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# Synergistic Effects of Combined High-Fat and High-Sugar Diets on Metabolic, Developmental, and Behavioral Dysfunction in *Drosophila melanogaster*

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## ABSTRACT

**Background:** The rising global prevalence of obesity and type 2 diabetes is closely linked to excessive consumption of dietary fat and sugar. Although *Drosophila melanogaster* is a well-established model for diet-induced metabolic dysfunction, most studies examine high-fat diets (HFDs) or high-sugar diets (HSDs) independently, leaving the combined metabolic effects of these major components of Western diets insufficiently characterized. **Aim:** To compare the metabolic, developmental, and behavioural effects of HFD, HSD, and a combined high-fat/high-sugar diet (HFD+HSD) in *Drosophila melanogaster*. **Methods:** Flies were reared on control, HFD, HSD, or HFD+HSD diets. Developmental timing, larval morphology, adult body weight, locomotor activity, and systemic biochemical parameters were assessed. Expression levels of Brummer (bmm) and Insulin-like peptide 2 (ILP2) were quantified using RT-PCR. Data were analyzed using ANOVA with Post-hoc Tukey's Honestly Significant Difference test in IBM SPSS (Version 23). **Results:** HSD markedly delayed eclosion (23 vs. 11 days in controls) and significantly reduced larval length (245.48 ± 34.81 µm vs. 724.21 ± 61.28 µm). The combined HFD+HSD diet produced the most pronounced metabolic disturbances, with significant elevations in systemic glucose (male: $p = 0.004$; female: $p < 0.001$) and lipid levels. Locomotor performance was also most severely impaired in the HFD+HSD group. Gene expression analysis revealed significant up-regulation of bmm and ILP2, suggesting enhanced lipid mobilization and disrupted insulin signalling consistent with early obesity-associated metabolic dysregulation. **Conclusion:** Combined exposure to high fat and high sugar induces a stronger metabolic dysfunction phenotype than either diet alone. This integrated *Drosophila* model offers a powerful platform for investigating the multifactorial mechanisms underlying obesity and related metabolic disorders.

**Keywords:** *Drosophila melanogaster*, High-fat diet, High-sugar diet, Metabolic dysfunction, Insulin signaling

## INTRODUCTION

Metabolic disorders, particularly obesity and type 2 diabetes (T2D), represent one of the most pressing global public health challenges of the twenty-first century. Their rapidly increasing prevalence has been strongly linked to major shifts in dietary patterns, especially the widespread consumption of energy-dense Western-style diets rich in saturated fats and refined sugars.[1] Excess intake of these macronutrients disrupts metabolic homeostasis and contributes to the development of insulin resistance, dyslipidemia, and chronic metabolic inflammation. Understanding how dietary fat and sugar interact to drive these pathological processes has therefore become a major focus of contemporary biomedical research. While mammalian models have traditionally been used to investigate diet-induced metabolic disease, they are costly, time-consuming, and raise ethical concerns, underscoring the importance of complementary model systems that enable rapid, controlled experimentation. [2-4]

The fruit fly, *Drosophila melanogaster*, has emerged as a powerful and widely accepted model organism for investigating metabolic regulation and diet-induced metabolic dysfunction. This model is particularly valuable because many fundamental metabolic pathways are

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evolutionarily conserved between flies and mammals, including those involved in insulin signaling, lipid metabolism, and energy homeostasis.[2-4] In addition, *Drosophila* offers several experimental advantages, including a short life cycle, well-characterized genetics, and the ability to perform high-throughput dietary and genetic manipulations. These attributes have enabled researchers to explore the molecular and physiological basis of metabolic disorders with remarkable efficiency. Dietary manipulation in *Drosophila* has been extensively used to model obesity- and diabetes-like phenotypes. Experimental exposure to high-fat diets (HFDs) or high-sugar diets (HSDs) can reproduce many features of metabolic disease observed in mammalian systems, including increased triglyceride accumulation, altered carbohydrate metabolism, insulin resistance, and shortened lifespan.[5-8]

High-fat feeding in flies has been associated with abnormal lipid storage and systemic metabolic disruption,[5,6] while high-sugar diets have been shown to induce hyperglycemia-like states and impair insulin signaling pathways.[7] These diet-induced phenotypes demonstrate that *Drosophila* provides a reliable platform for investigating the metabolic consequences of excessive nutrient intake. Despite these advances, important inconsistencies remain in the literature regarding the metabolic effects of single-macronutrient dietary models. For example, while several studies report that HFD exposure leads to pronounced metabolic abnormalities, others have observed more variable outcomes, including inconsistent effects on circulating glucose levels and insulin resistance.[5,6,8] Similarly, the metabolic consequences of HSD exposure are not always uniform across experimental settings, with evidence suggesting that the degree of insulin resistance induced by high-sugar feeding may vary depending on genotype, sex, and environmental conditions.[9] These discrepancies complicate the interpretation of findings and highlight the complexity of nutrient–metabolism interactions.

A key limitation of many existing experimental models is that they focus primarily on the effects of a single macronutrient in isolation. In reality, however, modern human diets rarely involve excessive consumption of only fat or only sugar. Instead, Western dietary patterns typically contain high levels of both macronutrients simultaneously. The combined intake of dietary fat and sugar may therefore exert interactive effects on metabolic regulation that cannot be adequately captured by single-diet models. Although some studies in *Drosophila* have explored "Western diet" paradigms that incorporate multiple nutrient components,[10-12] comprehensive comparisons of the independent and combined effects of HFD and HSD across multiple physiological endpoints remain relatively limited. Consequently, it remains unclear whether the metabolic consequences of combined diets reflect simple additive effects or produce synergistic interactions that exacerbate metabolic dysfunction.

In addition to metabolic disturbances, dietary imbalance can also influence developmental and physiological processes in *Drosophila*. Nutritional composition during larval development has been shown to affect growth, developmental timing, and adult physiological traits. Similarly, dietary excess can alter behavioral outcomes such as locomotor activity, reflecting the close integration between metabolic regulation and neural function. These diverse phenotypic outcomes underscore the importance of examining diet-induced effects across multiple biological levels rather than focusing solely on metabolic markers.[10-12] Given these considerations, a systematic evaluation of combined exposure to dietary fat and sugar is essential to improve the translational relevance of *Drosophila* metabolic models. In the present study, we addressed this gap by directly comparing the effects of a high-fat diet (HFD), a high-sugar diet (HSD), and a combined high-fat/high-sugar diet (HFD+HSD) in *Drosophila melanogaster*. Using a comprehensive experimental framework, we assessed developmental timing, larval morphology, adult body weight, locomotor behavior, and key biochemical indicators of metabolic function. Through this integrated approach, our study aimed to clarify the relative contributions of dietary fat and sugar to metabolic dysfunction and to determine whether their combined exposure produces enhanced pathological effects.

