments may also account for the observed differences, especially given the knowledge that in *Drosophila* the response to dietary manipulation varies by gender and reports suggest that sex may influence insulin-signaling [26,63–65]. Differences in the degree of phosphorylation may have also been influenced by the length of time flies were placed on sucrose diet (our 7 day treatment versus the Na *et al.* 21 day exposure), since, as has been shown for high-fat fed flies, AKT phosphorylation becomes reduced overtime [26,27]. Despite these experimental differences, both studies demonstrate that excess sucrose consumption is associated with disrupted insulin responsiveness in adult *Drosophila* [26].

In addition to insulin resistance, HSD adult females also exhibit impaired *dILP* gene expression. However, work done in HSD fed *Drosophila* larvae reported increased *dILP* expression, suggestive of the hyperinsulinemia commonly associated with insulin resistance

in humans [42]. Differences in developmental stages (adult versus larvae) may be a factor for this discrepancy since it is known that *dILP* gene expression, even independent of dietary intervention, changes during development [66]. Additionally, a previous study by Morris *et al.* showed that, while *dILP* gene expression increased in adult females fed a 10% (w/v) sucrose diet, at higher sucrose concentrations, *dILP* gene expression decreased [58]. Interestingly, sucrose concentrations resulted in impaired insulin signaling regardless of the degree of increased dietary sugar content [58]. The current study, in which we report insulin resistance and decreased *dILP* expression in HSD females, exposed adult female flies to a 72% (w/v) sucrose diet. These studies suggest a threshold may exist whereby excess dietary sucrose levels halt the further promotion of *dILP* gene expression.

High-fat diet promotes the excess accumulation of lipids in the cumulus-oocyte complexes of female rodents and correlates with decreased fertility and an altered mitochondrial phenotype [12]. The current data demonstrate that in *Drosophila,* high-sucrose feeding results in excess lipid and cholesterol accumulation in the ovary and decreased egg production. It remains to be known whether, in rodents, high-fat diet promotes cholesterol accumulation. Insects require sterols for growth and survival; however, they lack the enzymes to synthesize sterols and therefore must obtain them through diet [67]. Based on this, we can conclude that the excess cholesterol present in HSD fly ovaries was derived from diet rather than by *de novo* synthesis. HSD and LSD diets contain identical amounts of lipids and sterols, thus the accumulation of cholesterol in HSD ovaries may be due to increased uptake of sterols and/or decreased sterol utilization. Excess lipid accumulation in non-adipose tissues promotes oxidative stress, which contributes to cellular and organelle dysfunction [45]. In addition to increased lipid levels, ovaries of HSD females exhibit signs of oxidative stress, which is in agreement with previous studies in high-fat fed female rodents and together suggest oxidative stress may serve as one means through which lipid accumulation disrupts normal ovarian function [14].

Our observation that the ovaries of HSD females are smaller compared to LSD controls is in agreement with previous *Drosophila* studies that have demonstrated overall ovary size, egg production, and proliferation of stem cells in the ovary are highly controlled by nutritional availability, as well as insulin-dependent and independent signaling pathways [30–32,34]. Oogenesis in *Drosophila* is extremely well studied and occurs in the ovarioles, a group of tubules that make up the fly ovary. Each ovariole consists of a strand of egg chambers that mature in 14 stages as they progress posteriorly down the tubule [29]. We observed fewer mature eggs (stage 14) in HSD female ovaries compared to LSD controls; however, whether this was due to a blunting in egg maturation during oogenesis or other factors is the subject of future studies.

Analysis of ovaries from HSD females showed an increase in mitochondrial DNA copy number and support previous findings in a diabetic mouse model and high-fat fed mouse maternal obesity models, which concluded that increased mitochondrial DNA copy number may be a compensatory response to mitochondrial dysfunction [13,14,68]. Unlike a high-fat diet female rodent study that showed increased expression of PGC-1α, we demonstrate that high-sucrose feeding caused a decrease in the *Drosophila* PGC-1α homolog *Spargel*, highlighting the fact that different nutrients cause differential cellular effects [13].

Additionally, in contrast to mitochondria from high-fat diet rodent ovaries, mitochondria from the ovaries of HSD *Drosophila* failed to show any morphological changes [13]. This observation suggests that, unlike fat, high-sucrose consumption alters mitochondrial number and gene expression, but does not affect mitochondrial morphology under the tested conditions. However, it is possible that increasing the time of exposure to high-sucrose diet may further disrupt mitochondrial function and lead to a change in mitochondrial morphology.

