Dietary Restriction Induces a Stable Metabolic Obesity Phenotype in Drosophila Melanogaster

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Research note

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Abstract

Objective

Challenges associated with current nutritional models to induce obesity in Drosophila melanogaster created a rationale for this study. The objective of the study was to investigate biochemical changes associated with high-fat diet (HFD), high sucrose diet (HSD), and a protein-restricted diet (DR) to induce a healthy metabolic obesity state. Drosophila melanogaster were fed to four experimental diets: regular food (control), HFD, HSD, and DR, for four weeks. Peristaltic waves were measured on 3rd instar larvae, while negative geotaxis, body mass, catalase activity; and total triglycerides, sterol, and protein were measured in adult Drosophila melanogaster.

Results

DR produced a Drosophila melanogaster phenotype which had superior adaptive advantages than that generated from HFD and HSD. HFD was the best phenotype during larval stages; however, locomotory, body mass, triglyceride, sterol concentrations, and catalase activity were highest in the DR phenotype during adulthood. High catalase activity and high triglyceride content demonstrated a balanced and healthy metabolic obesity status than in other phenotypes in the adult stage. Evolutionary changes are responsible for the selective advantage of the DR phenotype over the HFD phenotype. Prospective studies to guide therapy and community behavior should place more emphasis on the DR phenotypes in Drosophila melanogaster.

1. Introduction

Drosophila melanogaster (Drosophila) animal disease model is increasingly being used because of the high cost of maintaining mammalian models (Coogan, 2013). Drosophila accumulates lipids in both the fat and non-fat tissue in a dose-dependent manner in a shorter period, unlike mice which take months to become obese (1). Most of the major metabolic enzymes in mammals are conserved in Drosophila, and the genes that regulate lipid metabolism are well conserved (2). Lipids in Drosophila are stored as triglycerides in the fat tissue, similar to triglyceride storage in mammals' adipose tissue (3, 4). Additionally, the molecular mechanism that controls the metabolism of neutral lipids in cellular lipid droplets (lipophorins) resembles that in mammalian pathways of lipoproteins (5). Like other insects when fed a high-fat diet (HFD), Drosophila stores other forms of sterols such as ergosterol, stigmasterol, zymosterol, and campesterol depending on the dietary fat source and the sterol requirements (6, 7). The excess sterols from dietary sources are interconverted to triglycerides as the main circulating and storage lipid energy reserves depending on the energy balance and metabolic requirements for specific body processes (8).

Drosophila has proved to be a great model of obesity (9, 10); however, studies on diet-induced phenotypes are scarce. For example, dietary protein restriction has been associated with increased lifespan in adult and larval stages of Drosophila (11). Furthermore, dietary restriction has been associated with increased expression of antioxidant genes, increasing stress resistance and locomotory activity (12). In Drosophila
larvae, restricted nutrition has been associated with higher fat reserves in adult flies due to increased expression of fat mobilizing genes and lipid storage droplet-2 in mature flies (13). However, effects of coconut and sucrose as feed additives on the *Drosophila* phenotype have not been explored. This study’s objective was to assess effects of high fat and sucrose diets on larval and adult fitness, mass, sterol and triglyceride content in *Drosophila melanogaster*.

### 2.0 Methods

#### 2.1 Laboratory animals

The study was done using Yellow white strain *w*^{118} of *Drosophila Melanogaster* obtained originally from the National Species Stock Center (Bowling Green, OH, USA). Roughly 1200 adult flies (with equal numbers of females and males were mated and maintained for two generations at the Institute of Biomedical Research of Kampala International University Western Campus, Uganda. The flies were divided into four groups exposed to regular food (control), high-fat diet (HFD), high sucrose diet (HSD), 15% protein-restricted diet (DR = 15% less yeast than in regular feed) for two weeks under 70% humidity, 24-26°C temperature and 12:12 h light/dark cycles. Flies at the second generation, which were age-matched flies, were transferred and placed in 500 ml plastic flasks containing 25 ml of fly food with extra yeast added and left there for 15 h at 25°C. Eggs laid on the food surface and the container wall were removed by using a tender brush and left on the food to grow in the incubator (14). First instar larvae were collected within 2–3 hours of egg hatching. Thirty third instar larvae were collected into fresh vials on day three, placed on new experimental specific media and allowed to grow to adulthood.

#### 2.2 Experimental design

*Drosophila* regular food contained cornmeal 7% w/v, dextrose 7.5% w/v, yeast 1.5% w/v, nipagin 2.33% v/v, agar base 1.05% w/v, propionic acid 0.37% w/v in a liter of food (control group). HFD was prepared by adding 10% w/v food-grade coconut oil to the regular cornmeal food (15). HSD was prepared by adding 150 mM sugar to regular cornmeal food (10, 15). DR was prepared by reducing the yeast composition by 15% (11). The larval media contained the same feed composition at a concentration of 50% except for agar and nipagin. Each group contained ten third instar larvae. Subsequently, ten adults were kept in each vial and experiments were conducted in triplicates for four weeks from the date of egg collection (larval experiments for one day while adults emerged after day 10).