## MATERIALS AND METHODS

### *Drosophila* Media Preparation

A standard cornmeal-based medium was prepared by boiling agar-agar in distilled water, mixing in cornmeal and yeast, and then adding methyl paraben dissolved in ethanol as a preservative once the mixture had cooled to approximately 65°C. Dietary modifications were incorporated into this base medium. For the HFD, 2% (w/w) coconut oil was added. For the HSD, 30% (w/w) sucrose was included. For the combined HFD+HSD, portions of HFD and HSD media were mixed to achieve

final concentrations of 1% coconut oil and 15% sucrose. All diets were dispensed into sterile *Drosophila* vials to a depth of 3–4 cm and allowed to solidify at room temperature before use.

## *Drosophila* Stocks

*Drosophila* stocks (Harwich strain) were obtained from the *Drosophila* Laboratory of the Centre for Advanced Medical Research and Training (CAMRET), Usmanu Danfodiyo University, Sokoto, Nigeria.

## Experimental Procedure

Adult flies were transferred into freshly prepared cornmeal medium and allowed to mate and lay eggs for 24–48 hours before being removed. The eggs were reared to adulthood and, soon after eclosion, flies were randomly assigned to one of four experimental diets using a random number allocation approach: (i) standard cornmeal (control), (ii) HFD, (iii) HSD, and (iv) Combined high-fat/high-sugar diet (HFD+HSD). Randomisation was performed by assigning sequential numeric codes to newly eclosed flies and using a computer-generated random number table to allocate each fly to a dietary group. All subsequent biochemical and gene expression analyses were conducted with the analyst blinded to group allocation; vials were labelled with numeric codes, and the allocation key was withheld until after data collection was complete.

The flies were maintained on their assigned diets under standard laboratory conditions (25°C, 60% relative humidity, 12–14 h light/10–12 h dark cycle). The flies were allowed to mate freely and lay eggs. After 5 days, the adult flies were removed. Development of these eggs was monitored across larval, pupal, and adult stages. Larval morphology, including length and width, was measured and recorded at the third instar stage. The number of days to eclosion from each dietary condition was recorded. Adult flies were separated by sex, maintained on their respective diets for seven days, and then grouped in batches of ten for further assessment. They were thereafter weighed, and locomotor activity was assessed using the negative geotaxis assay. Flies were then homogenized for biochemical analyses of glucose, triglycerides, and total cholesterol, as well as gene expression studies. Three independent biological cohorts of 10 flies per dietary group were used (n = 3), giving a total of 30 flies per group per sex.

## Biochemical Assays

### Test for Glucose

Drosophila glucose levels were measured using the Glucose Oxidase (GO) method, a colorimetric-based enzymatic assay.

### Test for Lipids

A coupled colorimetric method was used for triglyceride assaying, while the Amplex Red Cholesterol Kit was used for total cholesterol estimation.

## Nucleic Acid Extraction

Total RNA was extracted from homogenised fly samples using the DAAN Gene Extraction Kit, following the manufacturer's guidelines. RNA integrity and concentration were assessed prior to downstream analysis.

### Reverse Transcription and Real-Time PCR

Extracted RNA was reverse-transcribed to complementary DNA (cDNA) using a one-step RT-PCR approach (SYBR chemistry). The housekeeping gene *rp49* (*RpL32*; NCBI Reference Sequence: NM_079505) was used as the endogenous reference gene for normalisation, consistent with its widely validated use in *Drosophila melanogaster* gene expression studies. Prior to use, the stability of *rp49* expression was confirmed by calculating the coefficient of variation (CV) of Ct values across all experimental dietary groups; a CV of less than 1% was observed, confirming its suitability as a stable reference.

### RT-PCR Procedure

Target genes and their respective forward and reverse primers are listed in Table 1. PCR primers for all target genes (both forward and reverse) were designed using the NCBI Primer-BLAST tool and Primer3 software, and were synthesized by Inqaba Biotec Nigeria. Gene-specific RT-PCR cycling conditions were as follows. For *ILP2*: annealing at 58°C for 30 seconds, 40 cycles. For *Bmm*: annealing at 60°C for 30 seconds, 38 cycles. For the reference gene *rp49*: annealing at 57°C for 30 seconds, 40 cycles. Conditions common to all assays were: pre-denaturation at 95°C for 30 seconds; denaturation at 95°C for 15 seconds; and extension at 72°C for 30 seconds. For each RT-PCR run, each reaction comprised 2 µl template nucleic acid, 10 µl one-step RT-PCR master mix (SYBR chemistry), 2 µl synthetic primer, and 6 µl molecular-grade PCR water (total reaction volume: 20 µl). The

analysis threshold was set at 0.02. All experiments were performed in triplicate. Ct values were analyzed using the $2^{-DD}Ct$ (Livak) method to quantify fold changes in mRNA levels, normalized to the *rp49* reference gene.

### Data Analysis

Data were collected, cleaned, entered, and analyzed using SPSS version 23 and Microsoft Excel (Office 16). A one-way analysis of variance (ANOVA) was used to determine statistically significant differences among the four dietary groups for each outcome variable. Where a statistically significant omnibus ANOVA result was obtained ($p < 0.05$), pairwise comparisons were performed using Tukey's Honest Significant Difference (HSD) post-hoc test to identify which specific group pairs differed significantly. This post-hoc test was selected for its robustness in controlling the family-wise error rate when making multiple comparisons across groups. Detailed results of the post-hoc comparisons are presented in the Results section. All data are reported as mean ± standard deviation (SD). A p-value of $< 0.05$ was considered statistically significant.