Maternal mitochondria are inherited by the offspring and reports have demonstrated that a high-fat maternal diet causes mitochondrial dysfunction in zygotes and, recently, offspring oocytes and muscle [69,70]. Our work is the first to demonstrate that a high-sucrose maternal diet results in a mitochondrial phenotype in the offspring, characterized by decreased DNA copy numbers and disrupted expression of key mitochondrial regulators. The inheritance of dysfunctional mitochondria past the F1 generation has been shown in high-fat maternal obesity models [70]. While the current study did not examine the transgenerational impact of HSD on mitochondria past F1, we previously reported that F2 generations exhibit increased whole-body glucose and trehalose levels, suggestive of disrupted metabolism. The potential influence the F1 mitochondrial phenotype on metabolism and subsequent impact on the F2 generation is the focus of future studies.

In summary, the current study shows that a maternal diet high in sucrose produces an ovarian phenotype characterized by excess lipid and cholesterol accumulation, altered mitochondria, and reduced egg maturation. These features may play a role in the decreased fertility observed in our maternal obesity model and in the disruptions in offspring mitochondria and metabolism [39]. Whether these features of a high-sucrose diet compound the effects of high-fat consumption on maternal and offspring health remains to be explored and will further our understanding of maternal obesity.

Acknowledgments

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Abbreviations

PGC-1α	peroxisome proliferator-activated receptor-ϒ coactivator 1α
dILPs	Drosophila insulin-like-peptides
TAG	triacylglycerides
mt-TFB1	mitochondrial transcription factor binding protein 1
FSC	follicle stem cells
HSD	high-sucrose-diet
LSD	low-sucrose-diet

Highlights

- High-sucrose maternal obesity disrupts ovarian lipid and cholesterol homeostasis

- Excess sucrose increases mitochondrial DNA copy number while decreasing mitochondrial regulatory genes in the ovary

- Mitochondrial disruptions are relayed to the offspring of obese females
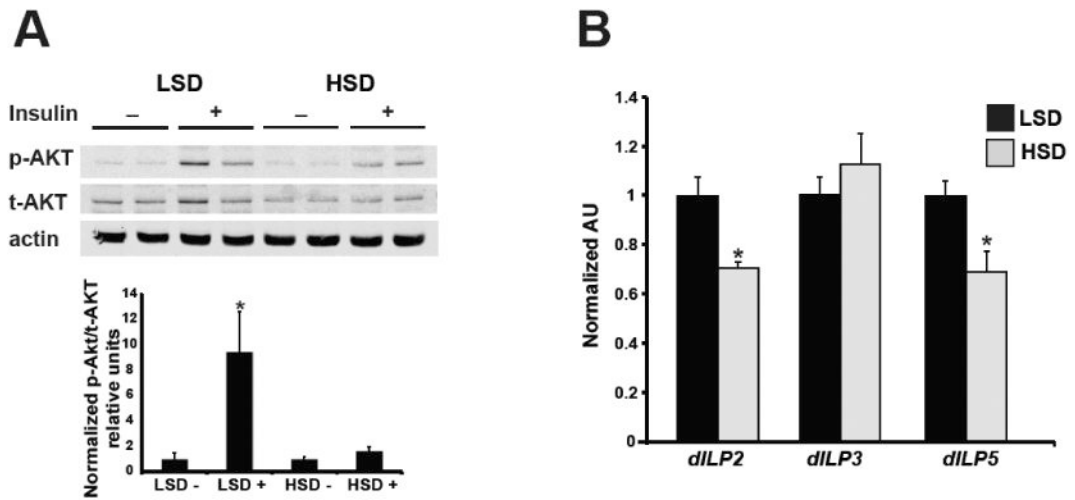
Fig. 1. Insulin responsiveness and *dILP* expression are altered in HSD females

