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Transgenerational inheritance of insulin resistance in offspring of white rice-fed female fruit flies

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ABSTRACT

Diabetes mellitus is a global pandemic that is also fast rising in Africa, including Nigeria. Chronic consumption of white rice (WR) increases the risk of developing insulin resistance (IR) and type 2 diabetes (T2D). Similarly, *Drosophila melanogaster* is a suitable model organism for studying metabolic disorders. We hypothesized that maternal WR consumption could alter offspring's metabolic health and predispose to IR. *Drosophila melanogaster* (fruit fly) eggs were laid and developed on either Control, 50 % WR and 50 % brown rice (BR); and female adults maintained on these dietary regimens for 7 days. F1 and F2 offspring of these flies were fed either a normal diet or high sugar diet (HSD). The effects of maternal WR consumption on indices related to IR and T2D such as weight, locomotor activity, glucose, trehalose, glycogen and triglyceride (TG) were assessed in the parent and offspring generations. Similarly, the mRNA expression levels of *ILP2, IRS, PEPCK* and *ACC* were investigated. We found that WR consumption induced IR in the parent as evidenced by weight gain, significant increases in glucose, trehalose and TG levels, and reduced glycogen levels. Similarly, maternal WR consumption appeared to program for elevated glucose, trehalose and TG levels in F1 offspring with significant increases in trehalose and TG levels in the F2 offspring. These metabolic perturbations were accompanied by overexpression of *ILP2, IRS, PEPCK* and *ACC*. Offspring reared on HSD exhibited worsened conditions. In contrast, the levels of these metabolic signatures in the BR groups were comparable with the control. Taken together, our study demonstrates the potential of WR but not BR to program for transgenerational inheritance of IR-T2D-like phenotypes. This suggests that offspring of chronic WR consumers may be at risk of developing IR, affecting the development and health of future generations.

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Introduction

Energy-dense diets affect the metabolic health of individuals and are often associated with the development of obesity, insulin resistance (IR), and type 2 diabetes (T2D) [34]. The effects of these diets are manifested in the form of altered expression of metabolic genes. Increasing evidence suggests that phenotypes acquired during the life of the parent impact offspring's health across generations [20,65]. Particularly, it has been shown that maternally acquired metabolic perturbations can alter the metabolic health of subsequent generations [15,24,31]. IR is a critical pathological factor in several metabolic disorders, including T2D and often characterized by reduced insulin sensitivity, impaired glucose utilization, hyperinsulinemia and abnormal lipid accumulation [36,79]. The prevalence of IR tends to parallel that of obesity and T2D, affecting over half a billion of the global population [11,63] and 3.6 million Nigerians [29]. One of the most implicated risk factors for the current T2D epidemic is the modern diabetogenic lifestyle such as excess energy intake, usually in the form of carbohydrates [26].

Rice continues to be a widely consumed staple food for over half of the world's population reflecting substantial consumption across households of diverse socioeconomic status [46,76]. The current per capita rice consumption in Nigeria is 32kg which is marginally less than the global average of 53.9 kg [3]. In 2021, Nigeria's rice production rose to 9 million metric tonnes up from 2 million metric tonnes in 2015 [3]. After harvest, rice grain undergoes several processing stages including drying, milling, and packing for easy consumption. The milling process begins by removing the husk from the rice grain or paddy, resulting in the brown rice (BR) grain with a brown outer bran layer [57]. Secondly, the outer bran layer is removed to obtain polished or white rice (WR). WR primarily consists of carbohydrates and exhibits a notably high glycaemic index (GI) and glycaemic load (GL) leading to elevated postprandial glucose [42,73]. Furthermore, rice milling removes the bran layer, leading to the depletion of many beneficial bioactive compounds including γ-aminobutyric acid (GABA), γ-oryzanol, flavonoids, phenolic acids, α-tocopherol, and γ-tocotrienol [60]. Thus, chronic consumption of WR has been associated with increased risk of IR and T2D due to the absence of these essential compounds [2,6,14,26].

It is established that the parental germline (sperm and egg) can be directly affected by environmental factors and altered nutrition, leading to reprogramming of cell epigenomes and subsequently transmitting susceptibility for disease to succeeding generations through epigenetic mechanisms of transgenerational inheritance [35,51]. Despite variations in study design, the typical outcomes of excessive maternal carbohydrate intake include weight gain and increased hepatic lipid accumulation in programmed offspring [59]. Defective insulin signaling is among the earliest signs that an individual is prone to developing IR and T2D [32]. The offspring's resistance to insulin and leptin hormones predisposes the individual to obesity and diabetes [7]. Epigenetic mechanisms such as DNA methylation, genomic imprinting, and histone modifications are likely involved in offspring's metabolic programming following maternal hyperglycaemia [7,19]. Intergenerational inheritance refers to the transmission of traits from one generation to the next. Specifically, when the parent generation (F0, male or non-pregnant female) is exposed to an environmental or external stimuli (such as a high carbohydrate diet, high fat diet, or stress) that causes epigenetic changes in the parent (F0) and F1 germlines [69]. For epigenetic effects to be transgenerational, alterations in the F0 generation must be seen in the F1 and F2 generations and perhaps beyond [65]. Evidence exists for intergenerational inheritance of diet-induced IR in *Drosophila* [9], mice [71] and rats [23]. Offspring of WR-fed rat dams were reported to develop IR later in life, suggesting intergenerational inheritance [28]. Our previous study revealed that locally grown Nigerian WR cultivar could induce IR in the fruit fly (*Drosophila melanogaster*) [56]. Thus, whether WR-induced IR could be transgenerationally inherited remains unknown. The *Drosophila* model has distinct advantages such as advanced genetics, easy diet manipulation, and a short life cycle, which is useful in understanding the molecular mechanisms of parental programming [8]. The fruit fly is therefore considered a valuable model for transgenerational study of IR and other metabolic diseases [9,75]. In this study, we examined the impact of prolonged maternal WR consumption on body weight, locomotor activity, glucose, trehalose, glycogen, and triglyceride levels of F1 and F2 offspring generations. Additionally, the transgenerational effects of chronic maternal WR consumption on the expression of insulin signaling genes such as insulin-like peptide 2 (*ILP2)* and insulin receptor substrate (*IRS*) were evaluated. The mRNA expression levels of phosphoenolpyruvate carboxykinase (*PEPCK*) involved in glucose metabolism and acetyl coA carboxylase (*ACC)* that drives lipid synthesis were also investigated in the parent and offspring.

Materials and methods

Reagents and chemicals

Distilled water was prepared with a distiller machine (Laboid International Solan, Pradesh, India). Phosphate buffered saline (PBS) tablet was obtained from Dulbecco A (PBS, Oxoid), BR0014G, Selangor, Malaysia. Ethanol was purchased from Merck, Sigma Aldrich, Germany. Baker's Yeast was bought from STK Royal Ltd, Lagos Nigeria. Agar-Agar powder (Bacteriological grade-B1CA1HV01) was purchased from Titan Biotech Ltd, Rajasthan, India. Nipagin (Methyl Paraben) was purchased from Molychem, Mumbai, India. Corn flour was obtained from Faso Farine Mais Blanc (FFMB), Ouagadougou, Burkina Faso. Sucrose, which was the sugar used in this study, was purchased from LOBA Chemie Pvt Ltd., Mumbai, India.hom Glycogen assay kits were obtained from Solarbio Life Science, Beijing, China. Nucleic acid extraction kit was purchased from Liferiver ®, Shanghai ZJ Bio-Tech Co., Ltd. SYBR-Green One-Step qRT-PCR Master Mix kit was purchased from TransGen Biotech Co., Ltd., Haidan District, Beijing, China. All the solvents used were of analytical grade.

Sample preparation

Baburashi, one of the popularly consumed rice cultivars in Sokoto, North West Nigeria and reported to induce IR [56] was used in this study. The rice cultivar was obtained from farmers from Sokoto, Nigeria upon harvest and thereafter transferred to the laboratory for storage at room temperature before the commencement of the research. The rough rice (paddy) was then subjected to a de-husking process using a Paddy Rice Husking Machine (Reeyor Machines, Nigeria) to generate BR and a subset of these cultivars were also polished to produce WR. Following that, the BR and WR were ground into a fine powder with a blender, then sifted using a laboratory sieve and placed in sealed polythene bags at ambient temperature for investigations performed within the study period. The rice cultivars were subsequently stored at 4°C for longer duration.

Fly stock and husbandry

Harwich strain of *Drosophila melanogaster* was acquired from the fly laboratory of the center for Advanced Medical Research and Training (CAMRET), Usmanu Danfodiyo University, Sokoto. The flies were cultured in vials and maintained at fly optimum temperature (22–25 °C) and controlled humidity (55 ± 5 %). They were provided with a standard cornmeal diet and subjected to a natural light-dark cycle. The fly media was regularly replaced every three days to avoid contamination.

Experimental design

Virgin female flies were mated with males on a standard cornmeal diet. Then, gravid (3-4 day old) flies were placed on either control, 50 % WR or 50 % brown rice (BR) diets [56] for 24 h and the laid eggs were allowed to develop on the respective diets until adulthood. The adult female flies were then categorized into three groups ($n = 30$/group) each in triplicate: Control, WR and BR; and further maintained on their respective diets for 7 days. The nutritional composition of the experimental diets is given in Table 1. The virgin flies were then mated with their male counterparts on a control diet to produce the first filial (F1) and second filial (F2) generations. Subsequently, the male and female offspring were raised on either a control or a high sugar diet (HSD) for 7 days of adulthood. After the intervention, changes in body weight, locomotor performance, glucose, trehalose, glycogen and triglyceride levels were evaluated ($n = 30$) in the parent (F0) and subsequent generations (F1 and F2). The effects of chronic maternal WR consumption on the expression of metabolic target genes including *ILP2, IRS, PEPCK* and *ACC* across multiple offspring generations were also examined. The schema of the experimental design is provided in Fig. 1.

