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A protein restricted diet induces a stable increased fat storage phenotype in flies

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ABSTRACT

Background: Scientific evidence has revealed possible confounders in diet induced obesity models of *Drosophila melanogaster*. High Sugar Diet (HSD) induction of obesity in flies has been associated with fly hyperosmolarity and glucotoxicity, while High Fat Diet (HFD) induction has been associated with lipotoxicity. The objective of this study was to assess for a healthy obesity phenotype by comparison of fly survival, physio-chemical and biochemical changes associated with HSD, HFD and Protein Restricted Diet (PRD) obesity induction models of male *Drosophila melanogaster*. Here, we provide information on a PRD as the plausible option in obesity research not involving cancer, diabetes, glucotoxicity and lipotoxicity studies.
Methods: Obesity was induced by exposing *Drosophila melanogaster* white mutant w^{1118} to four experimental diets for four weeks. Group 1 was fed regular food (control), group 2 was fed a 0.5% less yeast than in regular feed (PRD), group 3 was fed a 30% w/v sucrose to regular cornmeal food (HSD) and group 4 was fed a 10% w/v food-grade coconut oil to regular cornmeal food (HFD). Peristaltic waves were measured on 3rd instar larvae of all experimental groups. Negative geotaxis, fly survival, body mass, catalase activity, triglycerides (TG/TP), sterol, and total protein were measured in adult *Drosophila melanogaster* after four weeks.
Results: Triglycerides (TG/TP) and total protein levels were significantly higher in HSD phenotype. Sterols were higher in HFD phenotype. Though catalase enzyme activity was highest in PRD phenotype, this activity was not statistically significant when compared to that of HSD and HFD phenotypes. However, PRD phenotype had the lowest mass, highest survival rate and the highest negative geotaxis, thus demonstrating a balanced, stable and more viable metabolic status in the experimental model.
Conclusion: A protein restricted diet induces a stable increased fat storage phenotype in *Drosophila melanogaster*.

1. Introduction

A protein restricted diet (PRD) is nutritional intervention of limiting protein intake without causing malnutrition [1,2]. The benefits of PRD such as increased lifespan, remediation of chronic illnesses and improvement of whole organism wellbeing has been recorded in

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Abbreviations: BMI, Body mass index; HFD, High Fat Diet; HSD, High Sucrose Diet; PRD, Protein Restricted Diet; w/v, Weight by volume; v/v, Volume by volume.

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previous studies [3–7]. Longevity dietary patterns such as the Guangxi dietary pattern and the Okinawan dietary pattern (85% carbohydrate and 9% protein) have been found to have similarities with PRD [4,8]. The disposable soma theory attributes pro-longevity effects of PRD to the re-allocation of limited energy resources from reproduction to somatic maintenance [9,10]. However, recent studies continue to indicate that reproduction and lifespan under PRD respond to the balance of dietary macronutrients and not the overall energy content of diet [11–16]. More so, studies show that the remedial and beneficial effects of PRD could be an indirect result of a limitation in certain amino acids such as methionine, tryptophan and branched chain amino acids [16–22]. The interaction of these amino acids with other amino acids and/or metabolites may compromise multiple organ and/or whole organism molecular and physiological homeostatic functions, leading to disease and reduced lifespan [16]. Suggested mechanisms of action for the remedial and beneficial effects of PRD include; stimulated hydrogen sulfate (neurotransmitter) production [23], increased carbohydrate metabolism and accelerated tri-carboxylic acid cycle (TCA) activity [24], increased mitochondrial biogenesis and antioxidant activity [25, 26], increased cellular proteostasis and autophagy [7,27,28], reduced signaling of growth hormone/insulin like growth factor-1 (GH/IGF-1) pathway [29], reduced signaling of amino acid sensitive mechanistic target of rapamycin complex 1 (mTORC1) pathway [30–33] and increased activation of fibroblast growth factor 21 (FGF21) hormone [34,35].

