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Parental Diet Exposure and High-Sugar-Fat Intake Effect on Glucose, Triglyceride and Cholesterol Hemolymph Level of *Drosophila melanogaster* across Five Generations

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Abstract. Metabolic syndrome (MetS) is influenced by parental traits and diet. *Drosophila melanogaster* is a potential disease model organism, sharing physiology and genetic similarities with humans. Previous research had focused on *Drosophila melanogaster* as a model organism for obesity and diabetes, but not for MetS. This research aimed to determine the effect of both parental diet and high sugar fat (HSF) intake on glucose, cholesterol, and triglyceride hemolymph levels of *Drosophila melanogaster*. Wild flies were purified in either control (standard) or MetS media (extra 3% sucrose and palm oil). Seventy-five pairs were divided into 5 groups, according to parental origin and feeding media, and maintained in five generations (F_1-F_5). Glucose, triglyceride, and cholesterol levels were measured using a colorimetric assay in three replications of each generation per group. Glucose, triglyceride, and cholesterol levels were significantly different in all treatment than control groups, and across generations in each group ($p<0.05$). Higher glucose and triglyceride levels appeared in the youngest generation (F_5) of all groups, and in the flies reared on HSF diets. Maternal HSF-exposed groups demonstrated a more pronounced impact of parental metabolic-state on the glucose and triglyceride levels of the earlier generation. These findings highlight that parental exposure to HSF and prolonged HSF intake independently and synergistically lead to persistent and amplified metabolic dysregulation across generations. *Drosophila melanogaster,* modeled in this study, represents a novel experimental organism that is suitable for studying the epigenetics of MetS, gaining more consideration for the metabolic health consequences of long-term dietary habits and parental metabolic-state.

Keywords: parental diet; epigenetics; fruit flies; metabolic syndrome; model organism

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INTRODUCTION

Metabolic syndrome (MetS), the underlying risk factors for the development of cardiometabolic diseases such as type 2 diabetes (T2D), stroke, and cardiac attack, continues to receive public concerns (Peterseim et al., 2024; Zibaeenezhad et al., 2022). This is due to the increasing trend of its prevalence worldwide (Jamali et al., 2024). Nearly 25% of the global population suffers from metabolic syndrome, affecting both developed and developing countries (Mohamed et al., 2023). In Indonesia, the prevalence of MetS reached approximately 21.66% of the population, occurring in 28% Indonesian men and 46% Indonesian women (Sigit et al., 2020). These numbers are in line with the rapidly increasing cases of non-infectious diseases such as T2D, stroke, and coronary heart disease, leading to prominent morbidity and mortality (Mohamed et al., 2023; Noubiap et al., 2022).

Metabolic syndrome consists of interrelated metabolic abnormalities, including hypertension, central obesity, insulin resistance, and atherogenic dyslipidemia (Zila-Velasque et al., 2024). There are several diagnostic criteria of MetS, two of which are commonly applied are the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) and the International Diabetes Federation (IDF) criteria (Peterseim et al., 2024). Basically, they are only slightly different in terms of waist circumference cut points, but both criteria agree that if there are any 3 of 5 measures (elevated fasting glucose, elevated triglyceride, reduced High Density Lipoprotein C, elevated blood pressure, and elevated waist circumference) occur concomitantly, then MetS can be established (Villegas-Abrill et al., 2022). The cause of MetS is multifactorial (Guerra et al., 2021), hence, lifestyle change (high caloric intake, fatty diet, sedentary living) accounts for most cases, despite other associated factors such as genetic, aging, and hormonal imbalance (Lobene, 2023).

The changes in lifestyle, specifically the dietary pattern, can be nurtured down from parents to their offspring (Onu et al., 2025). On the other hand, genetic factors also play crucial roles in metabolic diseases (Rana et al., 2022). Therefore, it is important to investigate how the combination of these factors simultaneously influences the development of MetS since linking genetic and environmental exposure to the pathogenesis of metabolic dysfunction is increasingly gaining more attention through research in epigenetic modifications (Kaimala et al., 2025). One prospective way to enable that experimental design is by using a disease-model organism, namely the fruit fly (*Drosophila/D. melanogaster*) (Graham & Pick, 2017).

Fruit flies are reported to be among the most detrimental pests damaging horticultural crops, leading to economic loss (Agustina et al., 2025). Nonetheless, they provide a potential benefit to be used as a disease-model organism, comparable to other organisms such as mice and rats. It is known that approximately 60% of the *D. melanogaster* genome is homologous to the human genome, while 75% of genes that cause human disease have homologous genes in this model organism (Mirzoyan et al., 2019). *D. melanogaster* also has many physiological aspects that are like mammals, such as the organ function that works and responses to various stimuli. Moreover, its rapid life cycle and large number of offspring enable the use of *D. melanogaster* as a transgenerational model organism in various experimental biology and medicine (Yamaguchi & Yoshida, 2018).