**Table 1: Target genes with forward and reverse primers**

| Gene (NCBI Reference Sequence) | Annealing Temp. | Forward Primer (5'3') | Reverse Primer (5'3') |
|---|---|---|---|
| ILP-2 (NM_079288.3) | 58°C / 40 cycles | GTGAAGTTGGCCCAAGGAAC | CAAACTGCAGGGGATTGAGG |
| Bmm (NM_140466.2) | 60°C / 38 cycles | GCTGTCTCCTCTGCGATTTG | TCACCACCCTGAAGAAGTCC |
| *rp49/RpL32 (NM_079505) [Reference Gene]* | 57°C / 40 cycles | GACGCTTCAAGGGACAGTATC | AAACGCGGTTCTGCATGAG |

***Key:*** *HFD: High Fat Diet; HSD: High Sugar Diet; HFD+HSD: High Fat/High Sugar Diet*

## RESULTS

### Length and Width of the Third Instar Larva

The lengths of ten (10) third instar larvae feeding on the different diets were taken. The means of the length were 724.21±61.28, 776.34±119.26, 245.48±34.81, 378.03±44.75 for the control diet, high fat diet, high sugar diet, and high fat/high sugar diet, respectively. The mean of the control diet is similar to that of the high-fat diet ($p=0.118$) but significantly higher than that of the high sugar diet ($p<0.001$), and high-fat/high sugar diet ($p<0.001$). The mean of the high-fat diet is significantly higher than that of the high-sugar diet ($p=0.000$), and the high-fat/high-sugar diet ($p=0.000$), while the high-sugar diet is significantly lower than that of the high-fat/high-sugar diet ($p=0.000$).

The means of the widths are 171.52±27.80, 164.61±15.03, 51.10±6.72, and 77.43±10.47 for the control diet, high-fat diet, high-sugar diet, and high-fat/high-sugar diet, respectively. The mean of the control diet is similar to that of the high-fat diet ($p=0.369$) but significantly higher than that of the high sugar diet ($p<0.001$), and the high-fat/high sugar diet ($p<0.001$). The mean of the high-fat diet is significantly higher than that of the high-sugar diet ($p=0.000$), and the high-fat/high-sugar diet ($p=0.000$), while the high-sugar diet is significantly lower than that of the high-fat/high-sugar diet ($p=0.001$). In both length and width, the High Sugar diet produced the smallest larvae overall, indicating it is the most detrimental diet for physical development in this stage, followed by HFD+HSD (Table 2 and Figure 1).

### Eclosion

The number of days before eclosion of the *Drosophila* differs significantly with the control diet (11 days), high-fat diet (12 days), high-fat/high-sugar diet (14 days), and high-sugar diet (23 days).

### Weight by Diet and Sex

In both sexes, consumption of the High-Fat Diet (HFD), High-Sugar Diet (HSD), and their combination (HFD+HSD) led to increased weight compared with the Control group. Females exhibited significantly higher absolute weights across all categories compared to males. The average weight of the adult male fly in the control group range from 4.8mg to 5.1mg with a mean of 4.97±0.15mg while that of the flies in the HFD group range from 5.4mg to 6.1mg with a mean of 5.77±0.35mg, and that of the High-fat/high-sugar diet (HFD+HSD) group range from 6.4mg to 7mg with a mean of 6.60±0.35mg. High sugar diet adult male flies' weight ranges from 6.0mg to 6.7mg with a mean of 6.34±0.38mg

(control<HSD with p=0.001, HFD<HSD with p=0.076, and HSD<HFD+HSD with p=0.641). There are significant differences in the means of the fly diet groups (control<HFD with p=0.037, control<HFD+HSD with p=0.000, and HFD<HFD+HSD with p=0.030). The average weight of the female fly in the control group range from 6.4mg to 7.3mg with a mean of 7.00±0.51mg while that of the flies in the HFD group range from 8.4mg to 9.5mg with a mean of 9.07±0.58mg, and that of the High-fat/high-sugar diet (HFD+HSD) group range from 8.4mg to 9.5mg with a mean of 8.87±0.56mg. High sugar diet ranges from 7.5mg to 8.2mg with a mean of 7.8±0.36mg (control<HSD with p=0.037, HSD<HFD with p=0.002, and HSD<HFD+HSD with p=0.008). There are significant differences between the weight of the female flies in the control diet group and that of HFD or HFD+HSD (control<HFD with p=0.000, control<HFD+HSD with p=0.000), but there is no difference in the means of the HFD and HFD+HSD (p=0.577) (Figure 2).

## Negative Geotaxis by Diet and Sex

The values recorded for the three trials per group indicate that the control group exhibits consistently high performance, with a score of 10 in most trials and an average score of 99%, suggesting normal motor and neurological function. The HFD shows a similar score range, 8 to 10, with an average of 97% (p=0.709), while the HSD shows a range of 6 to 9, with an average of 75% (p=0.009). The HFD+HSD groups exhibit a more significant decline in performance, with scores ranging from 7 to 10 and an average of 83%. This suggests that this group has a more pronounced negative effect on the flies' climbing ability compared to the control (p=0.032) and the HFD (p=0.057). The values recorded for the three trials per group indicate that the control group exhibits consistently high performance, with a score of 10 in most trials and an average score of 99%, suggesting normal motor and neurological function.

The HFD+HSD show similar scores, ranging from 7 to 10, with an average of 93% (p=0.087), while the HSD shows scores ranging from 6 to 9, with an average of 73% (p=0.000). The HFD groups exhibit a greater decline in performance among female flies, with scores ranging from 7 to 10 and an average of 88%. This suggests that this group has a more pronounced negative effect on female flies' climbing ability than the control (p=0.015) and the HFD+HSD (p=0.284). However, the High Sugar Diet (HSD) caused the most drastic drop in climbing ability (p<0.01) in both sexes (Figure 3).

## Metabolic Measurements in Male *Drosophila*

Control males showed an average glucose level of 66.40 mg/dl, while HFD males had an average glucose level of 78.79 mg/dl. The HFD+HSD male flies have an average glucose level of 90.82 mg/dl, and the HSD flies have an average glucose level of 30.30 mg/dl. In general, the mean glucose level of the control group of flies in males is less than that of the HFD male group (p=0.094) and that of the HFD+HSD male group (p=0.004), although there is no significant difference exists between the HFD group and that of the HFD+HSD group of flies (p=0.102). Control males had an average triglyceride level of 114.29mg/dL, while HFD males had an average triglyceride level of 161.22mg/dL. The HFD+HSD and HSD male flies have lower average triglyceride levels of 93.88mg/dL and 102.04mg/dL, respectively. The mean triglyceride level of the control group of flies in males is less than that of the HFD male group (p=0.071) but higher than that of the HFD+HSD male group (p=0.405).