Drosophila melanogaster (Drosophila) disease model is increasingly being used because of the high cost of maintaining mammalian models to model metabolic conditions [36–38]. *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 [36,39]. Most of the major metabolic enzymes in mammals are conserved in *Drosophila,* and the genes that regulate lipid metabolism are well conserved [40]. Lipids in *Drosophila* are stored as triglycerides in the fat tissue, similar to triglyceride storage in mammals' adipose tissue [41,42]. Additionally, the molecular mechanism that controls the metabolism of neutral lipids in cellular lipid droplets (lipophorins) resembles that in mammalian pathways of lipoproteins [43]. 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 [44,45]. 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 [46]. Unlike humans where the standard for obesity is a Body Mass Index (BMI) $\geq$ 30.0 Kg/m^2, obesity in flies is not exactly defined, and the term is used as an equivalent for increased fat storage [47]. In *Drosophila* obesity modelling research, PRD refers to protein restriction and yeast is the main source of protein [48,49], and male flies are often preferred because they consume more carbohydrate and, they also accumulate more fat when compared to female flies [50,51].

Obesity can be traced back to 30,000 years ago when accumulation of energy in periods of plenty conferred a survival advantage during periods of hunger and starvation ('thrifty gene' theory)[52–54]. Although a BMI $\geq$ 30 kg/m^2 is associated with an increase in all-cause mortality [55], a BMI 25 $<$ 30 kg/m^2 is associated with a decrease in all-cause mortality [56,57], and a better disease prognosis, thus the concept of 'Obesity paradox' [58–60]. Similarly, obesity research using *Drosophila melanogaster* continues to reveal the protective roles of adiposity and the independence of increased adiposity from the known adverse effects of obesity, such as increased obesity related co-morbidities and mortality [61,62]. These instead, have been attributed to lipid autotoxin accumulation during fly development, glucotoxicity, low fat turnover rate and ectopic lipid accumulation [63–66]. A low fat turnover rate, glucotoxicity, ectopic and autotoxin lipid accumulation disrupt cellular pathways involved in; energy homeostasis such as Adenosine Monophosphate Protein Kinase pathway, cellular stress regulation such as Jun-N-terminal kinase pathway, pro-inflammatory cytokine regulation such as Mitogen Activated Protein Kinase pathway, gene expression regulation such as the Calcium-Calmodulin dependent protein kinase II pathway and the Phosphoinositol-3-protein kinase/Akt pathway [65,67]. This in turn disrupts normal multi-organ cellular homeostatic functions due to increased mitochondrial and endoplasmic reticulum stresses resulting in multifactorial adverse effects of obesity such as insulin resistance, foam cell formation, increased cytokine production and hypoxia which manifest clinically as type II diabetes, dyslipidemia, osteoarthritis, some cancers, Coronary artery disease, stroke, hypertension, liver disease, psychological challenges and polycystic ovary syndrome [68–70]. (Fig. 1).

Drosophila has proved to be a great model of obesity [71,72]; however, comparative studies on diet-induced obese phenotypes are scarce. Previous studies show an increase in the lifespan of *Drosophila* under a PRD [2,73–75]. Furthermore, PRD has been associated with increased expression of antioxidant genes, decreased stress resistance and increased locomotor activity in *Drosophila* [76,77]. In *Drosophila* larvae, PRD has been associated with higher fat reserves in adult flies due to increased expression of fat mobilizing genes and lipid storage droplets in mature flies [78]. Challenges still abound in obesity disease modelling using *Drosophila.* The use of High Sugar Diet (HSD) to model obesity has been associated hyperosmolarity and glucotoxicity (hexose amine flux and polycomb gene complex activation which damage pericardial nephrocytes) in flies [61,79]; while the use of HFD has been associated with lipotoxicity resulting in activation of inflammatory pathways that compromise cell signaling pathways [39,80]. The need to develop a healthy and more reliable diet induced fly obesity model with less confounders created a rationale for this study. Therefore, this study's objective was to compare the effects of a Protein Restricted Diet, High Fat Diet and High Sucrose Diet on larval and adult fitness, survival, mass, catalase activity, total protein, sterol and triglyceride (TG/TP) content of obese *Drosophila melanogaster*.

2. Methods

2.1. Fly cultures

The study was done using *Drosophila melanogaster* white mutant w^{1118} [81] 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. The flies were divided into four groups exposed to regular food (control), High Fat Diet (HFD), High Sucrose Diet (HSD), 0.5% Protein Restricted Diet (PRD=0.5% less yeast than in regular feed) for two weeks under 70% humidity, 24–26 °C temperature and 12:12 h light/dark cycles. Adult 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 [82]. First instar larvae were collected within 2–3 h 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. Adult male flies were flipped into new fresh food vials after 3 days for experimental investigations.