Previous studies utilizing *Drosophila* as a model organism have been conducted for studies related to obesity and diabetes, mainly by modifying the feeding media (Alfa & Kim, 2016; Graham & Pick, 2017; Miao et al., 2022). For instance, *D. melanogaster* treated with a high sucrose or high-fat diet demonstrated increased numbers of hemolymph glucose and triglyceride content, compared to when they were fed with a standard diet (Birse et al., 2010; Diop et al., 2017; Huang et al., 2023). On the other hand, a study on parental obesity and F2 generation of *D. melanogaster* reported lower triglyceride levels in F2 progeny descended from obese grandfathers and heterozygous mothers (Palu et al., 2017). Overall, to our best knowledge, there were only limited studies that had addressed the transgenerational effect of both parental dietary origin and interventional diet simultaneously on the metabolic profile of *D. melanogaster* in five generations.

This research purposes to investigate how the combination of parental dietary origin (HSF exposure) and high sugar-fat (HSF) diet intervention (extra 3% sucrose and 3% palm oil into the standard cornmeal diet) influence the core metabolic profile of MetS, namely glucose, cholesterol and triglyceride hemolymph levels of *D. melanogaster* across five generations (F_1-F_5). In terms of the scientific world, this study is expected to establish a rapid and effective MetS organism model using *D. melanogaster,* which can be used to study the epigenetic (combination of genetic and environmental intervention) mechanisms of MetS as a multifactorial disorder. Furthermore, in terms of society, translating the significant results of this study provides more understanding of the complex pathophysiology of MetS by looking at genetics, hereditary and dietary patterns, which provide an important contribution to the prevention and management of MetS, targeting at the decrease of associated morbidity, mortality, and socioeconomic burden worldwide.

METHODS

Research Design

This experimental study was designed as a non-complete randomized design with 5 types of intervention groups (Table 1). The study was conducted from the parental (P) generation until

the 5th generation (F_1-F_5). Stocks of flies were provided prior to the study. Wild-type *D. melanogaster* was first purified and reproduced up to the 7th generation (F_1-F_7) on either standard cornmeal (control diet) or with the addition of extra 3% sucrose and 3% palm oil (HSF diet). The purified 7th generation from both control and HSF diets was then assigned as the parental origin. Seventy-five pairs (male and female) of fruit flies from different parental diets were then divided into 5 groups of 3 cultured bottles, consisting of 5 pairs each, and were fed with either control or HSF diet, according to their assigned group, as seen in Table 1. They were kept inside the culture bottle at a 12-hour light-dark cycle, room temperature, and room humidity of 60%, whereby they mated to produce the F_1 generation. After 14 days, five pairs of offspring they produced (F_1) were taken from each culture bottle, put into a new culture bottle, they mated to produce the new generation of offspring (F_2). This cycle was repeated up until the 5th generation of offspring (F_5). The observed parameters were hemolymph glucose, cholesterol, and triglyceride, which were measured in three biological replications per group per generation, as modified from (Baenas & Wagner, 2022).

Preparation of Feeding Media

Prior to the making of the feeding media, all tools were sterilized. The control and HSF diet recipes were determined during a preliminary study (Kuswandi et al., 2024). Briefly, a standard control diet was created by mixing 31.25 grams of cornmeal, 1.75 grams of white agar, 250 mL of distilled water, 19 grams of brown sugar, and 3 grams of yeast. They were stirred and heated on a low heat stove. Once the media were boiled and well mixed, they were distributed into 250 ml of sterile culture bottles. A sterile filter paper was then inserted into the bottle, before the bottle was closed with a foam plug and covered with aluminum foil and tied with a rubber band. They were then autoclaved at 121ºC and 1 atm pressure for 45 minutes. For the HSF diet, the control media were added with an extra 3% sucrose and 3% palm oil (fat).

Measurement of Metabolic Parameters

In this study, the levels of hemolymph glucose, cholesterol, and triglyceride were estimated using an enzymatic colorimetric assay as modified from (Wang et al., 2022) protocol with GOD-FS, CHOD-PAP, and triglyceride-FS kits (Diasys Lab, Indonesia), respectively. All parameters were assessed from the 1st to the 5th generation in three biological replications. For each parameter, a blank solution (distilled water), a standard solution, and a sample solution were prepared. A sample solution was made by grinding around thirty (for glucose) and forty (for cholesterol and triglyceride) *D. melanogaster* offspring, of which were left after five pairs were taken for mating to produce the next generation at day 14. They were ground with mortar, rinsed with 0.01M *Phosphate Buffered Saline* (PBS, Kairoz, Indonesia), and centrifuged at 4000 rpm and 4°C for 15 minutes to collect the hemolymph and transferred to a cuvette. The sample solution, alongside the blank and standard solutions, was added with the corresponding reagent and incubated at 37°C for 10 minutes. Afterwards, the absorbance/optical density (OD) of each solution was measured using a UV-Vis spectrophotometer at λ = 510 – 546 nm. Total glucose, cholesterol, and triglyceride concentrations are determined by entering the sample absorbance value into the regression equation formula, derived from the plot between the standard concentration and its absorbance. The values are expressed in mg/dl (1 mmol/l = 18 mg/dl).