Representative Western blot of total body protein with (+) and without (-) insulin stimulation for phosphorylated (P-AKT) and total (T-AKT) AKT and actin loading control for LSD and HSD adult females (A). Quantification shown is of 5 separate blots using Odyssey imaging software. n=5 pooled samples. qPCR measurement of mRNA expression levels for *dILP 2, 3,* and *5.* n=6 pooled samples (B). All data is expressed as mean + SEM. *p<0.05.
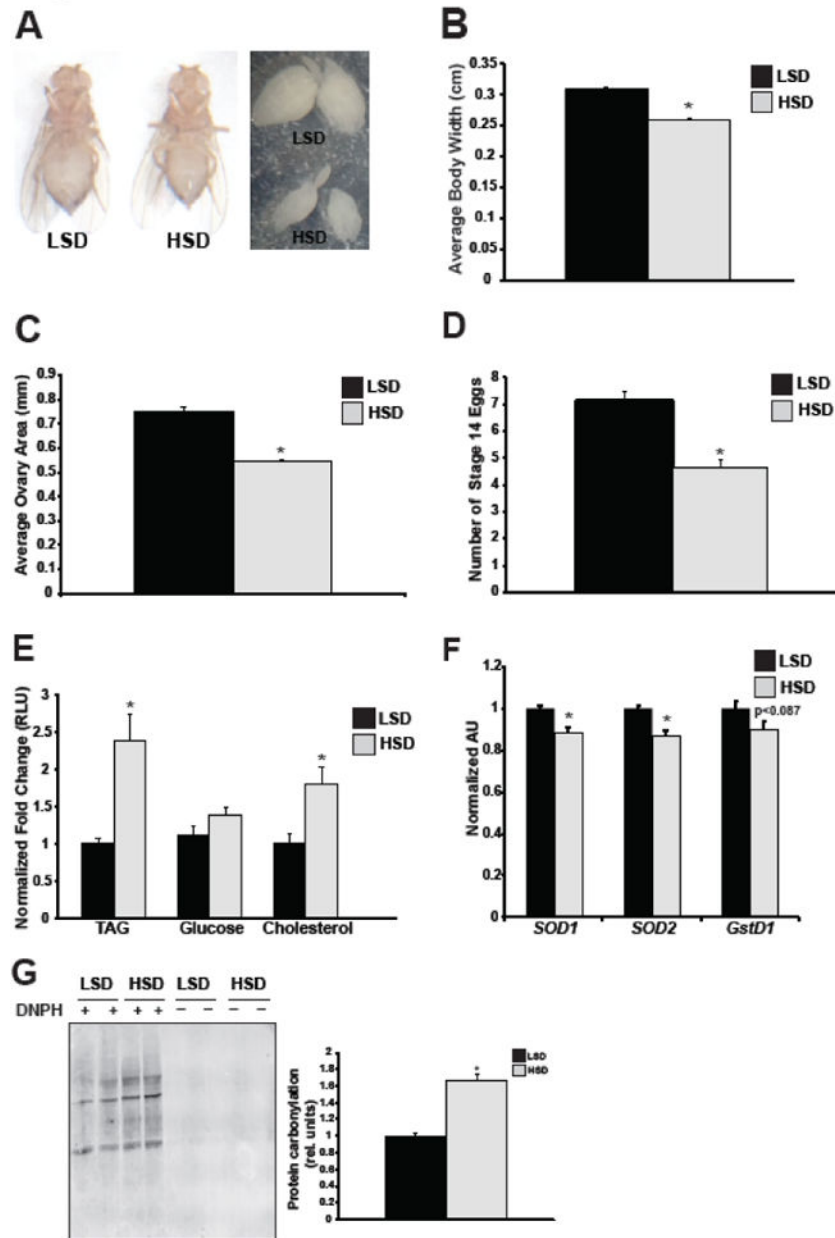
Fig. 2. HSD depletes mature egg production and elevates TAG and cholesterol levels in the ovary
Representative images of LSD and HSD adult females and ovaries are shown after 7 days on diet taken at 10X magnification (A), along with corresponding quantification of average body width (B), ovary area (C), number of stage 14 eggs present in ovaries (D), and ovarian TAG, cholesterol, and glucose levels (E). Body