2.2. Experimental design

Adult male *Drosophila* w^{1118} were exposed to 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). High Fat Diet (HFD) was prepared by adding 10% w/v food-grade coconut oil to the regular cornmeal food [83]. High Sugar Diet (HSD) was prepared by adding 30% w/v sucrose to regular

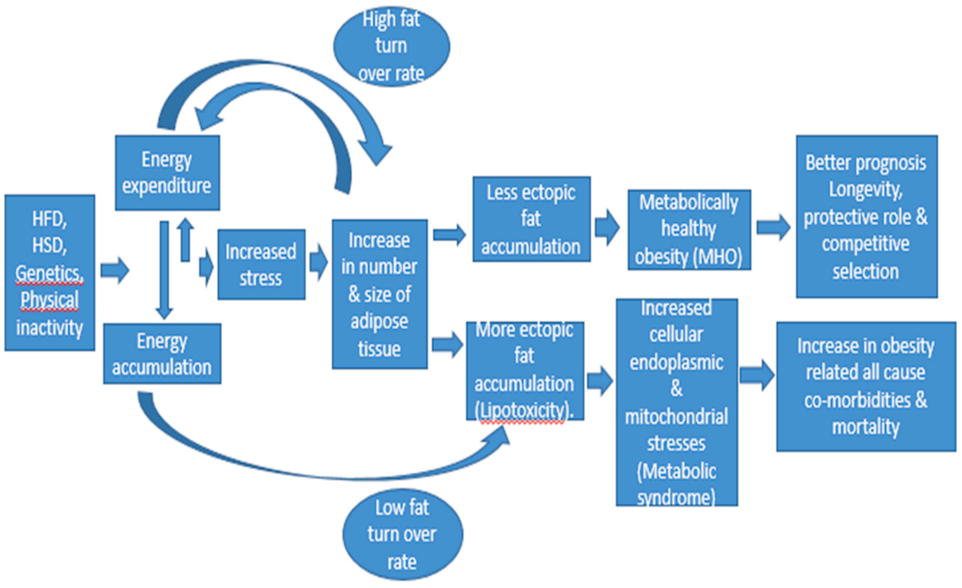

Fig. 1. A summary to elucidate the independence of adiposity from 'obesity' related co-morbidities and mortality (Obesity Paradox). A higher fat turnover rate and lesser ectopic fat accumulation results in a metabolically healthy obesity (MHO), thus conferring a protective role that improves prognosis, lifespan and competitive selection, while a lower fat turnover rate and higher ectopic fat accumulation results in an increase in obesity related all cause co-morbidities and mortality.

cornmeal food [72,83]. Protein Restricted Diet (PRD) was prepared by reducing the yeast composition by 0.5%, because reducing protein by nearly half enabled a higher feeding rate and accumulation lipid reserves. It also helps flies attain a pupation height similar to that of normal feed fed flies, probably ensuring successful eclosion and survival [84]. The larval media contained the same feed composition except for agar and nipagin. Each group contained ten third instar larvae and were exposed to the feed for 3 days. Subsequently, ten adults (40-day old males) 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

These were performed to assess larvae feeding and nervous activity on 1% agar in distilled water on Petri dishes (since this increased visibility and ease of recording movements, see [85]). 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 min, 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 [86] with minor modifications. Ten flies from the respective groups were immobilized under light anesthesia with ice. After 10 min of recovery, the flies were gently tapped to the bottom of the column, and the number of flies that reached the height of 8 cm in one minute were recorded [87]. The tests were repeated three times for each group at one-minute intervals and the mean number was taken as the number of flies which performed positively in the experiment, expressed as a percentage for each group.

2.5. Fly Body mass

Each fly was frozen, and its body weight measured using a Sartorius microbalance [88]. 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 g for 3 min [89]. 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 540 nm absorbance [90]. A fluorometric assay protocol was used to indirectly measure the sterol in the form of resorufin at 590 nm fluorescence as previously described [89].

2.7. Determination of total protein and catalase activity from the supernatant

The total protein was indirectly measured using Cypress Diagnostics kit following a Bradford assay protocol [86]. To 25 μL of standard bovine serum albumin and sample solutions, 1 ml of Bradford reagent solution was added. The solutions were mixed thoroughly and after two minutes, absorbance measured at 595 nm. Total protein (mg/dl) = Absorbance of sample/Absorbance of standard x Concentration of Standard.