Statistical Analysis

The levels of glucose, cholesterol, and triglyceride were analyzed descriptively and were compared among intervention groups and between generations in each group. Statistical analysis was carried out by using SPSS version 30 (IBM, Chicago, USA). One-way ANOVA or Kruskal-Wallis test was applied, based on the normality and homogeneity preliminary test, followed by Post Hoc Bonferroni Test if the results were significant ($p < 0.05$).

Table 1. Intervention group according to parental origin of diets and feeding media*

Intervention Group	Male Origin (♂)	Female Origin (♀)	Feeding Media
A	control diet	HSF diet	control diet
B	HSF diet	control diet	control diet
C	control diet	HSF diet	HSF diet
D	HSF diet	control diet	HSF diet
E	control diet	control diet	control diet

*Control diet: cornmeal basis, HSF diet: cornmeal with extra 3% sucrose and 3% palm oil

RESULTS AND DISCUSSION

Hemolymph Glucose Level of *D. melanogaster* in Five Experimental Groups Across Five Consecutive Generations

This study successfully modeled a combination of parental dietary exposure and HSF diet to observe its transgenerational effects across five generations of *D. melanogaster*. *D. melanogaster* had previously been proposed as a model organism for T2D (Graham & Pick, 2017). In addition to genetic manipulation, high sugar diet (HSD)(Morris et al., 2012; Musselman et al., 2019) or high fat diet (HFD) (Birse et al., 2010) had been previously reported to induce diabetes in fruit flies, showing the hallmark of T2D such as hyperglycemia, hyperinsulinemia (elevated *insulin-like peptides*), and insulin resistance. When a fruit fly eats, the sugars are then digested and transported through the digestive tract into its gut epithelial cells, whereby they are converted into trehalose, the major saccharide for a rapid source of glucose in *D. melanogaster*. Next, trehalose can be stored and/ or circulated through the hemolymph (Graham & Pick, 2017; Kazek et al., 2024; Miao et al., 2022). The comparison of HSD (with extra sucrose) and HFD (with coconut oil) treatment on the glucose level of *D. melanogaster* has been previously investigated for 10, 20, and 30 days (Baenas & Wagner, 2022). However, this study only found a significant increase in glucose levels in fruit flies exposed to HSD for 10 days and 20 days, but not to HFD at any duration. To our best knowledge, our study was among the rarest to combine high sugar (HSD) and high fat diet (HFD) into HSF diet for designing experimental *D. melanogaster* as a potential model organism for MetS.

Hemolymph glucose contents were measured in five experimental groups, consisting of treatment groups (A, B, C, and D) and a control group (E) across five generations (F_1-F_5). The average glucose levels of the treatment groups were significantly different than those in the control group (E) ($p<0.05$, Table 2). Particularly in F_2 and F_5, significantly higher glucose levels were found in Groups A, B, C, and D compared to Group E (Table 2, Figure 1). These findings demonstrate that both parental HSF exposure and sustained HSF diet can lead to significant glucose dysregulation in *D. melanogaster* progeny, leading to persistent and amplified effects over multiple generations.

The HSF exposure both on the parent's diet and on the maintained feeding media, elicited the consistent glucose elevation across generations in treatment groups (A, B, C and D), suggesting compounded impact of genetics and environmental factors, known as epigenetics (Masuyama et al., 2015), on metabolic state of *D. melanogaster.* Epigenetic studies have reported that parental diet influences offspring's health, such as body composition, reproductive output, behavior, immunity (Emborski & Mikheyev, 2019) and metabolic phenotype in mammals (Rando & Simmons, 2015). For example, larvae offspring from HS-fed maternal exhibited high glucose and trehalose concentration for at least two generations (Buescher et al., 2013). In our case, Group C represented the most suitable example of early epigenetic inheritance impact on glucose dysregulation, showing the highest glucose concentration among all experimental groups at least in two consecutive generations (Table 2, Figure 1).