measurements are n=5 flies; ovary area n=83-93 ovaries; stage 14 eggs n=40 ovaries. TAG, cholesterol, and glucose levels were quantified from isolated ovaries of adult females, n=9-14 pooled samples. qPCR measurement of mRNA expression levels of *SOD1* (*superoxide dismutase 1*), *SOD2* (*superoxide dismutase 2*), and *gstD1* (*glutathione S-transferase D1*) from ovaries. n=8 pooled samples (F). Protein carbonylation of ovary whole-cell lysates treated with (+) or without (-) DNPH were visualized by Western blot using Odyssey imaging software.
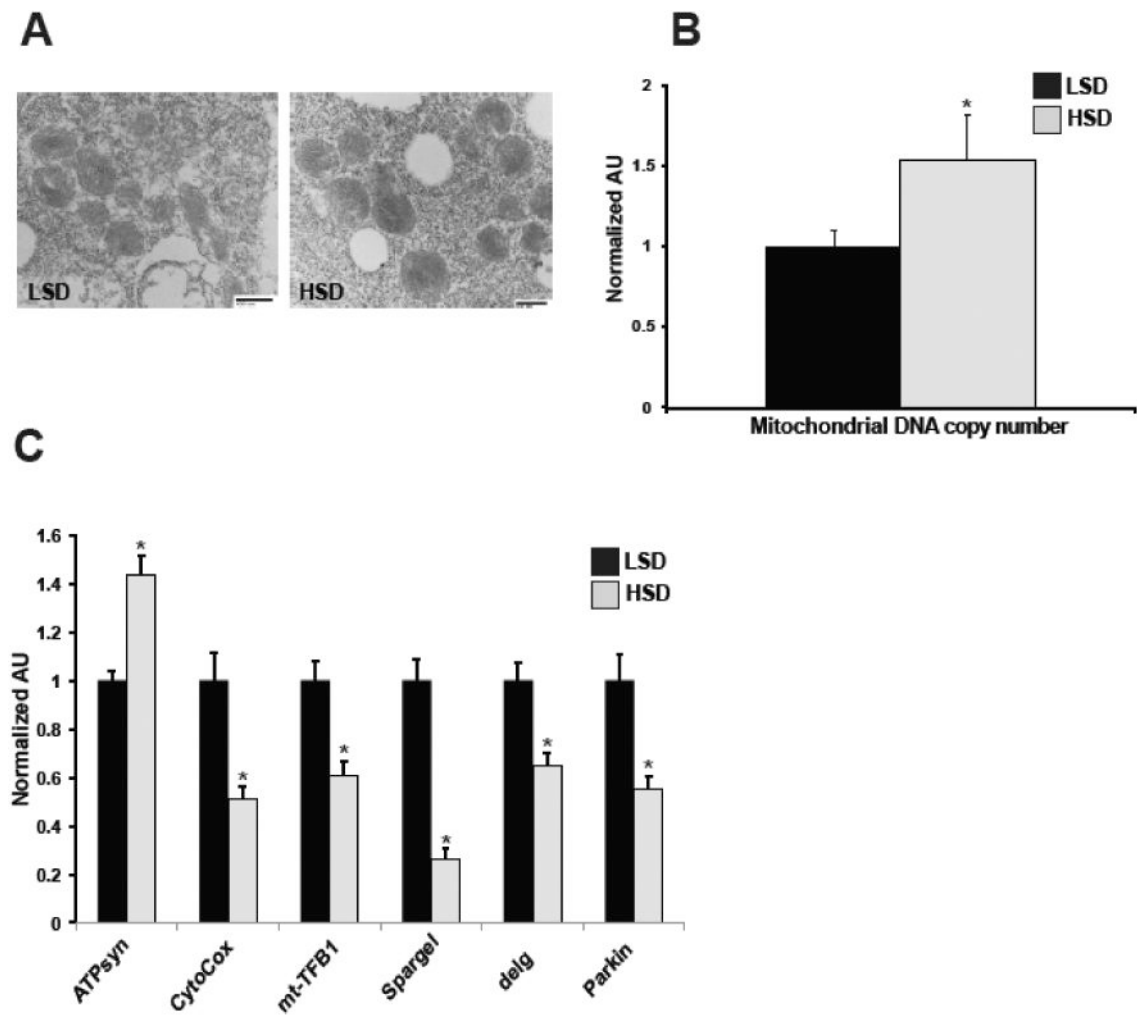
Representative blot is shown (left) as well as quantification (right). n=3 pooled samples (G). All data is expressed as mean ± SEM, *p<0.05.
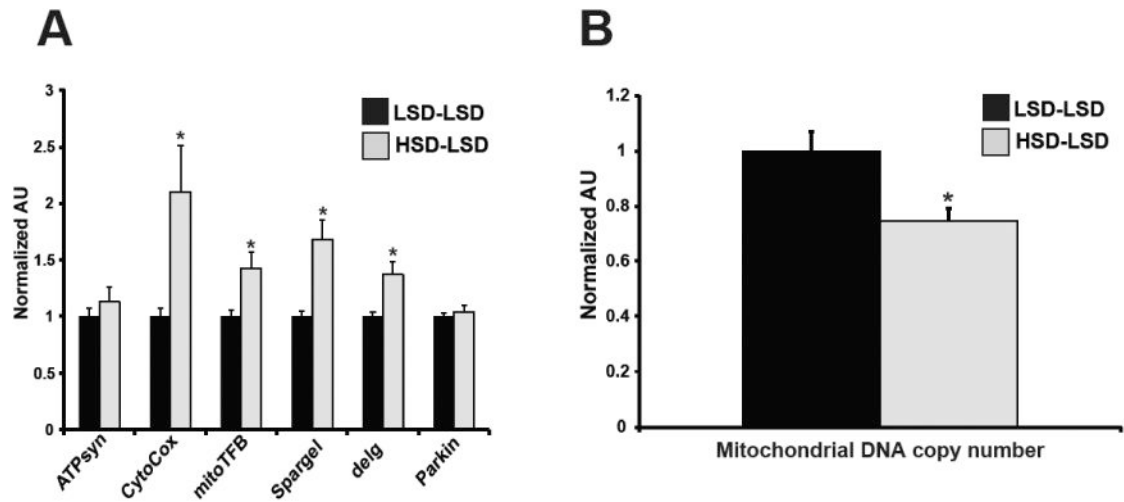
Fig. 3. Mitochondrial copy number and gene expression of key mitochondrial regulators are altered in HSD ovaries