Catalase activity to assess response to stressors was determined by following a protocol developed by [91] and customized by [86]. 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. 100 μL of catalase solution was pipetted in 13 mm diameter x 100 mm 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 O_2-forming foam that remained constant for 15 min 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 the control, PRD, HSD and HFD fed fly samples.

2.8. Fly survival analysis

Survival rate was determined across the four groups by recording the number of live and dead flies daily for 35 days. Survival was calculated as below.

Percentage survival = (Number of surviving flies/Total number of flies per vial x100) = (n/20) x100.

2.9. Statistical analysis

Graph pad prism version 6 software (Graph pad software, La Jolla, CA, USA) was used for statistical analysis of the biochemical assay tests. The results were reported as mean ± standard error of the mean (S.E.M) using figures and tables. Survival data was analyzed using Kaplan-Meier survival analysis and a Mantel-Cox was performed on the survival curves. A one-way ANOVA, followed by the post hoc Tukey's test, where a $p < 0.05$ was considered to represent a statistically significant difference in the metabolic and antioxidant assay results. Significance was represented using different superscripts a, b, c and d on the figures.

3. RESULTS

3.1. A protein restricted diet induced a stable increased fat storage phenotype in Drosophila melanogaster

Survival rates were highest under PRD group compared to HSD and HFD groups (Fig. 2A). In the larval stages, PRD was associated with significantly lower peristaltic movements compared to HFD (Fig. 2B). In adults, PRD was associated with decreased body mass (Fig. 2C) and significantly increased negative geotaxis (Fig. 2D). Also, HFD was associated with significantly higher peristaltic movements compared to other groups in the larvae stage, although no significant differences were observed between HSD and the control group on body mass and negative geotaxis (Table 1).

3.2. A protein restricted diet increases catalase enzyme activity and induces moderate levels of triglycerides and sterols in flies

In adult stage, triglyceride levels (Fig. 3A) and total protein levels (Fig. 3C) were significantly higher in HSD phenotype. HFD phenotype had significantly higher sterol levels (Fig. 3B) when compared to PRD and HSD phenotypes. Though PRD phenotype had a higher catalase enzyme activity, this activity was not statistically significant when compared to that of HSD and HFD phenotypes (Fig. 3D). Protein restriction increases catalase enzyme activity in flies.

4. Discussion

The study showed that a Protein Restricted Diet (PRD) induces an increased fat storage phenotype with a higher survival rate, higher negative geotaxis, higher catalase enzyme activity and a lower mass when compared to HSD and HFD phenotypes. These results are similar to those of previous studies on PRD [77,92–94]. In addition, relationships in larval nutrition and expression of adult phenotypic characteristics such as fat storage and the degree of resistance to starvation as previously described [78], were replicated in our study. PRD larvae showed the lowest peristaltic wave activity. This observation could have been due to PRD larvae compromised central nervous system protein synthesis and/or neural transmitter metabolism [20,85]. However, this may have favored a PRD induced increased production of hydrogen sulfate as a neural transmitter in the adult stage [23], and the development of dietary induced adaptive changes in line with the 'protein leverage hypothesis' whereby a reduction in diet proteins increases appetite for carbohydrates [95,96], thus a more active PRD increased fat storage phenotype. Coincidentally, there was a tradeoff between mass and locomotor activity in the adult stage since the PRD phenotype had the lowest mass but exhibited the quickest recovery time from light cold anesthesia and a higher locomotor activity. This demonstrated changes