There is a significant difference between the HFD group and the HFD+HSD group of flies (p=0.015). Control males had an average Total cholesterol level of 66.40mg/dL, while HFD males had an average Total cholesterol level of 78.79mg/dL. The HSD male flies have a lower average Total cholesterol level of 35.20mg/dl, while HFD+HSD have an average Total cholesterol level of 90.82mg/dl. The mean of the total cholesterol level of the control group of flies in males is less than that of the HFD male group (p=0.094) and that of the HFD+HSD male group (p=0.004), and there is no significant difference between the HFD group and that of the HFD+HSD group of flies (p=0.102). In males, the combination diet (HFD+HSD) showed a highly significant increase over Control (p=0.004). The High Sugar Diet (HSD) alone resulted in significantly lower glucose levels (p=0.000), the HFD increased triglycerides (161.22 mg/dl), but the addition of sugar (HFD+HSD) led to a significant reduction (93.88 mg/dl) compared to the fat-only group (p=0.015). Total cholesterol followed a pattern similar to glucose. Significant increases were seen in the HFD+HSD group for males (p=0.004) (Figure 4).

## Metabolic Measurements in Female *Drosophila*

The control female flies have an average glucose of 82.44 mg/dl, while the HFD females have a significantly higher average of 105.70 mg/dl, and the HFD+HSD female flies have an even higher average of 132.89 mg/dl. The HSD females have an average glucose level of 32.35 mg/dl. In the female group of flies, it is observed that the control group glucose value is significantly lower than that of the HFD group (p=0.005) and that of the HFD+HSD group of flies (p=0.000), while that of the HFD is less than that of the HFD+HSD (p=0.002). The control female flies have average triglycerides of 102.04mg/dl, while the HFD females have a significantly higher average of 190.48mg/dl, and the HFD+HSD female flies have an even higher average of 194.56mg/dl. It is observed in the female group of flies that the control group's triglyceride value is significantly lower than that of the HFD group (p=0.003) and that of the HFD+HSD group of flies (p=0.002), while that of the HFD is less than that of the HFD+HSD (p=0.866). HSD female flies have a lower average triglyceride level of 91.84mg/dl.

The control female flies have an average Total cholesterol of 82.44mg/dl, while the HFD females have a significantly higher average of 105.70mg/dl, and the HFD+HSD female flies have an even higher average of 132.89mg/dl. The HSD female flies have a lower average Total cholesterol level of 30.84mg/dl. It is observed that in the female group of flies, the control group's total cholesterol value is significantly lower than that of the HFD group (p=0.005) and that of the HFD+HSD group of flies (p=0.000), while that of the HFD is less than that of the HFD+HSD (p=0.002). Females were more severely affected, with both HFD (p=0.005) and HFD+HSD (p=0.000) showing dramatic increases.

The High Sugar Diet (HSD) alone resulted in significantly lower glucose levels (p=0.000), and HSD-only flies maintained significantly lower cholesterol levels compared to all other high-calorie groups (Figure 5).

## Brumer Gene Expression

Brummer (Bmm) gene expression differed by diet and sex in *Drosophila*. For the male *Drosophila*, there is a 1.7-fold increment in the expression of the bmm gene in those fed on HFD (p=0.703) and about a 3.8-fold increment in the expression of the bmm gene in those fed on HFD+HSD (p=0.141). While for the female *Drosophila*, there is a 1.7-fold increment in the expression of bmm gene in those fed on HFD (p=0.297) and about 1.4-fold increments in the expression of bmm gene in those fed on HFD+HSD (p=0.579). The bmm gene expression of flies fed on HSD was 10.0-fold and 13.0-fold significantly up-regulated in both male and female Drosophila, respectively (p=0.000) (Figure 6).

## Insulin-like Peptide 2 Gene Expression

For the male *Drosophila*, there is a 1.4-fold increment in the expression of the ILP2 gene in those fed on HFD (p=0.121) and about 1.7-fold increments in the expression of ILP2 gene in those fed on HFD+HSD (p=0.022) and 4.5-fold increments in the expression of ILP2 gene in those fed on HSD (p=0.000) only. While for the female *Drosophila*, there is a 1.4-fold downregulation in the expression of ILP2 gene in those that feed on HFD (p=0.100), and about 1.5-fold up regulation in the expression of ILP2 gene in that feed on HFD+HSD (p=0.069) and about 7.7-fold up regulation in the expression of ILP2 gene in that feed on HSD (p=0.000) (Figure 7).

**Table 2: Length and Width of the Third Instar Larva**

| Group | Length | | Width | |
|---|---|---|---|---|
| | Mean ± SD (µm) | P-value | Mean ± SD (µm) | P-value |
| Control (µm) | 724.21±61.28 | | 171.52±27.80 | |
| HFD (µm) | 776.34±119.26 | 0.118 | 164.61±15.03 | 0.369 |
| HSD (µm) | 245.48±34.81 | <0.001 | 51.10±6.72 | <0.001 |
| HFD+HSD (µm) | 378.03±44.75 | <0.001 | 77.43±10.47 | <0.001 |

F-Values are: (F=127.368, P=0.000 for length), (F=128.823, P=0.000 for width)