Measurement of body weight

The body weight of 7-day-old adult flies was measured across the experimental groups using a sensitive electronic weighing scale (Kern & Sohn Ltd., Balingen, Germany). Ten flies per group were anaesthetized on ice for 3–5 min. They were then placed in a pre-weighed empty 1.5 ml microcentrifuge tube (Wuhan Service BioTechnology Co., Ltd., Wuhan, China) and re-weighed in milligrams (mg). The same procedure was repeated for three biological replicates and the mean weight per group was taken.

Climbing assay

This is also known as locomotor performance assay and was performed according to the method described by Gargano and colleagues [21] with some modifications [72]. Briefly, thirty flies were placed in an empty 50 mL vial with a line drawn 6 cm from the bottom and allowed to acclimatize. The flies were carefully tapped to the base of the vial, and the number of flies capable of crossing the 6 cm mark in 10 s was recorded. This procedure was replicated thrice and the average percentage of flies that crossed the line was computed for the respective groups.

Biochemical assays

Haemolymph extraction

Seven-day-old flies were fasted for about one and a half hours. Upon cold anesthesia, a pooled population of thirty flies per group

Table 1
Nutritional composition of experimental groups.

	Control	White Rice	Brown Rice	High Sugar
Corn flour	100 g	50 g	50 g	100 g
Agar-agar	10 g	10 g	10 g	10 g
Baker's yeast	20 g	20 g	20 g	20 g
Distilled Water	1.5 L	1.5 L	1.5 L	1.5 L
Methylparaben	1 g	1 g	1 g	1 g
Others	–	50 % WR	50 % BR	30 % Sucrose

BR- Brown rice; WR-White rice.

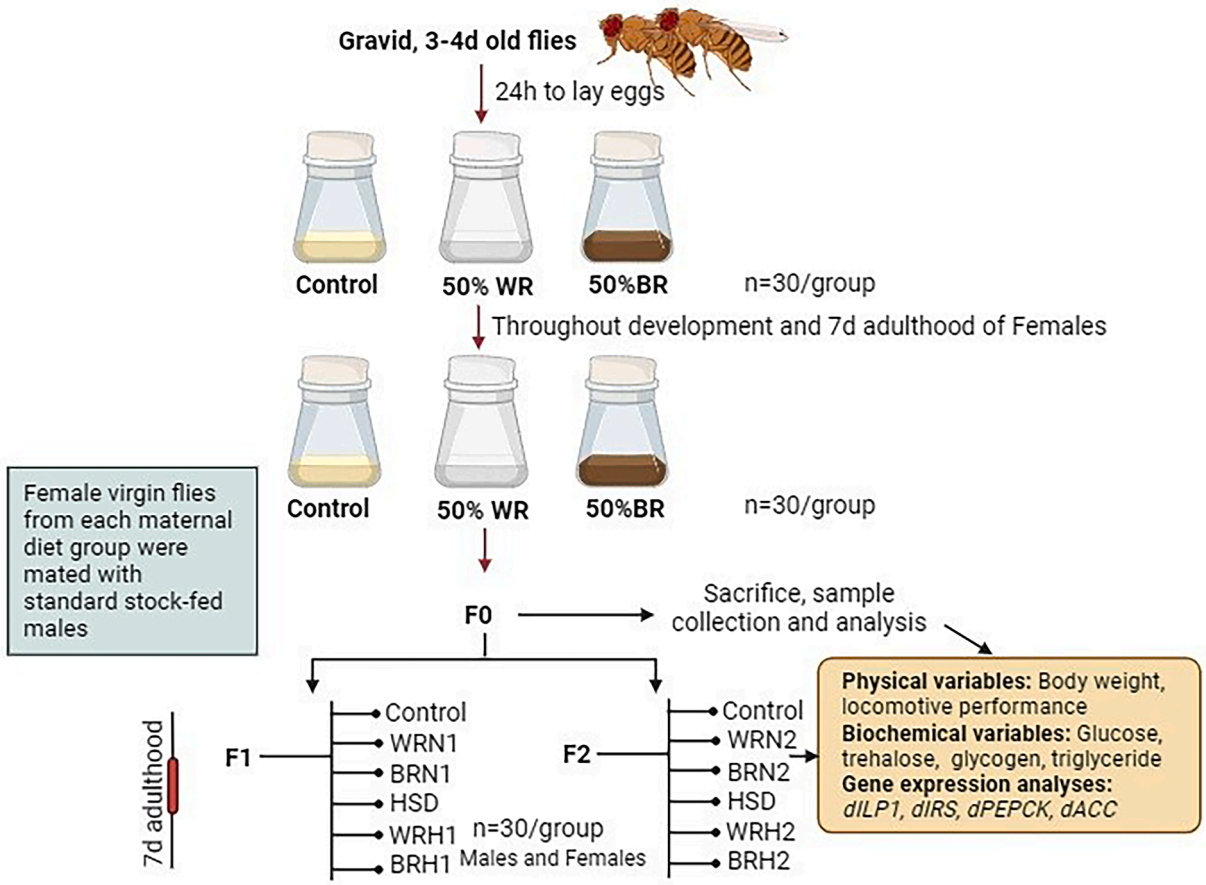


Fig. 1. Experimental design illustrating the study protocol. WR- White rice; BR- Brown rice; WRN1- F1 offspring of maternal WR group raised on normal control diet; BRN1- F1 offspring of maternal BR group raised on normal control diet; HSD- High sugar diet group; WRH1- F1 offspring of maternal WR group raised on HSD; BRH1- F1 offspring of maternal BR group raised on HSD; WRN2, BRN2, WRH2, BRH2- F2 offspring of respective diets as above; 24h-twenty four hours; 3–4d- three to four days, 7d-seven days.

were transferred into 1.5 mL Eppendorf tubes filled with 200 μL phosphate-buffered saline (PBS). The flies were homogenized using a hand-held homogenizer (Hangzhou Miou Instrument Co., Ltd., Hangzhou, China) and centrifuged at 8000 x g for 10 min at 4 °C (MX-301 High-Speed Refrigerated Microcentrifuge, TOMY Kogyo Co. Ltd, Japan). The supernatant was gently collected and then dispensed into a sterile 1.5 mL tube and kept at -20 °C for downstream biochemical analyses.

Quantification of glucose

Fasting glucose levels were measured by adding 98 μL of Glucose-TR reagent (Spinreact, Girona, Spain) into a 96-well microplate (Nest Biotechnology, Wuxi, China) that contained 2 μL of haemolymph samples. The reaction mixtures were incubated at ambient temperature for 20 min and the absorbance was measured at 505 nm in accordance with the manufacturer's guidelines.

Quantification of trehalose

To quantify trehalose, 100 μL of trehalose reagent (Solarbio Life Science, Beijing, China) was mixed with 25 μL of haemolymph and incubated at 95 °C for 10 min. The resulting solution was allowed to cool and the absorbance was measured at 620 nm according to the manufacturer's protocol.

Glycogen assay

For glycogen, 100 μL glycogen reagent (Solarbio Life Science, Beijing, China) was incubated with 25 μL of haemolymph at 100 °C for 10 min. The absorbance was subsequently measured at 620 nm according to the manufacturer's instructions.

Measurement of triglyceride

The levels of triglyceride were determined by adding 98 μL Triglyceride-LQ reagent (Spinreact, Girona, Spain) to 2 μL haemolymph. The mixture was then incubated at 37 °C for 5 min and the absorbance measurement was taken at 505 nm following the manufacturer's

manual.

Gene expression studies

Total rna extraction

Total RNA was isolated from whole flies ($n = 30$) using Liferiver® isolation kit (Shanghai ZJ Bio-Tech Co., Ltd., Shanghai, China) following the manufacturer's guidelines. The RNA was quantified using a Nanodrop spectrophotometer (Bioevopeak Nuclei acid analyzer, SP-MUV200F, Shandong, China). For gene expression analysis, RNA samples with A260/280 readings of 1.8 to 2.0 and A260/230 values of 2.0-2.2 were used.

Primer design and reverse transcription and quantitative polymerase chain reaction (RT-qPCR)

The gene-specific primers were designed using Primer Quest (Table 2). For mRNA expression, a one-step reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed according to TransScript® Green one-step qRT-PCR Supermix kit protocol (AQ211-01, TransGen Biotech, China) on a Rotor-Gene Q 5plex HRM qPCR machine (QIAGEN, Germany). All the reactions were carried out in triplicates. Following melting curve analysis, target mRNA expression was normalized to the RPL-32 mRNA reference gene according to the $2^{-\Delta\Delta ct}$ formula.

Statistical analysis

Shapiro-Wilk normality test was performed. All data were parametric. Data were expressed as means ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare variables in the parent (F0) generation while two-way ANOVA was used for comparisons among groups in F1 and F2 offspring followed by a Bonferroni *post hoc* test to compare the means. A p-value of < 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, California).