Table 2. The Hemolymph Glucose Level of *D. melanogaster* in Five Experimental Groups Across Five Consecutive Generations (in mg/dl)

Filial	Group A	Group B	Group C	Group D	Group E
F_1	$1.48 \pm 0.02^{*}$	$1.50 \pm 0.01^{*}$	$2.08 \pm 0.01^{*}$	1.80 ± 0.01	1.82 ± 0.01
F_2	$2.71 \pm 0.02^{*+}$	$2.11 \pm 0.01^{*+}$	$2.75 \pm 0.01^{*+}$	$2.53 \pm 0.01^{*+}$	$1.89 \pm 0.00^{+}$
F_3	$2.30 \pm 0.01^{*+}$	$1.83 \pm 0.01^{*}$	$1.81 \pm 0.04^{*+}$	$1.50 \pm 0.01^{*+}$	$2.15 \pm 0.02^{+}$
F_4	$1.54 \pm 0.03^{*+}$	$2.40 \pm 0.35^{+}$	$1.72 \pm 0.01^{*+}$	$2.48 \pm 0.01^{+}$	$2.21 \pm 0.02^{+}$
F_5	$3.97 \pm 0.01^{*+}$	$3.60 \pm 0.04^{*+}$	$3.38 \pm 0.01^{*+}$	$2.46 \pm 0.00^{*+}$	$2.59 \pm 0.00^{*+}$

Note: Levels of hemolymph glucose are calculated as the mean ± standard deviation of three biological replications per generation (Filial) per group. Group A (♂ control x ♀ HSF, control diet), B (♂ HSF x ♀ control, control diet), C (♂ control x ♀ HSF, HSF diet), D (♂ HSF x ♀ control, HSF diet), E (♂ control x ♀ control, control diet), *significant to E, $^{+}$significant to F_1 ($p<0.05$, Post Hoc Bonferroni test).

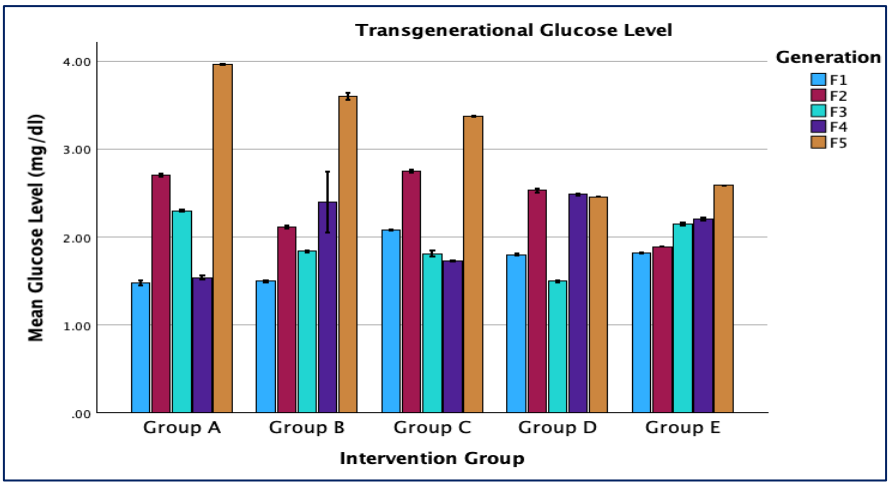

Figure 1. Comparison of glucose concentration obtained from the hemolymph of *D. melanogaster* among five intervention groups (Group A, B, C., D, and E) across five generations (F_1-F_5). Note: each bar represents the average glucose level in mg/dl with standard deviation; Group A (♂ control x ♀ HSF, control diet), B (♂ HSF x ♀ control, control diet), C (♂ control x ♀ HSF, HSF diet), D (♂ HSF x ♀ control, HSF diet), E (♂ control x ♀ control, control diet).

Changes in the parental metabolic status also play a key role in the probability of the appearance of metabolic disorders that may not be limited only to the first generation but also to subsequent generations, either exposed or unexposed to such metabolic interventions like the HSF diet (Gonzalez-Bulnes et al., 2014). Supporting that notion, this study succeeded maintaining our *Drosophila* experimental model until five generations and found that significant elevated glucose levels ($p<0.05$) were observed in F_2 onward, compared to F_1 (Table 2) with the highest glucose concentration was predominantly found in the youngest (F_5) generation of all groups (Figure 1). This finding indicates that repeated exposure to the HSF diet, either via parental inheritance or dietary intake, leads to a compounding metabolic burden over successive generations.

Regardless of their parental origin, the fruit flies fed with HSF diet (C and D) had significantly higher glucose levels compared to those in control diet (A, B and E), particularly in F_1 (only for C) and F_4 (only for D) (Table 2 and Figure 1). This significant finding reflects that the HSF diet also independently contributes to the alteration of glucose metabolism. In the absence of parental dietary exposure, a previous study on high-sugar-fed *Drosophila* larvae highlighted a direct link between high-sugar feeding and insulin resistance as evidenced by hyperglycemic and hyperinsulinemic larvae that exhibited a defective response to insulin (Musselman et al., 2011). On the other hand, the fluctuation of average glucose level, showing a spike and a drop, indicates a possible compensatory response to prior HSF diets, specifically for the case of Group D (Figure 1). In comparison, Group E showed relatively moderately elevated glucose levels, reflecting natural, varied heritability rather than the impact of experimental intervention.