**Key:** HFD: High Fat Diet; HSD: High Sugar Diet; HFD + HSD: High Fat/High Sugar Diet

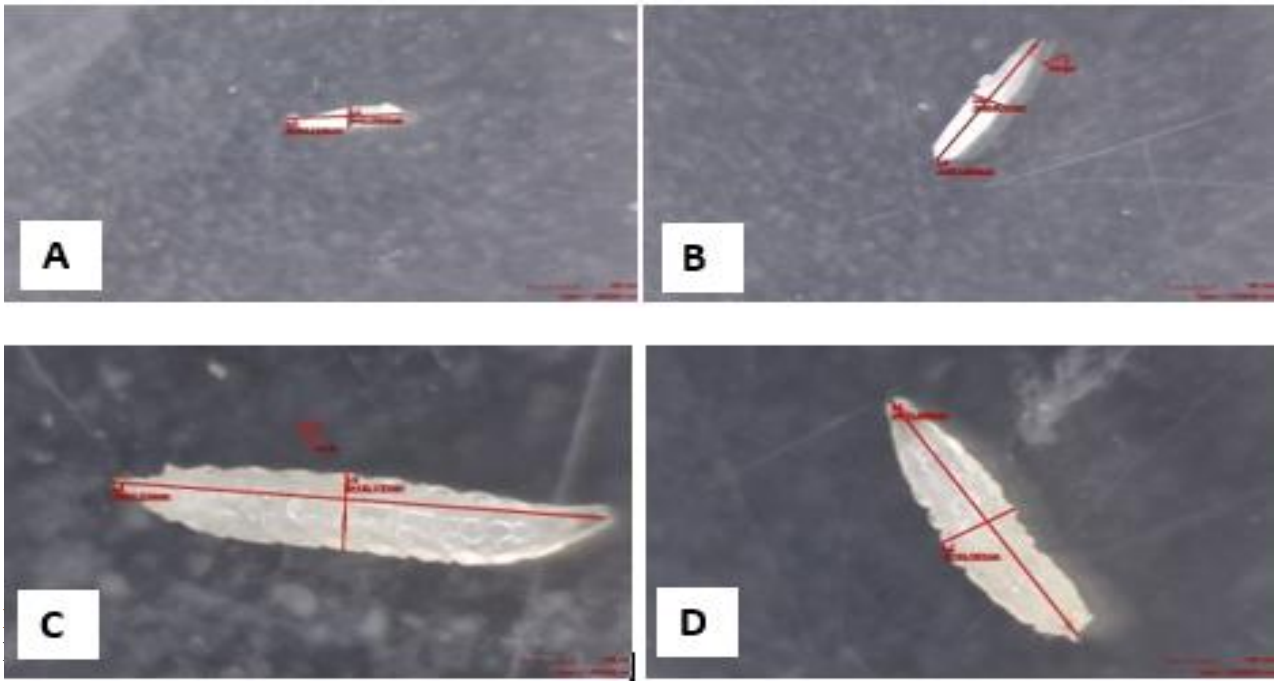


**Figure 1: Photomicrograph of the Third Instar Larva Showing the Length and Width of Different Diets**
**Key:** A: High Sugar Diet; B: High Fat Diet/High Sugar Diet; C: Control Diet; D: High Fat Diet

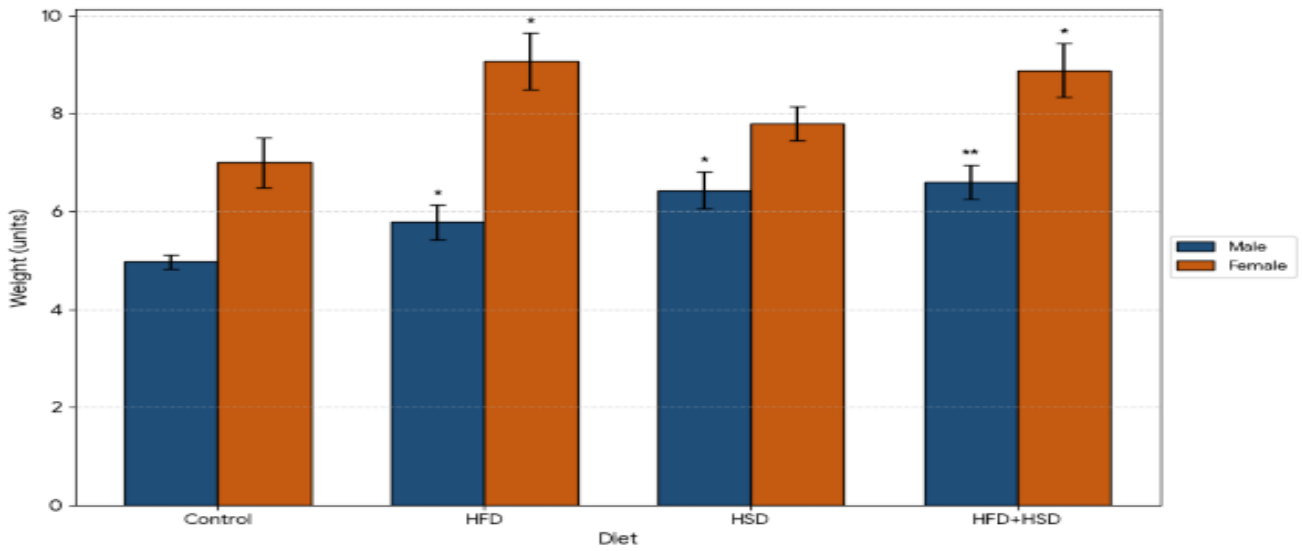


**Figure 2: Adult Weight by Diet and Sex**
F-Value is (F=33.669, P=0.000)
**Key:** HFD: High Fat Diet; HSD: High Sugar Diet; HFD + HSD: High Fat/High Sugar Diet