Results

Effects of maternal preconceptional WR consumption on physical variables

Effect of chronic WR consumption on offspring's body weight

At the end of the intervention, there was a significant increase ($p < 0.02$) in the terminal body weight of maternal flies on WR compared to the control (Fig. 2A). However, flies raised on BR had weight comparable to the control group. In contrast, the weights of the F1 and F2 offspring of maternal groups on different diets were not significantly different from each other (Fig. 2B, C).

Effect of chronic WR consumption on locomotor performance of the flies

A significant reduction in the locomotor performance of the WR fed parent flies ($p < 0.03$) was observed, however, those fed BR had comparatively similar climbing capacity with their control counterpart (Fig. 3A). The locomotor performance of the F1 offspring of maternal WR fed flies was not significantly different from the control groups ($p > 0.05$) but male offspring raised on HSD had reduced climbing activity compared to control ($p < 0.05$) (Fig. 3B). In the F2 generation, female offspring of WR fed maternal flies raised on a normal diet had reduced locomotor performance in comparison with the control groups on a normal diet (Fig. 2C).

Table 2
Primer Sequences for mRNA expression.

Gene	Primers	Annealing temperature
ILP2	F- ATCCCGTGATTCCACACAAG R- AGTTATCCTCCTCCTCGAACTC	60 °C
IRS2	F- CGGGCGCTTAATACATCACTA R- CTGCCGGTCAAATCTCCTATC	54 °C
PEPCK	F- GAAGGATAAGGTGGACGTGAAG R- ACCTGTGACTCGAAGTGGT	54 °C
ACC	F- ATCCCGTGATTTCCACACAAG R- AGTTATCCTCCTCCTCGAACTC	60 °C
RPL-32	F- GTCGTCGCTTTGTCATCT R- GCAGGTTGTGCCCTTCTT	60 °C

PCR was carried out as follows: 45 °C for 5 min (Reverse transcription); 94 °C for 30 s (pre-denaturation); 40 cycles of 94 °C for 5 s (denaturation), annealing at the corresponding temperature above for 15 s, and 72 °C for 10 s (extension). ACC- Acetyl CoA carboxylase; ILP2- Insulin like peptide-2; IRS2- Insulin receptor substrate-2; PEPCK- Phosphoenol-pyruvate carboxykinase; RPL-32- 60S ribosomal protein large subnunit-32.

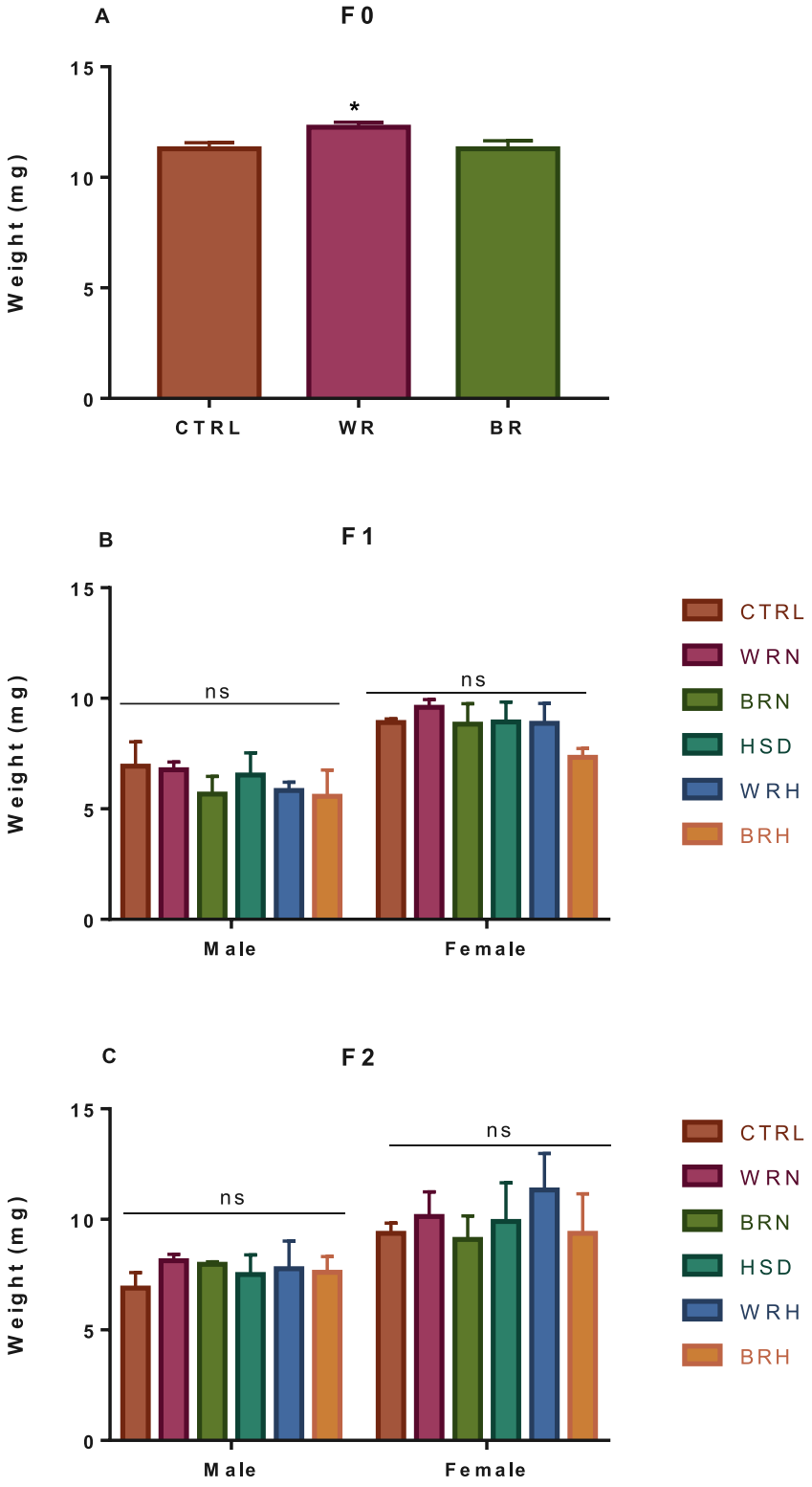


Fig. 2. Effect of chronic WR consumption on (A) maternal weight (B) F1 wt (C) F2 wt. CTRL- Control; WR- White rice; BR- Brown rice; WRN- WR offspring fed on a normal diet; BRN- Offspring of BR raised on a normal diet; HSD-High sugar diet; WRH- WR offspring fed HSD; BRH- BR offspring fed HSD. Data are expressed as Mean ± SD ($n = 30$). ns- not significant compared to control ($p > 0.05$). One-way ANOVA was used to analyze data in F0; two-way ANOVA was used to compare groups in F1 and F2.