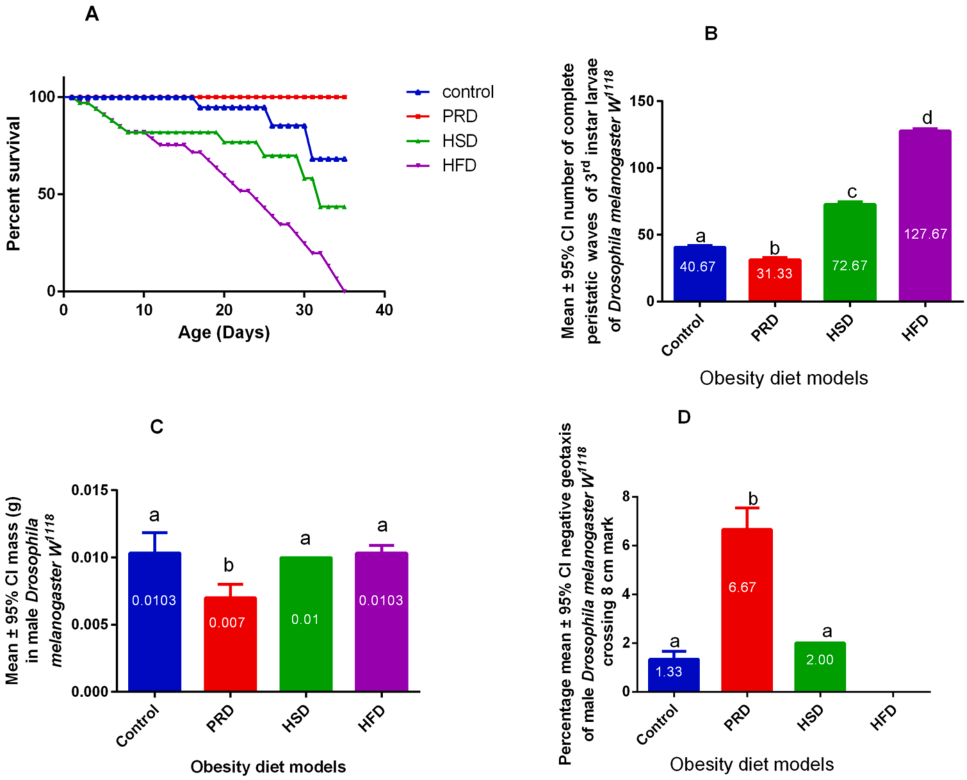

Fig. 2. PRD was associated with a higher survival rate (A), comparatively normal peristaltic larvae movements (B), low body mass (C), and a higher negative geotaxis in adult flies (D). This offers insights into a more agile, stable and increased fat storage phenotype associated with a PRD. Blank in graph (D) indicates slower fly recovery from light cold anesthesia to reach the 8 cm mark of the vertical column in one minute. Similarity of superscripts (a, b, c or d) represent no statistically significant differences ($p > 0.05$) between experimental groups.

Table 1
Tukey's multiple comparison test on peristaltic waves in 3rd instar larvae, body mass, negative geotaxis, triglycerides (TG/TP), sterols, and total protein, catalase activity and survival in adult male *Drosophila melanogaster*.

Tukey's multiple comparison test groups	N	Complete peristaltic waves Adjusted *p* Value	Mass	Negative geotaxis	TG/TP	Sterols	Total protein	Catalase	Survival analysis
Control vs. PRD	60	0.0236	0.0117	0.0002	0.2760	0.0788	0.2246	0.0003	Log-rank (Mantel-Cox) test for curve comparisons; X^2 (3) = 37.48, P < 0.0001
Control vs. HSD	60	< 0.0001	0.9723	0.7538	0.0120	< 0.0001	< 0.0001	0.0017	
Control vs. HFD	60	< 0.0001	> 0.9999	0.2641	0.7894	0.0008	0.0068	0.0010	
PRD vs. HSD	60	< 0.0001	0.0207	0.0005	0.1816	< 0.0001	0.0001	0.4308	
PRD vs. HFD	60	< 0.0001	0.0117	< 0.0001	0.7356	0.0258	0.1221	0.6816	
HSD vs. HFD	60	< 0.0001	0.9723	0.0665	0.0427	< 0.0001	0.0016	0.9645	