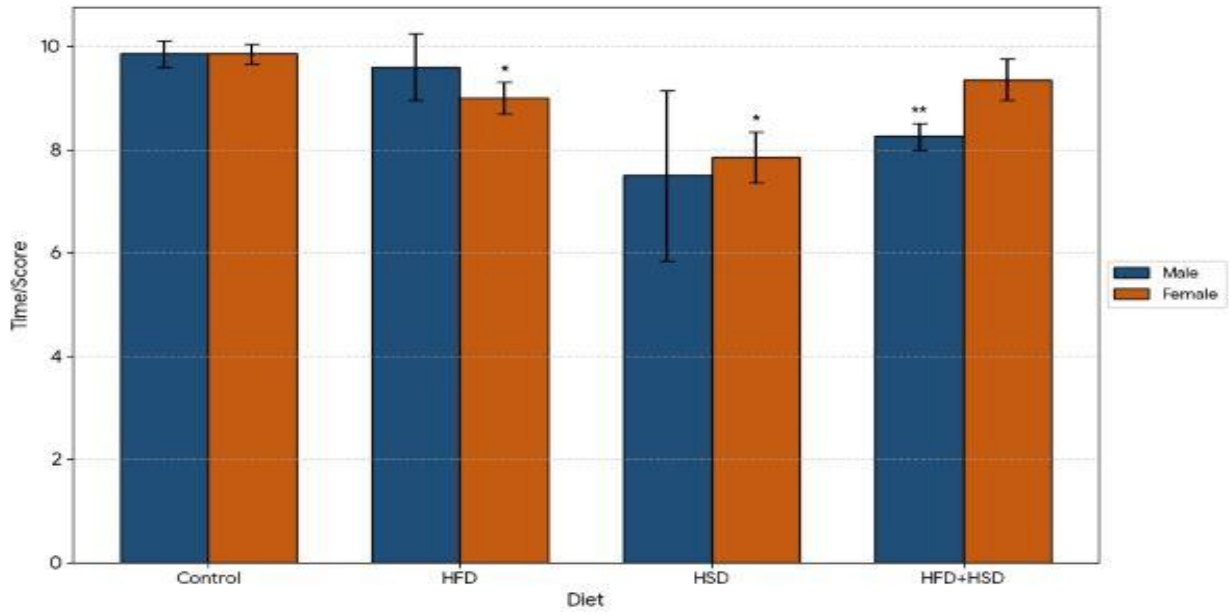


**Figure 3: Negative Geotaxis by Diet and Sex**
F-Values are (F=5.989, P=0.024 for Male), (F=17.393, P=0.001 for Female).
**Key:** HFD: High Fat Diet; HSD: High Sugar Diet; HFD + HSD: High Fat/High Sugar Diet

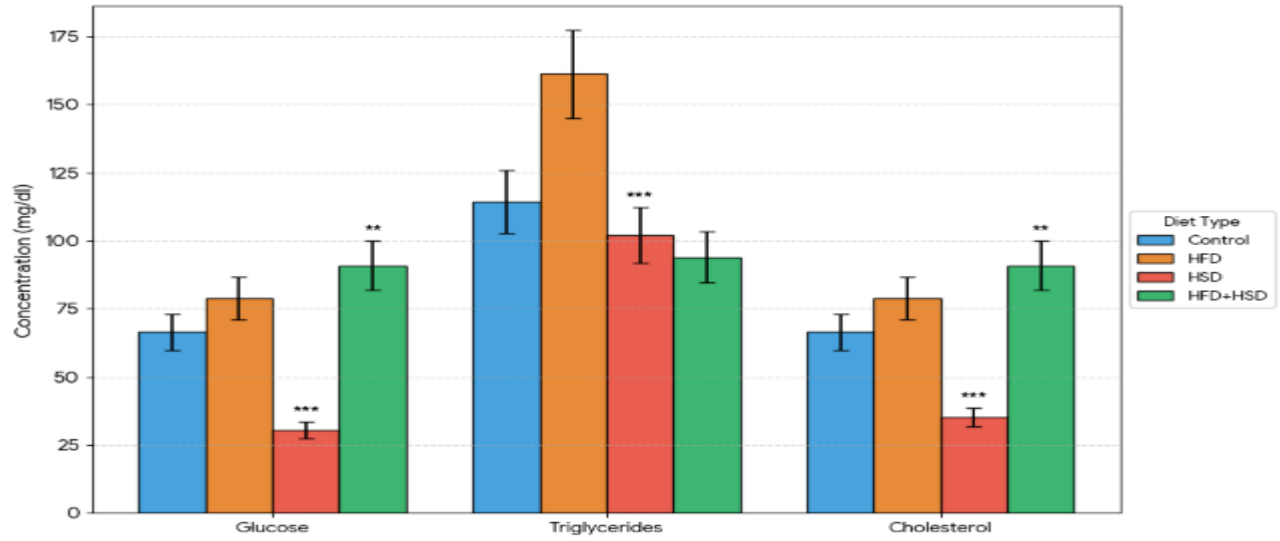


**Figure 4: Metabolic Measurements in Male *Drosophila***
F-Values are (F=23.992, P=0.000 for glucose), (F=7.259, P=0.002 for Triglycerides), (F=23.992, P=0.002 for Total Cholesterol).
**Key:** HFD: High Fat Diet; HSD: High Sugar Diet; HFD + HSD: High Fat/High Sugar Diet

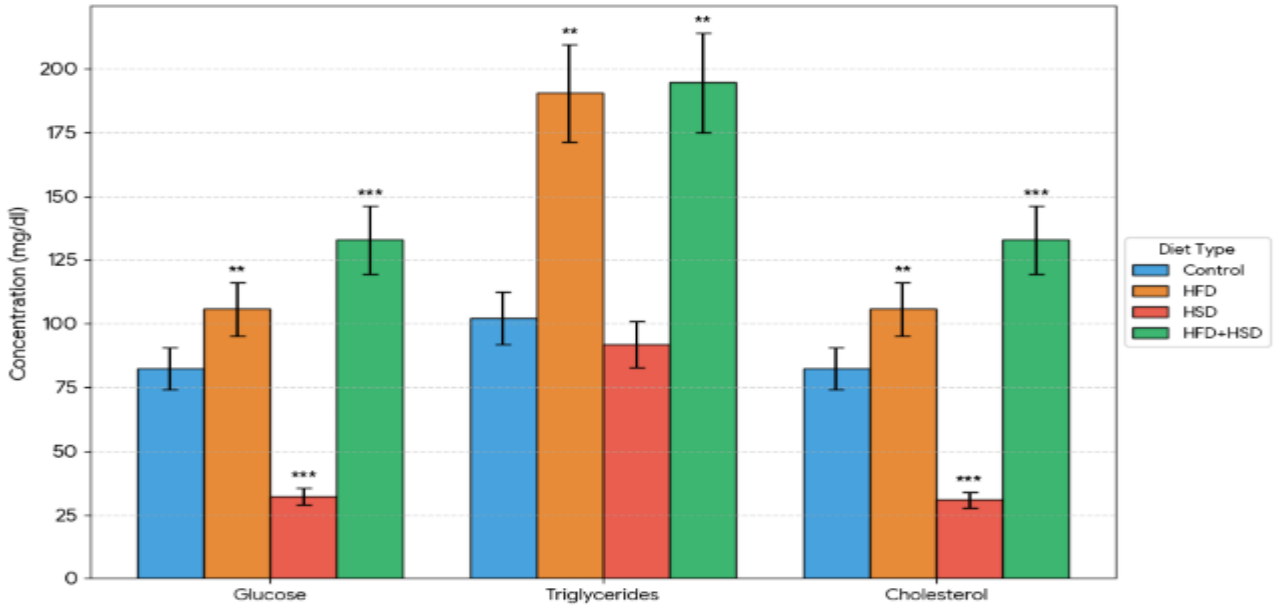


**Figure 5: Metabolic Measurements in Female *Drosophila***
F-Values are (F=23.992, P=0.000 for glucose), (F=7.259, P=0.002 for Triglycerides), (F=23.992, P=0.002 for Total Cholesterol).
**Key:** HFD: High Fat Diet; HSD: High Sugar Diet; HFD + HSD: High Fat/High Sugar Diet