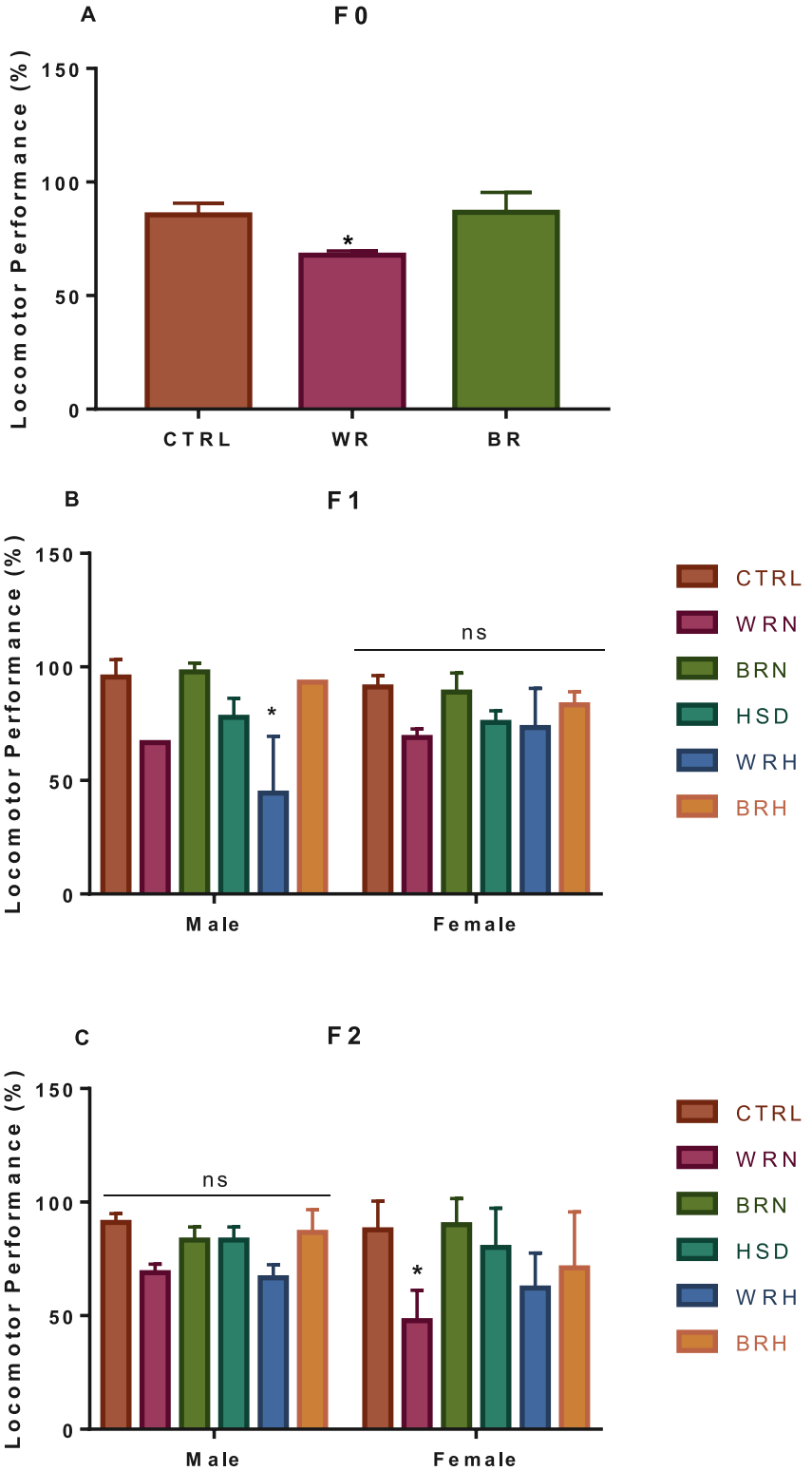


Fig. 3. Effect of maternal WR consumption on locomotor performance in (A) F0 parent, (B) F1 and (C) F2 offspring. CTRL- Control; WR- White rice; BR- Brown rice; WRN- WR offspring fed on normal diet; BRN- Offspring of BR raised on normal diet; HSD-High sugar diet; WRH- WR offspring fed HSD; BRH- BR offspring fed HSD. Data are expressed as Mean ± SD ($n = 30$). * represents $p < 0.05$ compared to control; ns- not significant compared to control ($p > 0.05$). One-way ANOVA was used to analyze data in F0; two-way ANOVA was used to compare groups in F1 and F2.

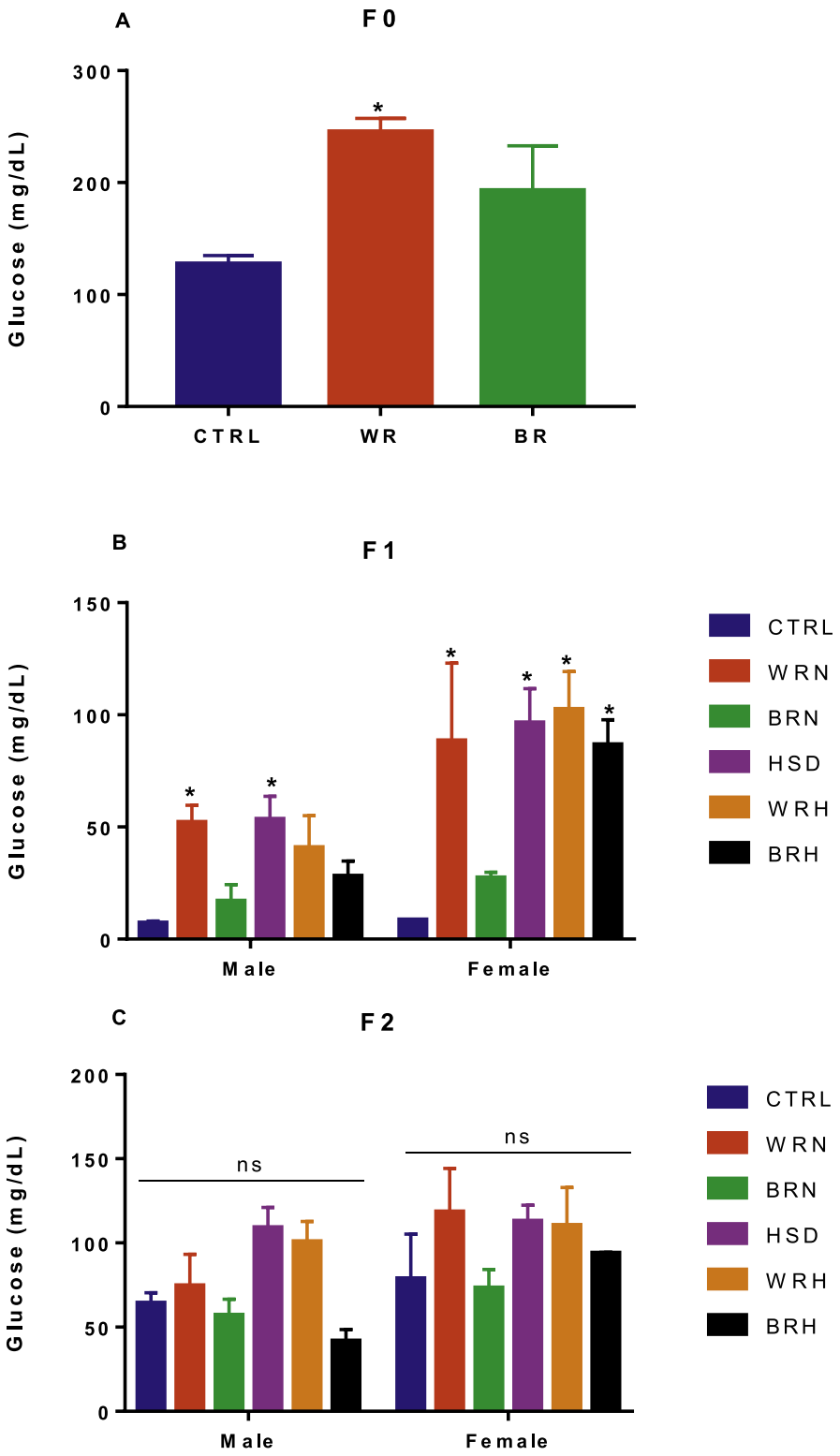


Fig. 4. Effect of chronic WR consumption on glucose levels in (A) F0 parent, (B) F1 and (C) F2 offspring. CTRL- Control; WR- White rice; BR- Brown rice; WRN- WR offspring fed on a normal diet; BRN- Offspring of BR raised on a normal diet; HSD-High sugar diet; WRH- WR offspring fed HSD; BRH- BR offspring fed HSD. Data are expressed as Mean ± SD ($n = 30$). * represents $p < 0.05$ compared to control; ns- not significant compared to control ($p > 0.05$). One-way ANOVA was used to analyze data in F0; two-way ANOVA was used to compare groups in F1 and F2.

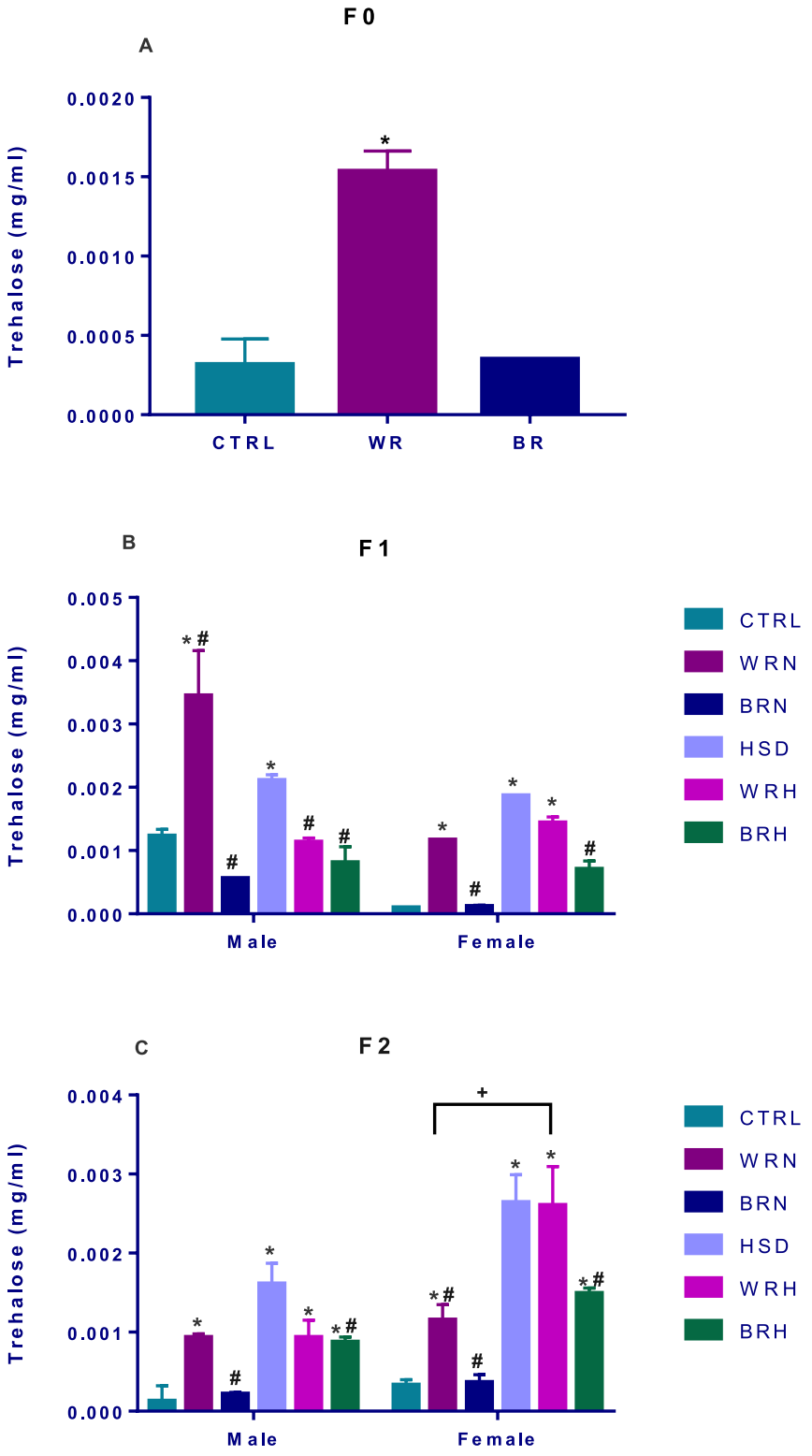


Fig. 5. Haemolymph trehalose levels in (A) F0 parent (B) F1, and (C) F2 offspring. CTRL- Control; WR- White rice; BR- Brown rice; WRN- WR offspring fed on a normal diet; BRN- Offspring of BR raised on a normal diet; HSD-High sugar diet; WRH- WR offspring fed HSD; BRH- BR offspring fed HSD. Data are expressed as Mean ± SD ($n = 30$). * represents $p < 0.05$ compared to control; # indicates $p < 0.05$ compared to HSD control; + denotes $p < 0.05$ compared to WR offspring on normal diet; ns- not significant compared to control ($p > 0.05$). One-way ANOVA was used to analyze data in F0; two-way ANOVA was used to compare groups in F1 and F2.