The heritable metabolic risk is also epigenetically transmitted from parent to offspring over multiple generations, even in the absence of a continued exposure to the triggering stimuli (Stegemann & Buchner, 2015). In our study, Group A and B, which received control diets but with HSF-exposed parents, also displayed significantly higher glucose levels than Group E, particularly in F_2 and F_5 (Table 2), underlining the enduring metabolic influence of parental diet, even without direct exposure to HSF diet, possibly through epigenetic mechanisms. Although the exact mechanisms are still unknown, DNA methylation, histone modifications, and the influence of small RNAs are currently being investigated. (Jaeger et al., 2017; Stegemann & Buchner, 2015).

Furthermore, a more pronounced effect on the glucose levels was found when the fly offsprings had maternal parents that originated from the HSF diet, both when they were reared on the control or HSF diet. This finding indicates that although both parental lines might contribute to elevated glucose levels, the maternal metabolic status elicited a more sustained effect on offspring glucose metabolism. In fruit flies, a high-sugar maternal diet has been reported to alter the body composition of two generations of larval offspring

(Buescher et al., 2013), explaining sex-dependent differences in transgenerational inheritance of specific phenotype. Other supporting evidence also arises from the strong association between maternal obesity and/ or nutritional support provided by mothers, such as HSF diet during pregnancy, with the occurrence of T2D and heart diseases in children, and even premature mortality in later life (Bertossa et al., 2024; Lee et al., 2015).

Hemolymph Triglyceride Level of *D. melanogaster* in Five Experimental Groups Across Five Consecutive Generations

Mammals and *Drosophila* share similar metabolic functions, such as the maintenance of glucose and cholesterol homeostasis, lipid storage and mobilization, and the regulation of food intake (Graham & Pick, 2017). In mammals, excess sugar and fat intake are stored as glycogen (in skeletal muscle and liver) and as triglycerides (in adipose tissue and liver), whereas, in *D. melanogaster*, excess sugar and fat nutrients are stored in the fat body and peripheral oenocytes, mimicking the mammalian hepatic and adipose tissues (Morris et al., 2012). On the other hand, elevated triglycerides are one main criteria of MetS, indicating insulin resistance (Fahed et al., 2022). Insulin resistance occurs due to multifactorial causes, such as genetic susceptibility, obesity, and dietary patterns. Thus, increased triglyceride levels in hemolymph can be measured to show increased fat storage in *Drosophila*, as one indicator of insulin resistance.

It is known that heritability due to gene-environment interaction of metabolic traits such as levels of glucose, insulin, triglycerides, cholesterol, and blood pressure is quite similar (Reding-Bernal et al., 2017; Vattikuti et al., 2012). Like those of glucose, significant differences in hemolymph triglyceride levels occurred between almost all intervention groups (A, B, C, D) and control (E) across five generations, except for the 1st generation of group A, whereby the average triglyceride content was the lowest (Table 3). Although the transgenerational triglyceride level varied among different experimental groups (Figure 2), the levels of hemolymph triglyceride of younger generations (F_2-F_5) were significantly different when compared to the 1st generation, in all intervention groups, including control (Table 3). These findings suggest that both parental diet and sustained HSF intake influence triglyceride metabolism with persistent and sometimes compounding effects across generations. Our study supports the notion of other previous studies on the significant effect of high sugar and high fat diet exposure of various durations to increase the triglyceride level of hemolymph in fruit flies (Baenas & Wagner, 2022; Emborski & Mikheyev, 2019; Musselman et al., 2011).

Parental dietary pattern is one of the principal epigenetic factors, which plays a crucial role in shaping offspring's phenotype, such as body composition and metabolic parameters (Dew-Budd et al., 2016). Although we found significant transgenerational effects of parental nutrition exposure on triglyceride levels across five generations (Table 3), the pronounced effects of parental origin and HSF diet exposure on increased triglyceride levels were only observed in two generations, similar to the results from previous studies which demonstrated high sugar maternal diet effect on the body composition and obesity-like phenotype, only in two generations (Buescher et al., 2013; Palu et al., 2017). In line with these findings, the transgenerational effects of parental dietary origin on the triglyceride level of two offspring generations had also been demonstrated by (Emborski & Mikheyev, 2019) In a sex-specific manner, which was not assessed in our current study.

