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Full Length Research

Consumption of some Nigerian local rice varieties and effect on development of insulin resistance and obesity in *Drosophila melanogaster*

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ABSTRACT: There is evidence to suggest that the increased incidence of insulin resistance and obesity is associated with the consumption of energy-dense and highly processed foods like polished rice. This study, therefore, investigated the effects of consumption of selected local rice varieties on some markers of insulin resistance and obesity using *Drosophila melanogaster*. Five different local rice varieties (Jamila, Kwandala, Mai-bakin carki, Yar-katabore and Yar-washagi) were subjected to proximate composition analysis, which showed that the unpolished local varieties had a better deposit of nutrients. Thereafter, adult flies were raised on diets containing either polished or unpolished forms of the five different local rice varieties for seven days and thereafter, analysed for weight and locomotor ability, as well as the levels of glucose, trehalose, glycogen and triglycerides in the hemolymph and expression levels of ACC and Pepck genes. *Drosophila melanogaster* flies on the polished rice groups showed increased weight, hyperglycaemia and hyperlipidaemia, compared to those on the unpolished rice ($p<0.05$). Changes in gene expression were in line with the biochemical changes. The study, thus, suggested that the substitution of polished with unpolished local rice may reduce the risk of insulin resistance and obesity, as well as their related complications.

Keywords: Gene expression, insulin resistance, metabolic diseases, obesity, unpolished rice.

INTRODUCTION

The world is experiencing an ever-increasing burden of non-communicable chronic diseases (NCCDs) like type 2 diabetes and other metabolic diseases, which have been traditionally associated with the adoption of unhealthy Western lifestyle factors including the consumption of energy dense and highly processed foods (Imam and Ismail, 2017). Rice unarguably falls under this category of foods and it is such an important food crop in Nigeria that despite many policies of the Federal Government of Nigeria in the rice sector, rice production has not matched the growing domestic demand. Currently, 573 million adults are reported to be living with diabetes worldwide and it is expected to rise to 643 million by 2030 and 783 million by 2045 (IDF, 2019; IDF, 2022a). Nigeria is the second country with the highest prevalence of type 2 diabetes in Africa with estimated 3.6 million people with diabetes (IDF, 2022; IDF, 2022b). Imam *et al.* (2015) reported an increased risk of insulin resistance (IR) in rat offspring whose parents were exposed to Polished Rice (PR) as a staple. Another study provided evidence that PR consumption worsens antioxidant status in type 2 diabetic rats, while Unpolished Rice (UR) and germinated brown rice (GBR) maintain antioxidant status to varying degrees and improved glycaemia (Imam *et al.,* 2012). Although

effort has been made to prevent the burden of these NCCDs, there is a need for more action to prevent people from further suffering from the pandemic.

PR, which primarily consists of starch, is produced from whole rice through a series of mechanized processes including hulling and milling (Atkinson *et al*., 2008) and it is the predominant type of rice consumed worldwide (Nanri *et al*., 2010). The complete milling and polishing destroy many nutrients. This may form part of the reason that the glycaemic index values of PR are higher on average than those of whole grains (Foster-Powell *et al*., 2002). It is reported that PR is the primary contributor to dietary glycaemic load for populations that consume rice as a staple food (Nanri *et al*., 2010). However, rice consumers often prefer to take PR despite the superior nutritional value of the UR (Nirmala *et al*., 2015). Several studies on foreign rice varieties reported that PR consumption can predispose to metabolic disorders like type 2 diabetes and obesity while UR can counteract the effects. However, limited studies were done on our local rice varieties.

This study, therefore, evaluated five common varieties of rice grown locally and the risk of developing IR and obesity by consuming either PR or UR local varieties using *Drosophila melanogaster* (fruit fly) as a model organism. *Drosophila melanogaster* is a versatile model for studying human diseases due to its anatomic, physiological, pathological and genetic similarities with humans (Wangler *et al*., 2015). *Drosophila melanogaster* has been used in biomedical research for over a century to study a broad range of phenomena. There are many technical advantages of using *Drosophila melanogaster* over vertebrate models; they are easy and inexpensive to culture in laboratory conditions, have a much shorter life cycle, they produce large numbers of externally laid embryos and can be genetically modified in numerous ways (Jennings, 2011).

Although humans and flies differ greatly in terms of their gross morphological and cellular features, many of the molecular mechanisms that govern the development and drive cellular and physiological processes are conserved between both organisms (Ugur *et al*., 2016). It has been proven to be excellent in modelling human diseases, which has been supported by the fact that around 75% of human disease genes have orthologues in the fly genome (Wangler et al., 2015). *Drosophila melanogaster* genome encodes seven insulin-like peptides and the main insulin-producing cells analogous to the pancreatic β-cells are localized in the central brain and have a full set of lipogenic enzymes involved in triglycerides de novo biosynthesis from fatty acids (Liu and Huang, 2013)

METHODOLOGY

Sample collection and preparation

Five different local rice cultivars namely; Jamila, Kwandala, Mai bakin-carki, Yar-katabore and Yar-washagi were collected from different farmers at the point of harvest from different places within Sokoto and its environs and identified by other farmers. The seeds were sun dried, de-hulled and divided into two sets. One set was kept unpolished (UR) and the other set was polished (PR). Portions of the UR and PR samples were then ground to obtain rice flour suitable for analysis.