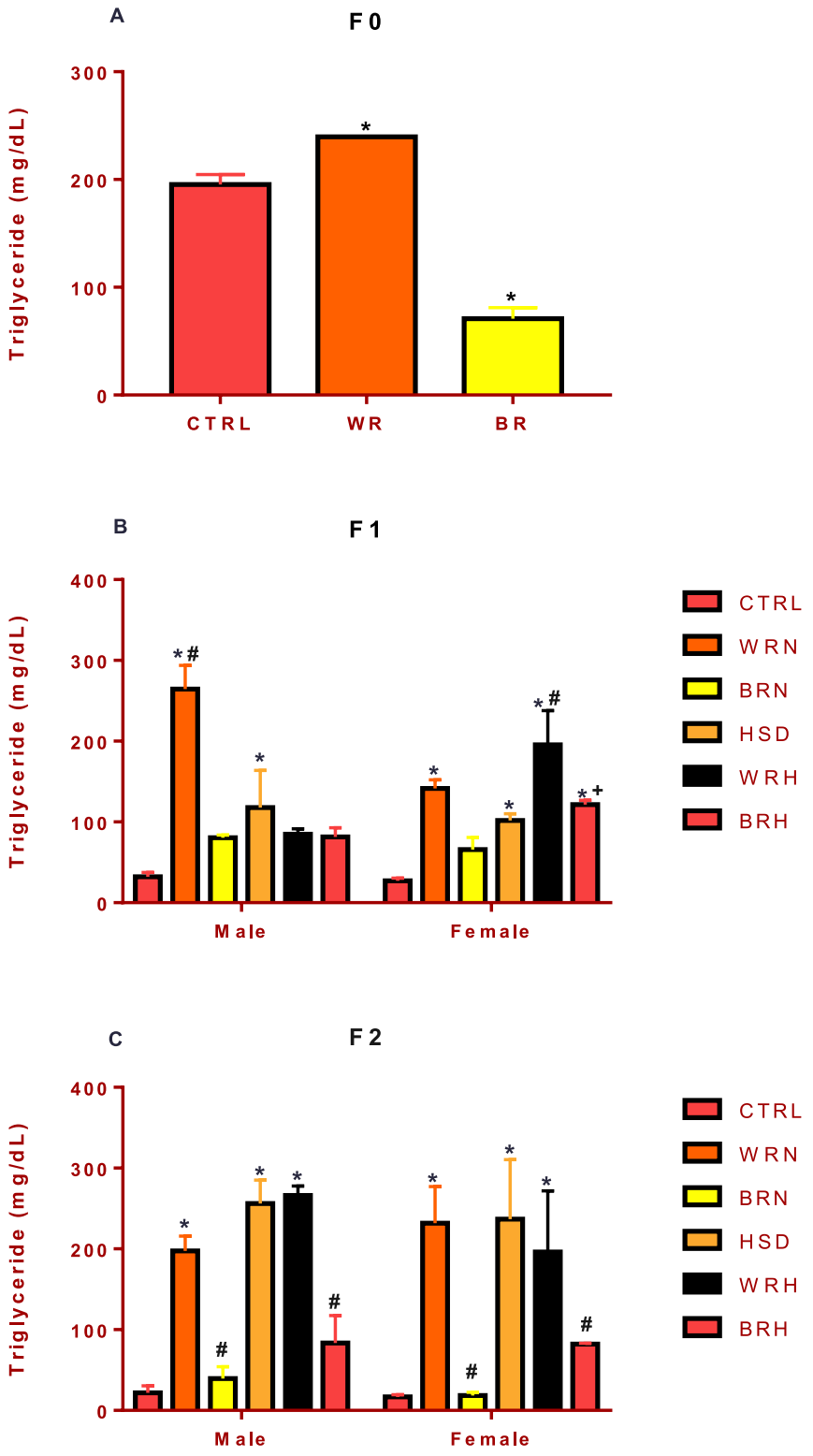
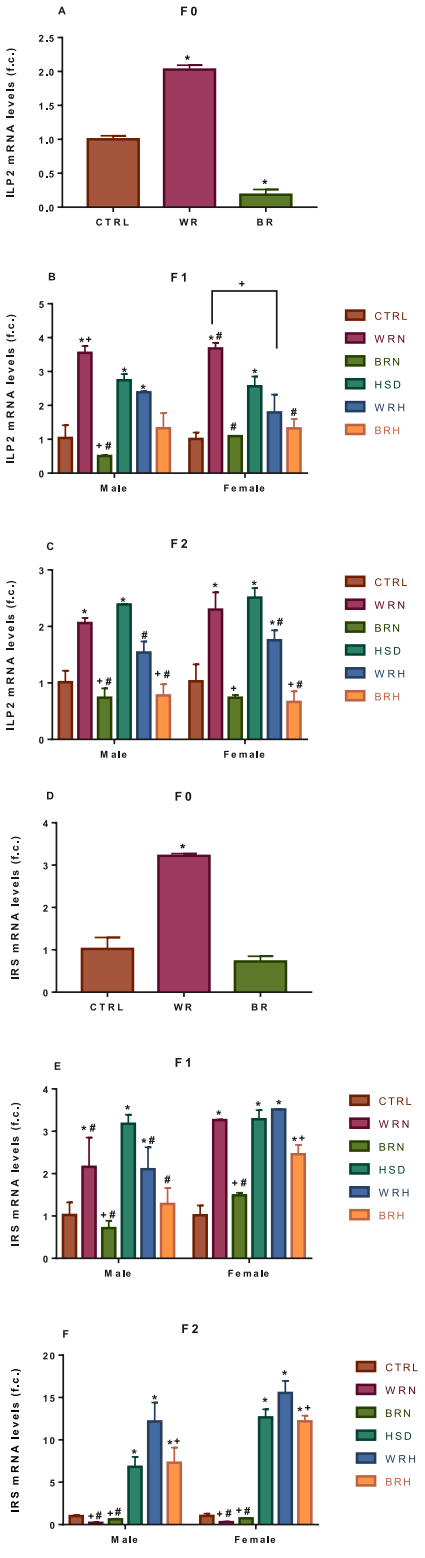


Fig. 6. Effect of chronic WR consumption on triglyceride levels in (A) F0 parent, (B) F1 and (C) F2 offspring. CTRL- Control; WR- White rice; BR- Brown rice; WRN- WR offspring fed on a normal diet; BRN- Offspring of BR raised on a normal diet; HSD-High sugar diet; WRH- WR offspring fed HSD; BRH- BR offspring fed HSD. Data are expressed as Mean ± SD ($n = 30$). * represents $p < 0.05$ compared to control; # indicates $p < 0.05$ compared to HSD control; + denotes $p < 0.05$ compared to WR offspring on HSD; ns- not significant compared to control ($p > 0.05$). One-way ANOVA was used to analyze data in F0; two-way ANOVA was used to compare groups in F1 and F2.



(caption on next page)

Fig. 7. Effects of WR consumption on the expression levels of ILP in (A) F0 parent (B) F1, and (C) F2 offspring. Changes in the expression levels of IRS in (D) F0 parent (E) F1, and (F) F2 offspring following consumption of WR in the parent. CTRL- Control; WR- White rice; BR- Brown rice; WRN- WR offspring fed on a normal diet; BRN- Offspring of BR raised on a normal diet; HSD-High sugar diet; WRH- WR offspring fed HSD; BRH- BR offspring fed HSD. Data are expressed as Mean ± SD ($n = 30$). * represents $p < 0.05$ compared to control; # indicates $p < 0.05$ compared to HSD control; + denotes $p < 0.05$ compared to WR offspring on HSD; f.c.-fold change; ns- not significant compared to control ($p > 0.05$). One-way ANOVA was used to analyze data in F0; two-way ANOVA was used to compare groups in F1 and F2.