Table 3. The Hemolymph Triglyceride Level of *D. melanogaster* in Five Experimental Groups Across Five Consecutive Generations (in mg/dl)

Filial	Group A	Group B	Group C	Group D	Group E
F_1	0.30 ± 0.01	0.68 ± 0.00*	0.86 ± 0.01*	0.44 ± 0.01*	0.35 ± 0.01
F_2	2.16 ± 0.02*$^+$	1.45 ± 0.01*$^+$	2.04 ± 0.01*$^+$	0.75 ± 0.01*$^+$	0.71 ± 0.01$^+$
F_3	0.49 ± 0.01*$^+$	1.60 ± 0.01*$^+$	1.86 ± 0.01*$^+$	1.43 ± 0.06*$^+$	1.78 ± 0.01$^+$
F_4	1.67 ± 0.00*$^+$	1.91 ± 0.01*$^+$	0.61 ± 0.01*$^+$	1.25 ± 0.00*$^+$	1.18 ± 0.01$^+$
F_5	1.41 ± 0.00*$^+$	1.82 ± 0.00*$^+$	1.82 ± 0.01*$^+$	1.66 ± 0.00*$^+$	1.34 ± 0.01$^+$

Note: The Level of triglyceride was calculated as the mean ± standard deviation of three biological replications per generation (Filial) per group. Group A (♂ control x ♀ HSF, control diet), B (♂ HSF x ♀ control, control diet), C (♂ control x ♀ HSF, HSF diet), D (♂ HSF x ♀ control, HSF diet), A (♂ control x ♀ control, control diet), *significant to E, $^+$significant to Gnr 1 ($p<0.05$, Post Hoc Bonferroni test).

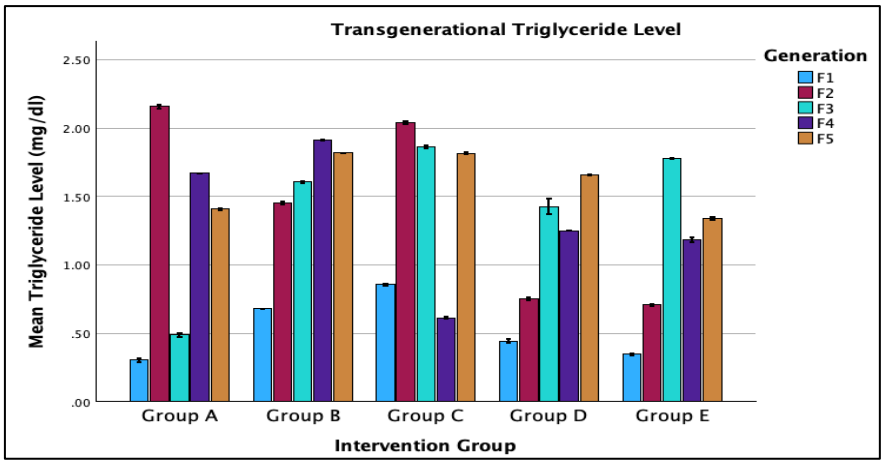

Figure 2. Comparison of triglyceride concentration obtained from the hemolymph of *D. melanogaster* among five intervention groups (Group A, B, C., D, and E) across five generations (F_1-F_5). Note: each bar represents the average glucose level in mg/dl with standard deviation; Group A (♂ control x ♀ HSF, control diet), B (♂ HSF x ♀ control, control diet), C (♂ control x ♀ HSF, HSF diet), D (♂ HSF x ♀ control, HSF diet), E (♂ control x ♀ control, control diet).

Hemolymph Cholesterol Level of *D. melanogaster* in Five Experimental Groups Across Five Consecutive Generations

Similar to those of glucose and triglyceride, we found that both parental HSF diet and continued HSF exposure contribute to the inheritance of cholesterol metabolic dysregulation. In almost all generations, the levels of hemolymph cholesterol of treatment groups (A, B, C, and D) were significantly different from they were in the control group E, except for the F_3 of Group D (Table 4). *Drosophila* studies related to the effect of either high sugar, high fat, or high sugar-fat diet on cholesterol level are relatively limited compared to other *Drosophila* studies on glucose and triglyceride levels. In this study, even in the first generation, HSF exposure and HSF diet immediately dysregulate lipid metabolism (Group D, Table 4).

Transgenerational cholesterol metabolism refers to how changes in cholesterol metabolism in one generation can affect the subsequent generations through epigenetic modifications, such as DNA methylation and histone modification, which alter gene expression and can be passed down through generations (Blanc et al., 2020; Wan et al., 2022). Utilizing genetic approaches in *D. melanogaster*, a previous study had successfully identified genes that regulate cholesterol metabolism and homeostasis, reinforcing the significant roles of *Drosophila* as a model organism for studying steroid hormones and cell membrane, leading to growth and development (reproduction), and to a wider context of sleep, memory and aging (Niwa & Niwa, 2011). In this study, significant transgenerational effects of cholesterol content were found in all experimental groups from F_2 onwards, indicating a developing transgenerational lipid imbalance (Table 4).