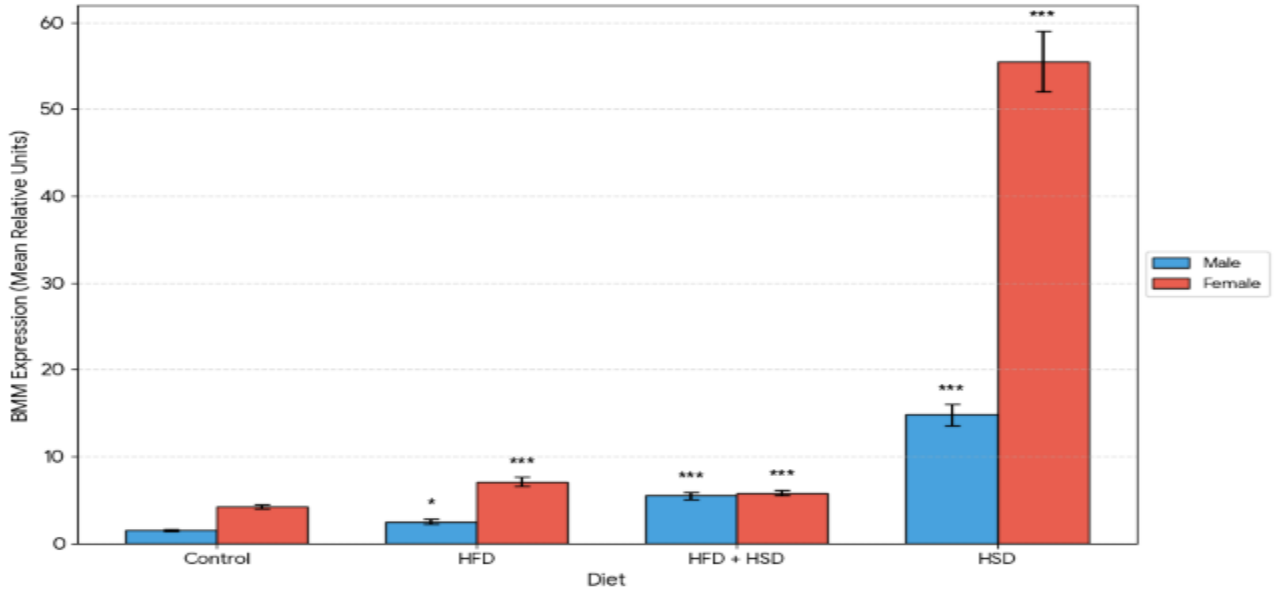


**Figure 6: Brummer (Bmm) gene expression**
F-Value is (F=1.336, P=0.314)
**Key:** HFD: High Fat Diet; HSD: High Sugar Diet; HFD + HSD: High Fat/High Sugar Diet

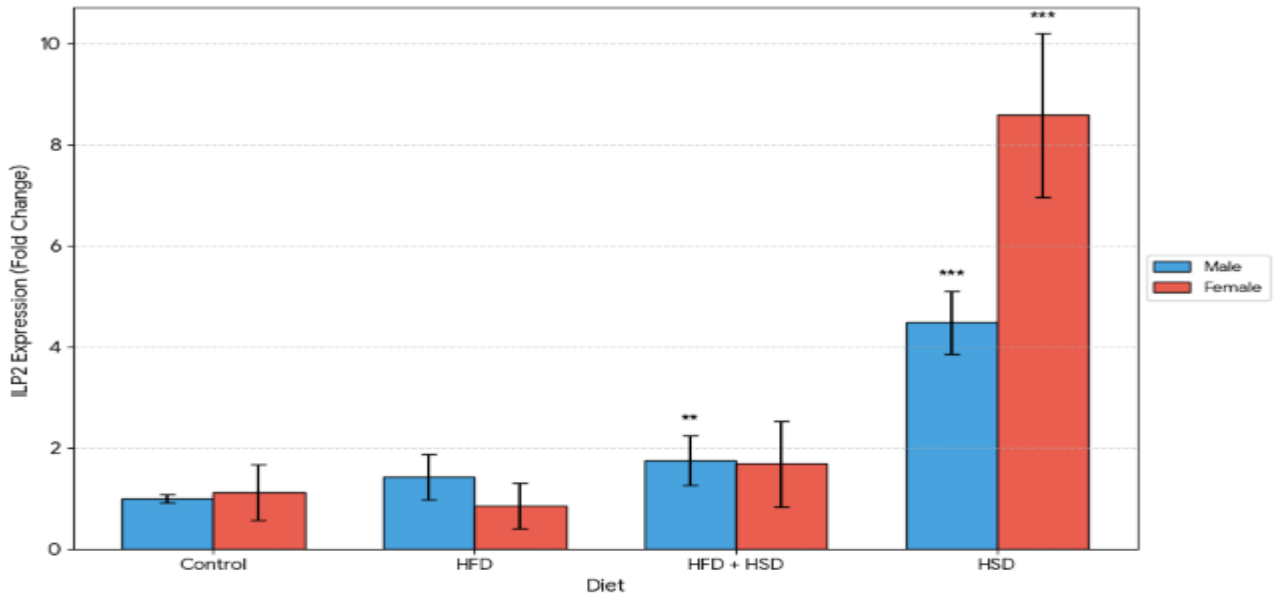


**Figure 7: Insulin-like Peptide 2 Gene Expression**
F-Value is (F=2.394, P=0.100)
**Key:** HFD: High Fat Diet; HSD: High Sugar Diet; HFD + HSD: High Fat/High Sugar Diet

## DISCUSSION

This study compared the metabolic, developmental, and behavioural effects of HFD, HSD, and a combined high-fat/high-sugar diet (HFD+HSD) in *Drosophila melanogaster*. Larval growth was significantly influenced by diet composition, as reflected by differences in the length and width of third instar larvae. Larvae reared on the high-sugar diet exhibited the smallest body size, while those exposed to the combined HFD+HSD diet also showed marked reductions compared with the control and HFD groups. In contrast, larvae fed the high-fat diet alone showed growth patterns closer to those of the control group. These observations align with previous studies describing the metabolic and developmental effects of high-fat and high-sugar diets in *Drosophila*.[8,13-15] Although both diets are associated with adverse physiological outcomes,[8] the high-sugar diet produced more pronounced effects on larval development, while the combined HFD+HSD diet showed indications of additive dietary stress.