Effects of maternal WR consumption on biochemical variables

Maternal WR consumption programs for hyperglycaemia in F1 but not F2 offspring

Prolonged WR consumption induced marked hyperglycaemia in the F0 exposed maternal flies ($p < 0.003$) compared to those fed a standard cornmeal diet (Fig. 4A). However, flies fed BR had similar glucose levels with the control. The increased glucose levels observed in F0 was also seen in F1 offspring fed a control diet ($p < 0.05$) (Fig. 4B). Increased glucose levels were also observed in high sugar diet (HSD) control groups and this is similar to levels in the F1 offspring of WR-fed maternal flies that were raised on a standard diet. Maternal BR however, did not induce hyperglycaemia in F1 offspring ($p > 0.05$) (Fig. 4B). Furthermore, female offspring from WR-fed maternal flies challenged with HSD also had increased glucose levels ($p < 0.05$). as shown in Fig. 4B. In the second generation (F2), maternal WR-fed offspring had increased glycaemic levels but were not significantly different from the control group ($p > 0.05$) (Fig. 4C).

Maternal WR diet transgenerationally programs for increased trehalose levels in offspring

Consumption of WR in the parent (F0) markedly increased the circulating trehalose levels ($p < 0.0001$) (Fig. 5A). The trehalose levels in BR-fed flies were not significantly different from that of the control group ($p > 0.05$). WR-induced increase in trehalose levels in the parent also programmed the same in male and female offspring of subsequent generations (F1 and F2) fed a normal diet (Fig. 5B, C). In the HSD control group, the trehalose level increased significantly ($p < 0.05$). Male F1 offspring from WR-fed parent that developed on a normal diet had higher trehalose levels when compared to the HSD control ($p < 0.05$) (Fig. 5B). In contrast, female F2 WR offspring challenged with HSD for 7 days in adulthood had elevated trehalose levels compared to their counterparts on a normal control diet ($p < 0.05$) (Fig. 5C). On the other hand, maternal BR-fed offspring raised on a control diet did not develop increased trehalose levels relative to control (Fig. 5B, C).

WR consumption in the parent does not affect glycogen levels in the offspring

Glycogen levels in the F0 WR-fed flies were significantly lower ($p < 0.008$) compared to control (Supplementary Fig. S1A). Offspring from respective maternal diet groups were not significantly different from the control in F1 and F2 ($p > 0.05$) (Supplementary Fig. S1B, C) except for the female offspring of WR-fed female flies whose glycogen level was lower compared to the control group (Fig. S1C).

Maternal WR consumption programs offspring for excessive lipid accumulation

The TG levels of the WR-fed flies were significantly elevated ($p < 0.0015$) relative to the control while a marked reduction was seen in BR-fed flies ($p < 0.0001$) compared to the control group (Fig. 6A). Both F1 and F2 WR offspring fed a control diet developed hypertriglyceridemia (Fig. 6B, C). This was observed in both male and female offspring. The TG levels of HSD-fed flies were also significantly increased. F1 female offspring from WR-fed maternal flies raised on HSD had higher TG levels in comparison with the HSD group. Unlike the WR groups, offspring of BR-fed flies which developed on the control diet had TG levels comparable with control. In F1, the TG level was significantly reduced in the female offspring of BR-fed flies which developed on HSD compared to WR offspring on HSD. Finally, the TG levels in offspring of WR-fed flies raised on HSD were not significantly different from those raised on the standard diet.

Effects of WR consumption on gene expression

Maternal WR consumption alters the expression of insulin signaling genes (ILP2 and IRS) in offspring

By using quantitative RT-PCR, we found that WR diet caused a 2-fold increase ($p < 0.0001$) in the mRNA expression level of *ILP2* in the F0 flies but was downregulated in the BR group (Fig. 7A). The mRNA expression of *ILP2* was also upregulated in F1 and F2 offspring of maternal WR-fed flies (Fig. 7B, C). HSD-fed flies had significantly ($p < 0.05$) elevated expression of *ILP2* (Fig. 7B, C). The expression of *ILP2* in male and female F1 offspring of maternal WR diet-challenged flies raised on a normal diet was higher than their counterparts on HSD but was not significantly different in F2 (Fig. 7B, C). Again, the expression of *ILP2* in offspring of BR-fed maternal flies was at normal levels.

Similarly, WR-fed flies had elevated ($p < 0.0001$) expression of *IRS* compared to control (Fig. 7D). Increased expression ($p < 0.05$) of *IRS* was also observed in F1 but not F2 offspring of WR-fed flies (Fig. 7E, F). Groups fed HSD had increased ($p < 0.05$) expression of *IRS* (Fig. 7E, F). The WR offspring that developed on HSD had remarkably higher levels of *IRS* expression relative to the control. In F1 female and F2 offspring of BR-fed maternal flies raised on HSD, there was a significant ($p < 0.05$) reduction in *IRS* expression compared to their WR counterparts (Fig. 7E, F). The expression of *IRS* in offspring of BR-fed flies showed normal patterns.

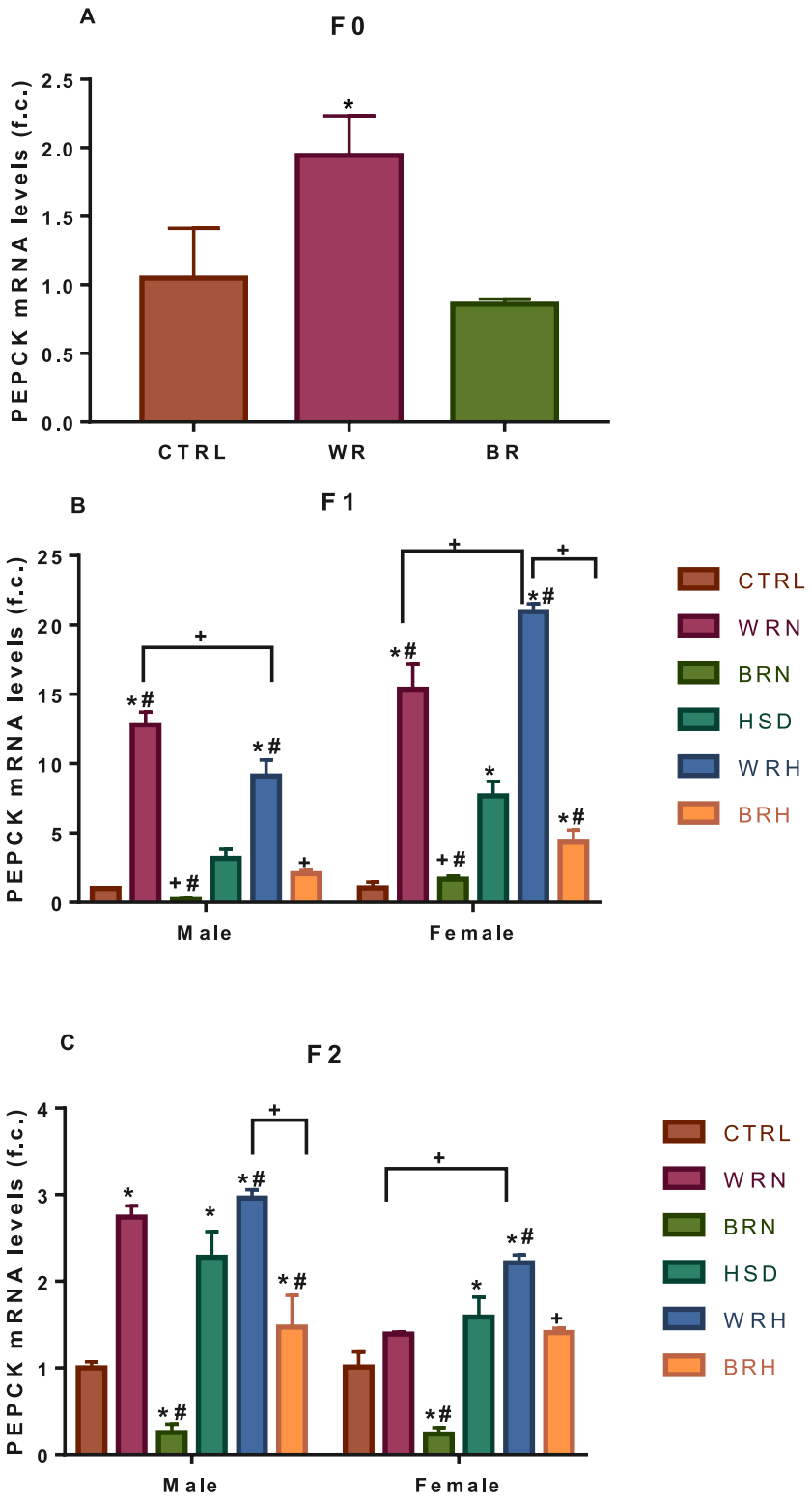


Fig. 8. Relative mRNA expression levels of *PEPCK* in (A) F0 parent following chronic WR consumption (B) F1 and (C) F2 offspring. CTRL- Control; WR- White rice; BR- Brown rice; WRN- WR offspring fed on a normal diet; BRN- Offspring of BR raised on a normal diet; HSD-High sugar diet; WRH- WR offspring fed HSD; BRH- BR offspring fed HSD. Data are expressed as Mean ± SD ($n = 30$). * represents $p < 0.05$ compared to control; # indicates $p < 0.05$ compared to HSD control; + denotes $p < 0.05$ compared to WR offspring on HSD; f.c.-fold change; ns- not significant compared to control ($p > 0.05$). One-way ANOVA was used to analyze data in F0; two-way ANOVA was used to compare groups in F1 and F2.