Table 4. The Hemolymph Cholesterol Level of *D. melanogaster* in Five Experimental Groups Across Five Consecutive Generations (in mg/dl)

Filial	Group A	Group B	Group C	Group D	Group E
F_1	1.38 ± 0.02*	1.27 ± 0.01*	1.30 ± 0.01*	1.82 ± 0.01*$^+$	1.68 ± 0.01$^+$
F_2	0.70 ± 0.01*$^+$	1.34 ± 0.01*$^+$	1.15 ± 0.01*$^+$	1.55 ± 0.01*$^+$	1.30 ± 0.01$^+$
F_3	1.53 ± 0.02*$^+$	1.37 ± 0.01*$^+$	1.40 ± 0.01*$^+$	1.58 ± 0.01$^+$	1.58 ± 0.02$^+$
F_4	1.57 ± 0.01*$^+$	1.66 ± 0.03*$^+$	0.92 ± 0.00*$^+$	1.30 ± 0.01*$^+$	1.49 ± 0.01$^+$
F_5	1.38 ± 0.01*$^+$	1.44 ± 0.00*$^+$	8.46 ± 0.05*$^+$	8.04 ± 0.00*$^+$	1.61 ± 0.01$^+$

Note: The Level of cholesterol was calculated as the mean ± standard deviation of three biological replications per generation (Filial) per group. Group A (♂ control x ♀ HSF, control diet), B (♂ HSF x ♀ control, control diet), C (♂ control x ♀ HSF, HSF diet), D (♂ HSF x ♀ control, HSF diet), A (♂ control x ♀ control, control diet), *significant to E, $^+$significant to Gnr 1 ($p<0.05$, Post Hoc Bonferroni test).

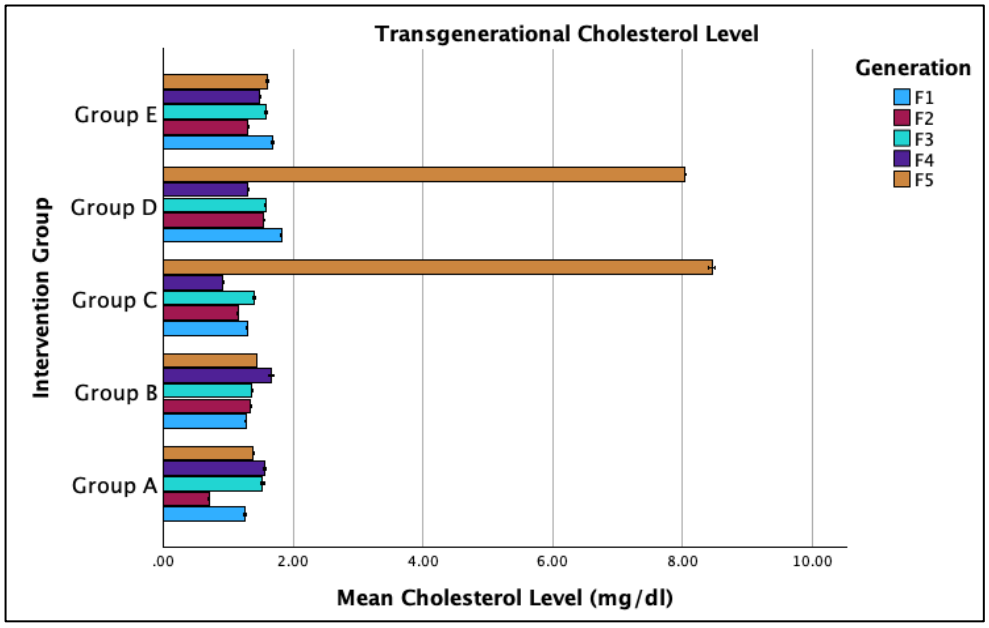

Figure 3. Comparison of cholesterol concentration obtained from the hemolymph of *D. melanogaster* among five intervention groups (Group A, B, C., D, and E) across five generations (F_1-F_5). Note: each bar represents the average glucose level in mg/dl with standard deviation; Group A (♂ control x ♀ HSF, control diet), B (♂ HSF x ♀ control, control diet), C (♂ control x ♀ HSF, HSF diet), D (♂ HSF x ♀ control, HSF diet), E (♂ control x ♀ control, control diet).