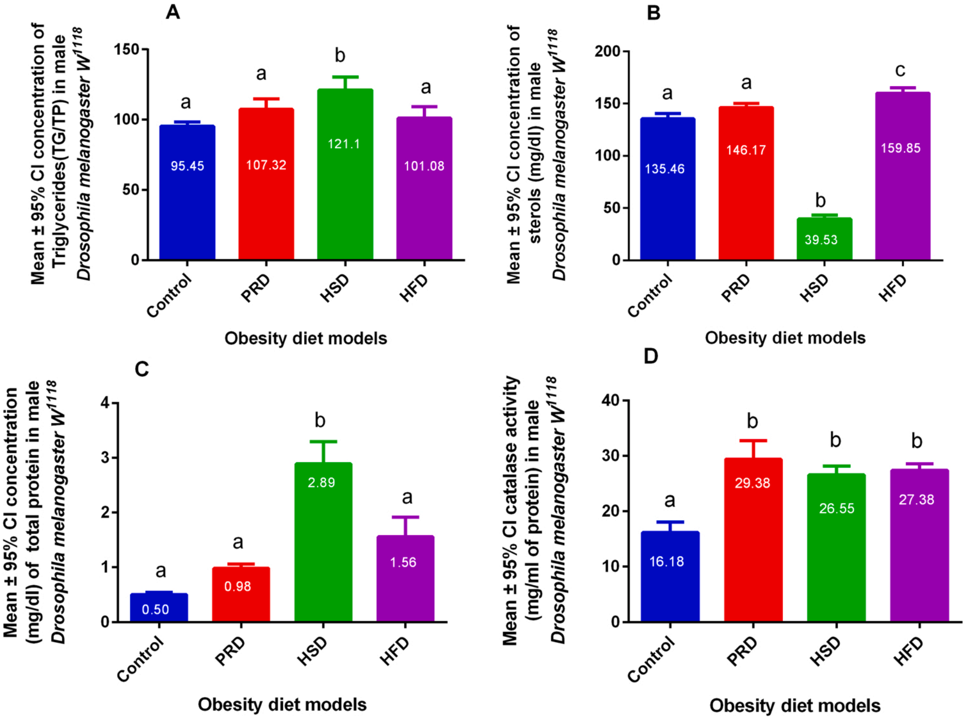

Fig. 3. PRD was associated with moderate triglyceride levels (TG/TP) (A), moderate sterol levels (B), moderate total protein levels (C), and a higher catalase enzyme activity (D). Similarity of superscripts (a, b, c or d) represent no statistically significant differences ($p > 0.05$) between experimental groups.

under PRD that favor quick environmental adaptation, a reduction in frailty and a higher locomotor activity during adulthood similar to results of previous studies on PRD [92,97,98]. Furthermore, a higher survival rate of PRD phenotype could have been due to a PRD induced epigenetic re-programming of lipid metabolism pathways which induced a higher fat turnover rate that improved the general efficiency in lipid metabolism and physiological function [78,92,97,99,100]. In addition, a higher survival rate of the PRD group indicated the improved health span and lifespan effects of a higher mitochondrial catalase enzyme activity against oxidative stress [26,101]. Interestingly, the HFD showed the highest locomotor activity of the third instar larvae due to the voracious uptake of the available high energy sources inform of fats in preparation for the pupa stage. However, this resulted in significantly higher sterol levels in the adult stage for the HFD group, thus lesser resistance to light cold anesthesia and locomotor activity in the adult stage when compared to PRD group. These results indicate the pathological outcomes associated with ectopic lipid accumulation (lipotoxicity) in *Drosophila* physiology due to a HFD. These observations were similar to previous studies where HFD showed a ≥ 50% reduction in adult *Drosophila* locomotor activity and survival [72,102,103]. Lower 3rd instar larvae locomotor activity of PRD group was a preparatory stage for exhibition of better nervous coordination of PRD adult flies as shown by a shorter cold stress recovery time and subsequent higher locomotor activity when compared to HFD and HSD flies. The findings of this particular study on PRD are in agreement with previous studies, which reported the independence of locomotor activity and antioxidant activity under increased fat storage conditions on the level and degree of adiposity [92,101,104]. Furthermore, this study demonstrates the versatility and reliability of *Drosophila* model for increased fat storage studies in healthy metabolic state and under disease conditions that could be relevant to humans [71,72].

The study also showed moderation in fly triglyceride and sterol accumulation under a PRD. Since *Drosophila* is a sterol auxotroph and yeast is the main source of sterols in fly diet, these findings demonstrate the improved metabolic state in the PRD phenotype in adapting to conditions of essential nutrient deprivation by enhancing fly locomotor activity and survival. Triglycerides provide energy, and these are markers of obesity in *Drosophila* [46]. Increased energy storage versus expenditure is associated with improved antioxidant balance, which is important to control reactive oxygen species arising from the oxidation of lipids and oxidative stress [105,106]. The protective role of lipid droplets in health and disease pathology states has been identified in previous studies [107,108]. More so, previous studies have also shown the signaling role of reactive oxygen species and oxidized lipids [107,109]. Thus, a higher locomotor activity and survival in PRD group could have been an indication of a beneficial hormetic response brought about by moderate reactive oxygen species and oxidized lipids that improved the general fly signaling mechanisms and wellbeing. The major storage forms of sterols in *Drosophila* are dehydrocholesterol and ergosterol [89]. With this moderate triglyceride and sterols level accumulation in the PRD phenotype when compared to HFD and HSD phenotypes, the mechanisms for these differences remain to be explored.