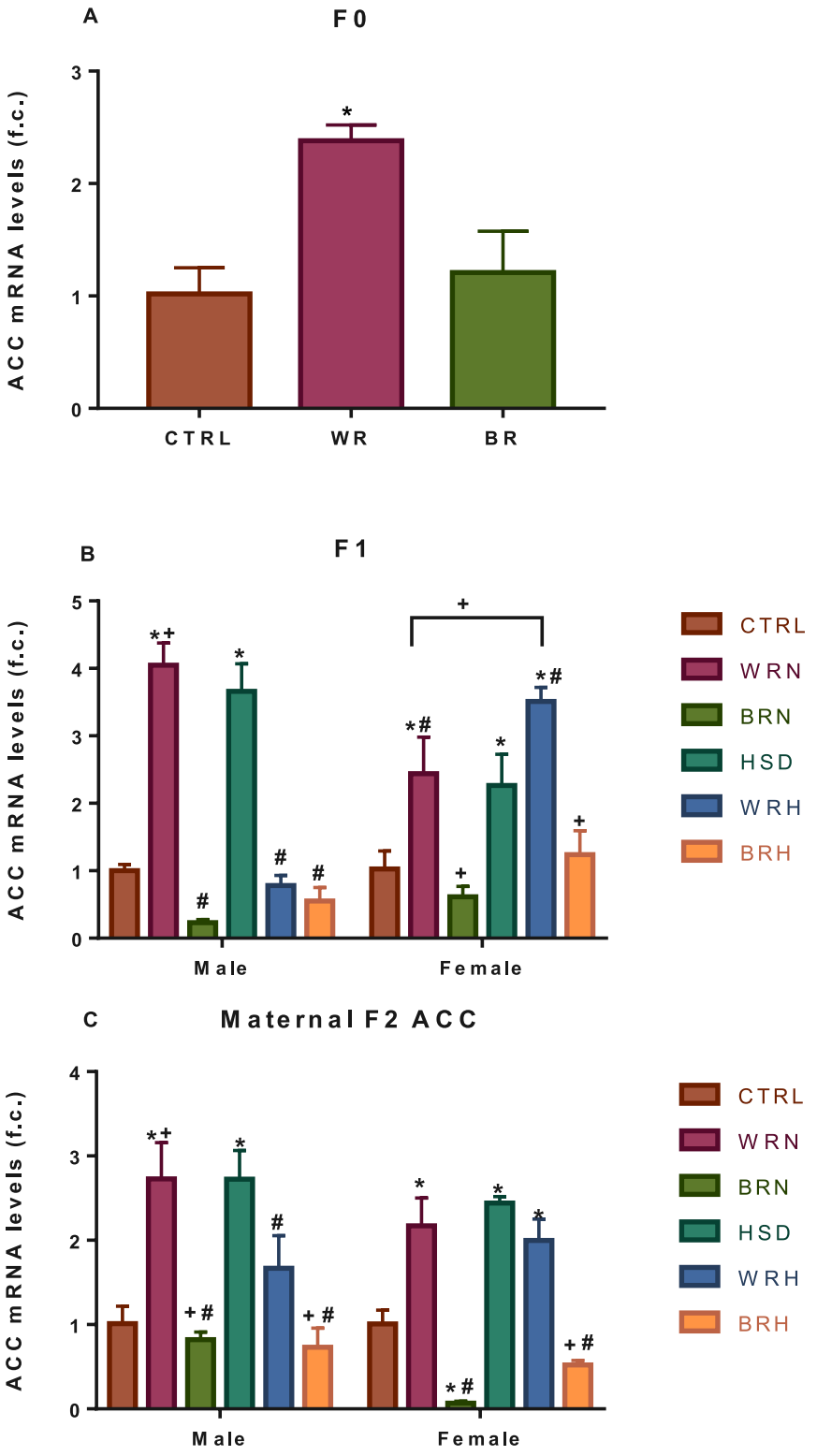


Fig. 9. Relative expression levels of the ACC gene in the (A) parent (F0) exposed to WR-diet (B) F1 and (C) F2 offspring. CTRL- Control; WR- White rice; BR- Brown rice; WRN- WR offspring fed on a normal diet; BRN- Offspring of BR raised on a normal diet; HSD-High sugar diet; WRH- WR offspring fed HSD; BRH- BR offspring fed HSD. Data are expressed as Mean ± SD ($n = 30$). * represents $p < 0.05$ compared to control; # indicates $p < 0.05$ compared to HSD control; + denotes $p < 0.05$ compared to WR offspring on HSD; f.c.-fold change; ns- not significant compared to control ($p > 0.05$). One-way ANOVA was used to analyze data in F0; two-way ANOVA was used to compare groups in F1 and F2.

Chronic maternal WR consumption programs offspring for enhanced gluconeogenesis

Following chronic WR feeding, *PEPCK* was upregulated ($p < 0.02$) in the F0 flies while the BR group showed an expression level comparable with the control (Fig. 8A). In F1 and F2, HSD induced increased mRNA expression of *PEPCK* (Fig. 8B, C). F1 offspring and F2 males from WR-challenged flies that developed on a control diet exhibited upregulation ($p < 0.05$) of *PEPCK* (Fig. 8B, C). Male F1 offspring of maternal WR flies on a normal diet had significantly ($p < 0.05$) higher expression of *PEPCK* compared with offspring of WR-fed female parent on HSD (Fig. 8B). In contrast, female F1 from maternal WR-fed flies raised on the HSD diet had increased ($p < 0.05$) expression of *PEPCK* than their counterparts on the standard diet. Also, maternal WR-fed flies F2 offspring that were challenged with HSD had significantly ($p < 0.05$) increased *PEPCK* expression when compared to those that were fed the standard diet (Fig. 8C). Additionally, F2 offspring of BR that were fed HSD were spared from overexpression of *PEPCK* when compared with their WR counterparts on HSD.

ACC is overexpressed in the offspring of WR-fed flies

In WR-fed flies, there was a significantly higher expression of the *ACC* compared to the control ($p < 0.02$) (Fig. 9A). This phenotype is steadily transmitted across the offspring spanning two generations (F1 and F2) (Fig. 9B, C). On the other hand, HSD control flies overexpressed the *ACC* ($p < 0.05$). The *ACC* expression in F1 female offspring of maternal WR-fed flies that developed on the HSD diet was significantly ($p < 0.05$) increased compared to their counterparts that developed on a normal diet (Fig. 9B). Furthermore, *ACC* expression in female F1 offspring that were fed HSD was higher ($p < 0.05$) compared to those on a normal diet. F2 female offspring from BR-fed maternal flies that developed on a standard diet had a significantly ($p < 0.05$) lower expression level of *ACC* (Fig. 9C). Similarly, F1 female and F2 offspring of maternal BR-fed flies that were raised on HSD had a reduced ($p < 0.05$) expression of *ACC* compared to their WR counterparts on HSD (Fig. 9B, C).

Discussion

Over the last couple of decades, cases of childhood diabetes have increased by 39.4 % [78] and this can be attributed to parental history of T2D [70]. Nutrient overload in the parent is accompanied by an altered metabolic state which can be passed to subsequent generations and could persist till adulthood [9,18], hence, contributing to the growing diabetes pandemic. We have previously established that WR consumption alters body composition, locomotor activity, energy homeostasis as well as other IR phenotypes in adult flies [56]. In this study, we hypothesized that these metabolic perturbations could be passed to multiple offspring generations. Here, our findings show that WR consumption produced obesity in the maternal parent but not the offspring. The weight gain in the maternal flies could be attributed to disturbances in normal metabolism potentially triggered by chronic consumption of WR. Given the high GI of WR, excess glucose might have been readily converted into fat in the presence of reduced energy expenditure, fostering adipose tissue expansion [4]. The observed lack of significant differences in the body weight of offspring is similar to a previous study in mammals where the body weight of WR-fed rat offspring did not differ from the control despite disrupted metabolism [28]. A similar finding was reported in a mouse model where F1 offspring of diet challenged parent developed IR but not obesity [50]. It was also observed that HSD-fed control flies did not exhibit any statistically significant differences in weight and this is not surprising because weight may not be an adequate metric of obesity in flies in the presence of other well established obesity features such as fat accumulation and reduced climbing ability [5].

Since walking speed is used as an indicator of human health [22], a frequently used health indicator for *Drosophila* is locomotor performance, which may be conveniently assessed by a negative geotaxis (climbing assay) experiment [30,62]. Studies have shown that IR and diabetic flies had decreased locomotor activity and displayed an impaired ability to climb up towards light [44,45]. This is consistent with our finding on WR-fed flies that showed reduced climbing ability compared to BR which showed better locomotor activity, and agrees with our previous study [56]. However, our study revealed that F1 offspring of WR-challenged flies on a normal diet (WRN) showed a tendency of reduced climbing ability compared to their BR counterparts. Although F1 male offspring of WR-fed flies on HSD showed significantly decreased locomotor performance, this was not observed in the females on the same diet. In F2, male WRN offspring showed a drastic reduction in locomotor performance. This suggests that the underlying tendency for reduced locomotor activity observed in F1 was prominently shown in F2 which may indicate an increased propensity for altered well-being.