Dietary composition also influenced developmental timing, particularly the duration to eclosion. Flies maintained on the control diet eclosed at approximately 11 days, whereas those reared on the high-fat diet showed a slight delay (12 days), and those on the combined HFD+HSD diet exhibited a longer developmental period (14 days). The most pronounced delay occurred in the high-sugar group, where eclosion was observed at approximately 23 days, more than twice that of the control. Previous studies have reported an inverse relationship between dietary sugar concentration and developmental rate in *Drosophila*, suggesting that elevated sugar intake may interfere with physiological processes involved in normal developmental progression.[16,17]

Adult body weight was also influenced by diet and sex. Both male and female flies exposed to high-calorie diets exhibited increased body mass relative to controls, with the largest increases observed in the high-fat and high-fat/high-sugar groups. Females consistently displayed greater body weight than males across all diets, highlighting sexual dimorphism in metabolic responses. Similar sex-dependent differences in fat accumulation and energy storage have been reported in *Drosophila* exposed to obesogenic diets.[6,10] Behavioral assessment using the negative geotaxis assay revealed that dietary excess adversely affects locomotor performance. Although flies on the high-fat diet showed only modest impairment,

those fed high-sugar or combined diets demonstrated more pronounced reductions in climbing ability, with the high-sugar group exhibiting the most severe deficits. These findings align with earlier reports that high-fat and high-sugar diets impair neuromuscular function and accelerate behavioral decline in *Drosophila.* [6,18]

Metabolic measurements further demonstrated diet-dependent alterations in glucose and lipid homeostasis. In both sexes, glucose levels increased markedly in flies exposed to high-fat and combined high-fat/high-sugar diets, indicating diet-induced metabolic stress and potential insulin resistance.[6,8] Interestingly, flies fed high-sugar diets alone exhibited significantly lower glucose levels. This unexpected pattern has been previously reported and may reflect reduced food intake or enhanced metabolic utilization under high-sugar conditions.[9,19] Genotype-specific metabolic responses have also been shown to influence glucose regulation in *Drosophila* exposed to high-sugar diets.[9]

Triglyceride levels also varied according to diet and sex. In males, the high-fat diet produced the highest triglyceride levels, whereas the addition of sugar reduced triglyceride accumulation relative to the fat-only diet. In contrast, female flies exhibited the highest triglyceride levels under the combined high-fat/high-sugar diet. These sex-specific patterns highlight the importance of considering sexual dimorphism in metabolic studies and are consistent with previous work demonstrating differential lipid metabolism between male and female flies.[6,10]

Total cholesterol levels followed a pattern similar to that of glucose, with the highest concentrations observed in flies exposed to the combined diet, particularly in females. These findings reinforce the notion that diets rich in both fat and sugar exacerbate metabolic disturbances more than single-nutrient excess, reflecting interactions between lipid and carbohydrate metabolic pathways. Gene expression analyses provided further insight into the molecular mechanisms underlying these metabolic changes. Expression of the *brummer* (*bmm*) gene, which encodes a key triglyceride lipase involved in lipid mobilization, increased in response to high-calorie diets. Up-regulation was particularly evident in flies exposed to high-fat and combined diets, suggesting activation of lipid-mobilizing pathways in response to excess energy intake. These observations are consistent with evidence that *bmm* plays a central role in regulating fat storage and breakdown in *Drosophila*.[20] Persistent metabolic overload, however, may overwhelm these compensatory mechanisms, leading to lipid accumulation and metabolic dysfunction.[21]

Similarly, expression of the insulin-like peptide 2 (*ILP2*) gene was altered by dietary interventions. *ILP2*, a key regulator of insulin signaling and metabolic homeostasis, was up-regulated under high-sugar and combined high-fat/high-sugar diets in both sexes. The strongest induction occurred in flies exposed to high-sugar diets, consistent with previous studies showing that excessive carbohydrate intake stimulates insulin signaling pathways in response to metabolic imbalance.[6,8,19] Notably, males displayed a greater increase in *ILP2* expression under combined dietary stress, whereas females showed a more moderate response and even mild down-regulation under the high-fat diet. These differences likely reflect sex-specific regulatory mechanisms in insulin signaling and energy metabolism. Taken together, these findings demonstrate that high-fat and high-sugar diets profoundly influence developmental, metabolic, and behavioral outcomes in *Drosophila melanogaster*. Excess sugar exerted particularly strong effects on growth and locomotor performance, whereas fat-rich diets primarily altered lipid metabolism. The combination of both nutrients produced the most severe metabolic disturbances, highlighting potential synergistic interactions between dietary fat and sugar in driving metabolic dysfunction.

## STUDY LIMITATIONS

The following limitations should be considered when interpreting the findings of this study. Metabolic assessment was limited to a small set of biochemical markers and may not fully capture the complex metabolic alterations associated with high-fat and high-sugar diets in *Drosophila melanogaster*. In addition, the genetic sequence of the Harwich strain used was not verified, which may limit the interpretation of potential genotype-specific responses. The study also did not include lifespan analysis or functional assays to directly assess insulin resistance, and gene expression results were not validated at the protein level. Despite these limitations, the study still provides useful insights into the metabolic, developmental, and behavioral responses of *Drosophila melanogaster* to diets enriched in fat and sugar and highlights potential interactions between these dietary

components in the development of metabolic dysfunction.

## CONCLUSION

This study demonstrates that combined exposure to high-fat and high-sugar diets produces more pronounced metabolic, developmental, and behavioral disturbances in *Drosophila melanogaster* than either diet alone. The combined HFD+HSD condition was associated with greater alterations in growth, metabolic markers, locomotor performance, and diet-responsive gene expression, indicating an amplified metabolic stress response. These findings highlight the value of the combined high-fat/high-sugar dietary model for investigating the interactive effects of excess dietary fat and sugar and for studying mechanisms underlying metabolic disorders such as obesity and type 2 diabetes.

### Source of support

Nil.

### Conflict of interest

None declared.

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