#### 2.3 Complete peristaltic waves of third instar larvae

This was performed on 1% agar in distilled water on Petri dishes to increase visibility and ease recording of movements (16). Each larva was washed in distilled water to clean them of any food and transferred using a smooth brush to a fresh plate. The larvae were allowed to acclimatize for 1 minute, and video recordings were conducted from the top. The number of peristaltic waves per minute was counted in 3 consecutive trials.

#### 2.4 Negative geotaxis assay and locomotor activity assays
After two weeks, negative geotaxis was investigated as previously described (17) with minor modifications. Ten flies from the respective groups were immobilized under light anesthesia with ice. After 10 minutes of recovery, the flies were gently tapped to the bottom of the column, and the time they took to reach the height of 8cm was recorded. The tests were repeated three times for each group at one-minute intervals.

2.5 Fly Body mass

Each fly was frozen, and its body weight measured using a Sartorius microbalance (18). Whole fly samples were then homogenized in 100 µl of cold 0.05 % phosphate-buffered saline tween solution, and the homogenate centrifuged at 13,000 rpm for 3 minutes (19). The supernatant was immediately stored at 4°C for biochemical analysis.

2.6 Determination of total triglycerides and sterol concentrations from the supernatant

Total triglyceride and sterol levels were measured using Cypress Diagnostics triglyceride and cholesterol oxidase kits, respectively. A commercial coupled colorimetric assay (CCA) protocol was used to indirectly measure the triglyceride in the form of a quinone imine dye at 540nm absorbance (20). A fluorometric assay protocol was used to indirectly measure the sterol in the form of resorufin at 590nm fluorescence (19).

2.7 Determination of total protein and catalase activity from the supernatant

The total protein was indirectly measured using Cypress Diagnostics kit and a Bradford assay protocol (17).

Catalase activity was determined by following a protocol developed by (21) and customized by (17). A calibration curve was generated in the form y = mx + c using standard catalase concentrations for which the corresponding foam heights were determined with the defined unit of catalase activity. 100µl of catalase solution was pipetted in 13mm diameter x 100mm height test tubes, 100 µl of 1% Triton X-100 and 100 µl of undiluted hydrogen peroxide (30%) were added to the solutions, mixed thoroughly, and incubated at room temperature. After reaction completion, the height of O₂-forming foam that remained constant for 15 minutes in the test tube was measured using a ruler. The corresponding catalase activity was ascertained and expressed as mg/ml of protein. A standard curve was constructed from which an equation was generated in the form y = mx + c i.e., Absorbance (y) = 0.0432 concentration (x) + 0.013.; \( R^2 = 0.9973 \). The above experimental protocol and the standard curve generated were used to determine catalase activity for HFD, HSD, and yeast supplemented base food fed fly samples.

2.8 Statistical analysis

Graphpad prism version 6 software (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis and the results were reported as mean ± SEM. A Tukey's test was used for inferential statistics, and p < 0.05 was considered to represent significance.
3.0 Results

3.1 Restrictive diets led to better phenotypes in *Drosophila melanogaster*

In the larval stages, DR was associated with significantly low peristaltic movements compared to HFD (Fig. 1A). In adults, DR was associated with increased body mass (Fig. 1B) and significantly increased locomotory activity (Fig. 1C). Also, HSD was associated with high peristaltic movements than the control feed in the larval stages, although no significant differences were observed between HSD and the control on body mass and locomotion (Table 1).

| Tukey's multiple comparisons test | N   | Complete peristatic waves | Mass | Negative geotaxis | Triglycerides | sterols | Total protein | Catalase |
|----------------------------------|-----|---------------------------|------|-------------------|---------------|---------|---------------|----------|
| Control vs. DR                   | 60  | 0.0236                    |      | <0.0001           | <0.0001       | <0.0001 | <0.0001       | 0.0003   |
| Control vs. HSD                  | 60  | <0.0001                   | 0.8452| 0.7538            | <0.0001       | <0.0001 | <0.0001       | 0.262    |
| Control vs. HFD                  | 60  | <0.0001                   | 0.1442| 0.2641            | <0.0001       | 0.0002  | <0.0001       | 0.0416   |
| DR vs. HSD                       | 60  | <0.0001                   |      | <0.0001           | <0.0001       | <0.0001 | <0.0001       | 0.0167   |
| DR vs. HFD                       | 60  | <0.0001                   |      | <0.0001           | <0.0001       | <0.0001 | 0.9988        | 0.0107   |
| HSD vs. HFD                      | 60  | <0.0001                   | 0.4141| 0.0665            | <0.0001       | <0.0001 | <0.0001       | 0.9863   |

3.2 Protein restrictive diet improved fat storage and antioxidant enzymes

In adult stages, DR was associated with significantly increased triglycerides (Fig. 2A), sterols (Fig. 2B), low total protein (Fig. 2C), and high catalase activity (Fig. 2D). Furthermore, HFD had high triglycerides and sterol levels only lower than those in the DR; however, HFD had the highest total protein levels and significantly comparable levels of total protein and catalase to HSF (Table 1).