Representative transmission electron microscopy images of LSD and HSD oocytes (A). qPCR measurement of mitochondrial DNA copy number in the ovaries. n=36 pooled samples (B). qPCR measurement of mRNA expression levels of the indicated mitochondrial genes from ovaries. *ATPsyn, ATP synthase; CytoCox, cytochrome C oxidase I; mt-TFB1, mitochondrial Transcription Factor B1.* n=12-17 pooled samples (C). All data is expressed as mean ± SEM, *p<0.05.
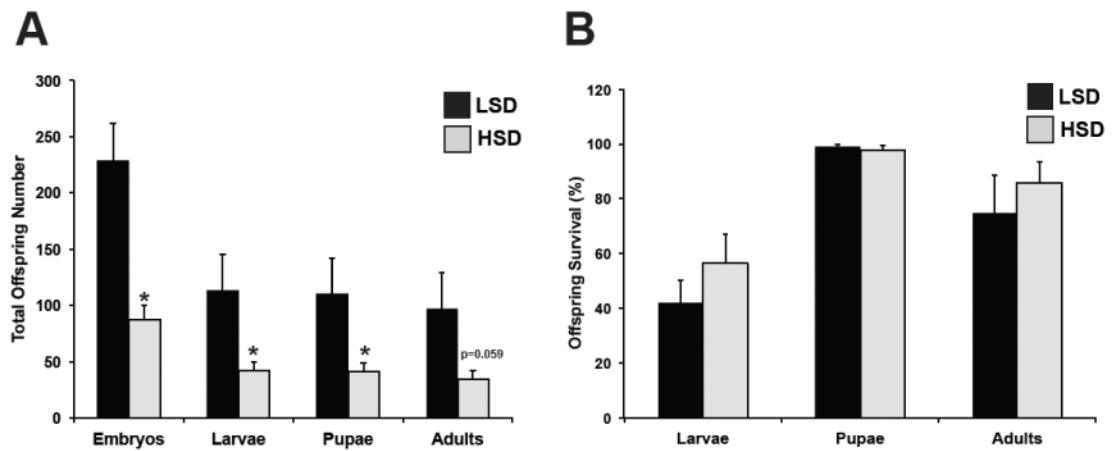
Fig. 4. High-sucrose maternal feeding decreases offspring production, but does not impact offspring survival

Average number of embryos and number of offspring that subsequently developed into wL3 stage larvae, pupae, and adults from the initial embryonic cohort (A) and percent survival of the offspring at each developmental stage (B). Each developmental group consisted of 9-10 biological replicates. All data is expressed as mean ± SEM, *p<0.05.

Fig. 5. Offspring of HSD females have altered gene expression and mitochondrial copy number
qPCR analysis of whole-body mitochondrial mRNA expression (A) and whole-body mitochondrial DNA copy number of wL3 stage offspring larvae (B). n=13-27 pooled samples. All data is expressed as mean ± SEM, *p<0.05.