Nevertheless, the transgenerational epigenetic effects of cholesterol metabolism display a more complex pattern (Figure 3) compared to other metabolic parameters. Similar to those of triglyceride level, the fluctuating transgenerational effects of cholesterol concentration could also indicate metabolic compensation or adaptive homeostatic response. Although the numbers varied, there was a tendency to accumulate cholesterol content in the youngest generation (F_5), particularly in *D. melanogaster* reared on HSF diet (C and D) (Table 4, Figure 3). Thus, persistent HSF diet in combination with parental exposure suggests cumulative cholesterol dysregulation through possible epigenetic modification (Kaimala et al., 2025; Wan et al., 2022), which may further lead to pathological hypercholesterolemia, a key risk factor for cardiometabolic diseases (Jamali et al., 2024).

In this study, we observed that Groups A and B (control diet) maintain moderate but elevated cholesterol levels (Figure 3), supporting that even in the absence of direct HSF exposure, parental metabolic state still exhibits a strong impact. Moreover, flies from group D, which also originated from paternal HSF diet, demonstrated a higher level of cholesterol than other groups in almost all generations (Table 4), different from the findings on glucose and triglyceride observations. Some previous transgenerational *Drosophila* studies reported that no significant effect of both maternal and paternal diets on offspring development and disease resistance (Dinh et al., 2021), no parental origin diet effect on the body composition of offsprings in two generations (F_1-F_2) (Dew-Budd et al., 2016) and that high fat diet is not a consistent predictor of its untreated descendants' pupal body weight in ten generations (Emborski & Mikheyev, 2019).

Metabolic syndrome (MetS) is defined by the existence of at least three of five risk factors: elevated glucose level, elevated triglyceride, low HDL-cholesterol and high LDL-cholesterol level, as well as increased waist circumference and high blood pressure (Sigit et al., 2020).The use of *D. melanogaster* as a model organism for MetS has been previously summarized by (Vatashchuk et al., 2022) based on several studies, either by modifying diet, such as HSD or HFD, and/ or inducing mutant *D. melanogaster.* The novelty of this research lays in the successful development of a MetS model organism using *D. melanogaster,* which was not only treated with HSF diet (sucrose and palm oil supplementation) but also was set to have parental HSF exposure as the ancestral origin

through various mating combinations. This model provides compelling evidence that both parental HSF exposure and direct HSF intake contribute to the persistence and progressive inheritance of metabolic dysregulation of glucose, triglyceride, and cholesterol, via epigenetic mechanisms. The compounded effects appear more severe when the maternal HSF and HSF diet are involved.

However, like other *Drosophila* studies on MetS, this study encountered several limitations. First, we could only demonstrate metabolic parameters such as glucose, triglyceride, and cholesterol hemolymph levels. In this regard, elevated blood pressure and central obesity are the MetS criteria in humans that are difficult to mimic in *Drosophila* (Vatashchuk et al., 2022). Second, one intervention group with parental mating from both HSF diets (male and female) was missing in this study due to a technical issue, and third, we also did not differentiate the metabolic effects based on sex differences of the offspring. Lastly, the HSF treatment could not also be longer than 14 days, adapting to the need to provide the parental mating and to sacrifice for the parameter measurement.

Nonetheless, the highlight significant findings on the changing of metabolic parameters in *Drosophila melanogaster* as an epigenetic human-disease model organism for MetS display potential benefits for science and society. This *Drosophila* epigenetic MetS-model organism can be used as a foundation research to further elucidate the mechanism and the genes involved in the development and heritability of MetS, to deepen investigation on the transgenerational effects of other metabolic parameters in several generations, to distinguish the sex-difference effects across multiple generations, as well as to test the potential of various metabolite agents derived from natural resources as alternative treatment for diseases related to MetS.

CONCLUSION

This study presents a potential model organism for epigenetic research related to metabolic syndrome in humans, by combining parental diet and modified high sugar fat diet on the metabolic parameters, namely glucose, cholesterol, and triglyceride hemolymph levels across five generations of *Drosophila melanogaster*. Regarding the use of our experimental design of *Drosophila melanogaster* as a model organism for metabolic syndrome, more studies are still needed to address the limitations encountered by our study, to further improve our experimental design, and to create more innovative experimental studies related to other clinical settings. Furthermore, by using this experimental model of *Drosophila melanogaster*, the potential results of future studies may contribute to the medicinal and health science world, particularly onto the prevention of cardiometabolic diseases through awareness on long-term metabolic health consequences of dietary habits and parental health status, rapid development of early detection tools, and the expansion for new target therapies.

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AUTHOR CONTRIBUTION STATEMENT

KRP, PCK, and RDA designed the study. PCK and RDA performed the preliminary study. KRP, PCK, and RDA performed experiments; KRP and HHM analyzed the data. KRP, PCK, and HHM contributed to the design of the manuscript. KRP and PCK wrote the manuscript while RDA and HHM critically revised the manuscript. All authors had final approval of the submitted versions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript.

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