4. Discussion
The study shows that protein-restricted dietary nutrition results in superlative phenotypes than high-fat diets. The study also shows that stress experienced during early developmental stages in life (larvae) could lead to reduced activity due to reduced impulse transduction (16). However, this favors the development of adaptive changes with increased foraging for food, resulting in an increased body mass, demonstrating evolutionary changes that would favor survival during adulthood (11). Furthermore, the study findings are similar to those of previous studies where diet restriction improved the ability to resist stress (13), increased mitochondrial density and respiratory activity, enhanced fat metabolism and physical activity (22). Besides, high fat diet-induced obesity showed a ≥ 50% reduction in Drosophila's competitive ability (10,23). On the other hand, overnutrition during childhood would lead to the development of laziness and reluctance to forage for food, and this would justify the decreased activity in adults exposed to a lot of food in childhood. DR-induced obesity modelling in Drosophila did not affect the nervous coordination potential of obese flies. Thus, it caused higher competitive ability, unlike HFD- and HSD-induced obesity models that negatively affected nervous coordination ability, causing lesser competitive ability. These results are supported by previous studies, which reported competitive ability under obese conditions is independent of the level and degree of adiposity (24). Findings in this study demonstrate the reliability of Drosophila as a research model to study metabolic and evolutionary conditions in humans (9,10).

We also showed that DR in adulthood caused increased triglycerides, sterols and high catalase activity. These findings demonstrate the improved metabolic state in the Drosophila phenotype. Triglycerides provide energy, and these are markers of obesity in Drosophila (8). Increased energy storage versus expenditure is associated with improved antioxidant balance, which is important to control reactive oxygen species arising from oxidation of lipids and oxidative stress (25,26). The major storage forms of sterols in Drosophila is dehydrocholesterol and ergosterol (19). The DR phenotype had the highest levels of sterols than the HFD and HSD fed obese flies, and mechanisms for these differences remain to be explored.

Total protein levels were highest in HSD, providing evidence that HSD-induced obesity in Drosophila could face major increased protein levels compared to HFD. This is because HFD has been associated with increased lipid peroxidation, which causes increased tissue death and reduced lifespan in Drosophila (27,28). Furthermore, HSDs are characterized by a hypertonicity—a condition that hastens autolysis (29). On the other hand, challenges observed with HFD and HSD models help identify them as better cancer research models (30). High catalase activity demonstrates the superlative advantage associated with DR for the induction of obesity in Drosophila. Increased endogenous catalase enzyme activity serves as a natural defense mechanism against oxidative stress in pathological conditions such as obesity (31). Our findings are similar to previous studies that reported DR association with increased mitochondrial density and respiratory activity, causing elevated antioxidant enzyme activity. This is unlike the HFD and HSD fed obese flies which were associated with high levels of oxidative stress and tissue death (15,22). Furthermore, sugar diets at either low or high supplementation alter physiological function (reproduction) due to body size changes and organ size (32).

In this study, induction of obesity by DR produced a competitively more active and obese fly phenotype compared to the HFD and HSD fed obese phenotypes. The study's findings demonstrate a need to explore
more active and appropriate models for modeling obesity, emphasizing evolutionary adaptive changes to promote knowledge on healthy metabolic obesity.

**Limitations**

Molecular markers describing the metabolism of triglycerides were not investigated in this study due to financial and infrastructural challenges. Further studies would emphasize the crosstalk between a balanced diet and obesity to generate knowledge to address metabolic obesity. This would enhance further understanding of the immunological modulators of inflammation, second messengers and modulators of gene expression that can lead to the development of novel therapeutical options and healthy lifestyles.

**Abbreviations**

HFD High-fat diet HSD High sucrose diet DR Restrictive diet w/v Weight by volume v/v Volume by volume

**Declarations**

**Ethical approval and consent to participate**

Ethical considerations were followed. This research model was selected based on following the replacement protocol for ethics in animal research and registered under number Nr.UG-REC-023/201916. Consent to participate was not applicable.

**Consent to publish**

Not applicable

**Competing interests**

The authors declare no conflicts of interest exist.

**Author contributions**

OHA and KIK conceptualized and designed the study, OHA and KIK conducted data acquisition and analysis while SSO, JJO, DO, JMN, LOO, AOO interpreted the data. KIK and OHA drafted the manuscript. All authors revised it critically for intellectual content, gave final approval for publication and all authors remain in agreement to ensure that questions related to the work are appropriately resolved.

**Availability of data and materials**
Information used in the study can be found at

**Funding statement**

Not applicable

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Figures

Figure 1

Changes in Drosophila melanogaster after induction of obesity (A) Complete peristaltic waves of third instar larvae (B) Mass and (C) Negative geotaxis.
Figure 2

Changes in Drosophila melanogaster after induction of obesity (A) Triglyceride (B) sterols (C) Total protein (D) Catalase activity. Different superscripts indicate significant differences in experimental groups.