Total protein levels were significantly higher in HSD flies, providing evidence that HSD induced increased fat storage in *Drosophila* could face major increased protein levels compared to HFD and PRD. This is important since HSD like HFD has been associated with increased lipid peroxidation, which causes increased tissue death and reduced lifespan in *Drosophila* [15,83]. Furthermore, HSD is characterized by hypertonicity in both the larvae and adult stages. This condition affects larvae food intake and development, hastens fly tissue autolysis and glucotoxicity in adult flies [64]. Thus, this may result into lean fly phenotypes and a decrease in lifespan [15,106]. On a positive note, challenges and outcomes observed in both HFD and HSD increased fat storage fly models help identify them as better cancer, lipotoxicity and glucotoxicity fly research models [110]. Although catalase activity was higher under the PRD phenotype, this activity was not statistically significant when compared to HSD and HFD phenotypes. However, this increased catalase enzyme activity indicates increased stress probably due to a limitation in protein intake under a PRD when compared to HSD and HFD. These results were similar to those of previous studies that showed increased antioxidant activity under a PRD [15,77].

Increased endogenous catalase enzyme activity serves as a natural defense mechanism against oxidative stress [111]. A previous study showed that an increase of mitochondrial catalase enzyme activity significantly increases the lifespan of model organisms [26]. Our findings are similar to those of previous studies that reported a PRD association with elevated antioxidant enzyme activity in *Drosophila melanogaster* [112], rodents [113], and humans [114]. The increased catalase enzyme activity in PRD group could have been due to a lower ratio of TG/catalase activity when compared to HSD and HFD groups [36]. This may have caused a re-programming of multiple anti-oxidant/nutrient sensing pathways and metabolic processes which improved the health span and lifespan of the PRD group [99,115,116]. Furthermore, sugar diets at either low or high supplementation alter physiological function (reproduction) due to body size changes and organ size [117]. A comparative study on high fructose and high glucose diets showed no significant differences in weights and gene expression, although both led to comparable levels of obesity in *Drosophila melanogaster* larvae, and physiological effects were associated with sugar concentration [118].

5. Conclusion

In this study, PRD produced a more active and a long lived increased fat storage phenotype. The lifespan extension under a PRD could have been due to the metabolic and physiological benefit of a reduction in protein derived metabolic waste, less ectopic lipid accumulation and an increased investment in somatic maintenance. More so, these results of an increased fat storage PRD phenotype in *Drosophila* could have been due to less production of fly lipid autotoxins and less disruptive effect on the fly gut integrity and microbiome. Thus, this study's findings demonstrate a need to explore more diet induced appropriate models of obesity, emphasizing nutritional adaptive changes to promote knowledge on healthy metabolic obesity. More so, prospective studies to guide therapy and healthy community nutrition could place more emphasis on the results of a PRD increased fat storage phenotype in *Drosophila melanogaster*.

5.1. Study limitations

Comparative genomics, transcriptomics, proteomics and metabolomics studies should be carried out to assess the fat turnover rate in diet induced obesity models of *Drosophila melanogaster*. More so, comparative oxidative stress resistance, lipid autotoxin production in fly development, histological and gut microbiota composition studies should be carried out in diet induced obesity models of *Drosophila melanogaster*. Feeding behavioral studies using the mouth hook contraction assay could help provide further evidence on the effects of the PRD under increased fat storage conditions. Comparative differences of calorie intake under the PRD, HSD and HFD should be carried out to ascertain their effects on fat storage. Furthermore, comparative studies on immunological modulators of inflammation, second messengers, molecular modulators of gene expression and studies using different control settings could help offer more insights to identify novel metabolic pathways at play.

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.

Funding statement

Not applicable.

Author contributions

OHA and KIK conceptualized and designed the study, OHA and KIK conducted data acquisition and analysis while SSO, JR, LOO 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.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Consent to publish

Not applicable.

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