IR is an underlying condition preceding the characteristic non-physiologic hyperglycaemic state, which is the principal clinical manifestation of T2D [36]. In the later stages of IR, there's a rise in insulin levels to counter the abnormal rise in blood glucose levels which ultimately results in chronic hyperinsulinaemia. Over time, this may result in the failure of the β-cells in the pancreas and ultimately lead to the development of T2D [33]. IR is further distinguished by increased glycogen synthesis, ectopic lipid accumulation, inhibition of lipolysis and hepatic glucose output [52,79]. In this study, we demonstrated that the offspring of WR-fed flies developed IR characterized by abnormal levels of measured biochemical parameters along with altered expression of assayed metabolic genes. Specifically, our data show that F1 offspring that were fed a normal diet exhibited increased haemolymph glucose, trehalose, and triglyceride with moderate reductions in glycogen levels. In a related study in mammals, F1 and F2 offspring of diet-challenged parents developed hyperglycaemia, hypertriglyceridemia, glucose intolerance and IR [50,66]. While the levels of glucose moderately increased in F2, significant elevation in haemolymph trehalose and triglyceride persisted. Trehalose is considered the most abundant circulating carbohydrate in *Drosophila* [40] and therefore its remarkably high levels further confirm insulin insensitivity in the flies. The hyperglycaemia, hypertrehalosemia and hypertriglyceridemia observed in the offspring reflect impairment in insulin signaling pathways and maladaptive responses to prolonged maternal WR consumption. Maladaptation in adipose tissue is closely linked to the onset of dyslipidaemia, IR, and T2D [12]. The consumption of energy dense diet like WR could trigger chronic inflammation in white adipose tissue, culminating in IR and dysfunction of fat cells [17,58]. One of the hallmarks of adipose

tissue maladaptation is the heightened accumulation of lipids, a trend consistent with our findings. We also established that the offspring of WR-diet-challenged maternal flies raised on HSD showed comparatively worsened metabolic states except in a few instances. This suggests that offspring born to chronic WR consumers and further challenged with a Western diet such as HSD may develop worsened metabolic disorders secondary to the underlying aberrant metabolic state.

Furthermore, these biochemical alterations in the offspring were also accompanied by a dysregulation of metabolic target genes. The offspring exhibited dysfunctional carbohydrate homeostasis. The substantial increase in circulating glucose and trehalose levels signals a failure to absorb and store carbohydrates. Diminished glucose transport activity leads to abnormal utilization of energy substrates and is linked to impaired glycaemic control [10] and may explain the hyperglycaemia observed in this study. The attempt to rescue the IR state in both the parents and offspring is evidenced by the upregulated expression of the insulin-signaling genes (*ILP2* and *IRS*) which was met with diminished response of peripheral tissues to exogenous stimulation of insulin given the elevated levels of the biochemical markers. Several studies have reported a similar increase in *ILP2* expression in IR flies [37,41,49]. While Saka et al. [56] reported downregulation of *IRS*, our findings demonstrated upregulated levels of *IRS* in F0 and F1 while the expression was reduced in F2. This discrepancy in the expression pattern may be attributed to the shorter duration of exposure in their study, which lasted just 7 days, and their use of adult-only flies as opposed to the current study. Increased expression of *IRS* may be an attempt by the IR flies to clear the haemolymph glucose levels but was probably hindered by defective downstream signaling as the hyperglycaemic state remained intact. The decreased expression of *IRS* in F2 is indicative of insulin insensitivity [38]. This suggests that insulin insensitivity may worsen down the line. In the BR groups, however, the expression of the insulin signaling genes was relatively normal corroborating the health benefits of BR as previously reported [1,28].

In T2D, the primary factor contributing to elevated hepatic glucose production and hyperglycaemia is thought to be heightened gluconeogenesis [54]. PEPCK is a crucial rate-regulating enzyme in the synthesis of glucose from pyruvate, lactate and alanine [25]. The ability of insulin to inhibit gluconeogenesis is impaired in T2D [64]. Previous studies have also demonstrated elevated expression of *PEPCK* in IR models [13,43] and in WR-fed adult *Drosophila* [56]. Here, we provide evidence that maternal WR consumption programs F1 and F2 offspring for increased gluconeogenesis. Although the expression was not pronounced in F2 female offspring, the potential to stimulate gluconeogenesis was pertinent. Again, the BR groups showed normal and, in some cases, reduced expression levels of *PEPCK* which further confirms its antidiabetic potential.

ACC is an enzyme that catalyses the conversion of acetyl-CoA to malonyl-CoA, a precursor for lipid synthesis [67]. Malonyl-CoA also doubles as an inhibitor of CPT-1 which transports long-chain fatty acid into the mitochondria for beta oxidation [68]. WR-fed flies accumulate abundant lipids in their fat body [56]. In line with this, we found that *ACC* mRNA was overexpressed in F1 and F2 offspring of WR-fed parent raised on a normal diet. Offspring maintained on HSD demonstrated remarkably higher expression levels than their counterparts on the control diet. In contrast, BR-fed flies exhibited expression levels similar to the control. Generally, the health benefits conferred by BR in this study may be a function of higher amounts of fiber, and bioactive compounds such as oryzanol, phenolic, and flavonoid contents of BR in comparison to the WR cultivar [56]. The major difference in the nutritional composition of WR and BR is reflected in the especially many active components in the rice bran which offer nutritional value that are beneficial to health [74]. This is evident in the anti-oxidant, anti-inflammatory, lipid-lowering effects and anti-diabetic effects of BR previously reported in the literature [56,57].

Overall, the variations in gene expression patterns observed in the offspring provide further evidence of significant differences in the metabolic gene regulatory program, potentially explaining the observed changes in biochemical variables measured in the flies. In addition, a thorough examination of the data reveals that both male and female progeny are almost equally affected by the detrimental consequences of chronic maternal WR consumption. A possible common mechanism behind these diet-induced modifications is via the programmed transmission of certain epigenetic marks [27,48,55]. Gene regulatory factors, including covalent chemical changes (such as methylation) to DNA, histone post-translational modifications, and various RNA species, may be passed from parental gametes to the zygote during both mitosis and meiosis [61]. In this study, chronic WR consumption might have altered the epigenome of the parental gametes which was then transferred to the offspring hence, programming their gene expression patterns via the principle of meiotic epigenetic inheritance [61]. A previous study in *Drosophila* highlighted the significance of meiotic inheritance of histone modifications such as H3K9me3 and H3K27me3 in response to dietary stressors [47]. The study revealed that paternal HSD altered the chromatin state of the embryonic, larval and metabolic genes in F1 progeny [47]. This was accompanied by elevated triglyceride and body weight. Similarly, there is evidence of maternally inherited histone mark H3K27me3 in F1 offspring of *Drosophila* [77]. Furthermore, the inheritance of metabolic phenotypes via transmission of DNA methylation marks [39,53] and small regulatory RNA species [16] to offspring has also been documented. Thus, environmental challenges including high energy diet could alter epigenetic signatures and program embryo development. This may eventually contribute to disease development not just in the exposed individual but also in succeeding generations [55]. We, therefore, postulate that the observed transgenerational effects of WR consumption occurred via one of the aforementioned epigenetic mechanisms to program metabolic dysfunction in the offspring.

Conclusion

Collectively, maternal preconception exposure to a chronic WR diet produced increased glycaemia, trehalose and triglycerides in F1 and F2 offspring fed a normal diet. This was accompanied by overactivation of insulin signaling genes (*ILP2* and *IRS*) coupled with enhanced expression of *PEPCK* and upregulation of *ACC*. Offspring challenged with HSD developed a worsened metabolic state. Unlike WR, the measured parameters in BR groups were similar to the control across offspring generations. This study underscores the metabolic programming potential of WR, but not BR consumption. The present study, therefore, demonstrates for the first time, the transgenerational risk of IR following chronic maternal consumption of WR. This is a strong indicator that individuals who are chronic

consumers of WR may develop IR and subsequently T2D and may equally be at risk of transmitting these traits to succeeding generations. Certainly, our study supports the findings in the existing literature, suggesting that the consumption of WR may have negative implications for metabolic health. The translational implication of these observations indicates that the consumption of WR may play a substantial role in the rising prevalence of IR and T2D. Furthermore, it is even more worrisome that the metabolic perturbations following maternal WR consumption could be passed down to the offspring. Hence, it is crucial to restrict the intake of WR in order to avert its deleterious effects on consumers. Further studies should focus on elucidating the underlying epigenetic mechanism of this transgenerational inheritance. Subsequent studies may also explore other mechanistic pathways such as investigating mitochondrial function assays, notable inflammatory pathways, and the crucial role of the gut microbiota in mediating dietary effects on host metabolism in the parent and offspring. Additional research is necessary to corroborate our findings in mammals and to carry out clinical trials in order to assess their significance in relation to the growing burden of IR and T2D in humans. Also, longer-term follow-up studies are required to evaluate the persistence of transgenerational effects.

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Institutional review board statement

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Informed consent

Not applicable.

CRediT authorship contribution statement

Kehinde Ahmad Adeshina: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Kasimu Ghandi Ibrahim:** Funding acquisition, Supervision, Writing – review & editing. **Murtala Bello Abubakar:** Supervision, Visualization, Writing – review & editing. **Mustapha Umar Imam:** Conceptualization, Project administration, Formal analysis, Funding acquisition, Resources, Supervision, Writing – review & editing.

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 is available upon reasonable request from the corresponding author.

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Supplementary materials

Fig. S1: Fig. S1: Haemolymph glycogen levels in (A) F0 parent (B) F1, and (C) F2 offspring following maternal WR consumption. Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2024.e02208.

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