Fly stock

Wild type (W^{118} strain) *Drosophila melanogaster* was acquired from Usmanu Danfodiyo University, Center for Advanced Medical Research and Training (CAMRET) and maintained in temperature-regulated (22-25℃) fly lab with access to normal diet and natural light: dark cycle in the same centre.

Fly husbandry and diet preparation

Stocks were maintained on a standard cornmeal-yeast-agar medium. For the experiments, a modified diet making 50% of the standard diet to have either PR or UR of the rice varieties was used while 10% HFD was made as a control as shown in Table 1.

Flies homogenization and haemolymph extraction

Flies were anesthetized using cooling effect as previously described by Reynolds and Orchad (2011) with slight modifications and weighed. Phosphate buffered saline (PBS) volume equivalent to the weight of the flies multiplied by 10 was mixed with the flies in a small Eppendorf tube and crushed mechanically using a plastic homogenizer. The homogenized sample was centrifuged at 4000 rpm for 1 minute and the haemolymph was collected.

Experimental design

To determine the effect of local rice consumption on the risk of developing IR and obesity, flies were grouped into 12 different groups of 90 flies each. Each group was fed different rice varieties while groups 1 and 2 were fed normal diets and HFD respectively which served as control groups. Adult flies (1-3 day old) were allowed to feed on their respective diets for 7 days (equivalent of 7 human-years) after which they were analysed for weight and negative geotaxis. Finally, 30 flies from each group were anaesthetized in ice, washed and crushed in PBS. Then, the haemolymph was collected and used for biochemical analyses while another 20 were crushed in lysis buffer and used for gene expression analyses.

Table 1. Dietary composition of *Drosophila melanogaster* diet.

Nutrients	Standard diet (1000g)	Test diet (1000g)	High fat diet (1000g)
Corn flour	100g	50g	90g
Baker's yeast	12g	12g	12g
Agar agar	20g	20g	20g
Methyl paraben	1.g	1g	1g
Water	1.5L	1.5L	1.5L
Others	-	50g Rice	10g Coconut oil

GROUP 1: Control flies fed with normal diets.
GROUP 2: Control flies fed with HFD.
GROUP 3: Flies fed with PR of Jamila rice variety.
GROUP 4: Flies fed with UR of Jamila rice variety.
GROUP 5: Flies fed with PR of Kwandala variety.
GROUP 6: Flies fed with UR of Kwandala variety.
GROUP 7: Flies fed with PR of Mai Bakin-Charki variety.
GROUP 8: Flies fed with UR of Mai bakin-Charki variety.
GROUP 9: Flies fed PR of Yar-katabore variety.
GROUP 10: Flies fed with UR of Yarkatabore variety.
GROUP 11: Flies fed with PR of Yarwashagi variety.
GROUP 12: Flies fed with UR of Yarwashagi variety.

Proximate analysis

The moisture contents were determined using the method of AOAC (2006). Briefly, the empty crucible was weighed (W_0), and 2 g of the sample was added to the empty crucible and weighed (W_1). The crucible containing the sample was then dried in a hot air-drying oven at 105°C for 24 hours. The crucible was then cooled in a desiccator, and the crucible with the dry sample was weighed (W_2). The crucible containing the dried sample was returned to the oven for further 24 hours to make sure the drying is complete. It was then cooled in a desiccator and weighed again. The percentage moisture was calculated based on dry weight using the relation;

$$\%\ \text{Moisture} = \frac{W1 - W2}{W1 - W0} x100$$

The ash contents were determined using the method of AOAC (2006). The empty crucible was weighed (W_0), and 2 g of sample was added to the empty crucible and both the crucible and sample were weighed (W_1). It was burnt to ash in a muffle furnace at 600°C for 3 hours and then cooled in a desiccator. The weight of the crucible and ash sample was weighed (W_2). The percentage ash was calculated using the relation;

$$\%\ \text{Ash} = \frac{W2 - W0}{W1 - W0} \times 100$$

The crude protein contents were determined using the method of Kjeldahl (1883). One gram of the sample was weighed into a dry 500 ml Macro-kjeldahl flask, and 20 ml of distilled water was also added. The flask was then rotated for a few minutes and allowed to stand for 30 minutes. One tablet of mercury catalyst was added followed by 10 ml of concentrated sulphuric acid. The flask was heated cautiously at low heat on the digestion stand. When the water has been removed and frothing has ceased, the heat was increased until the digest clears. The mixture was boiled for 5 hours.

Heating was regulated during this boiling so that the H_2SO_4 condenses about middle of the way up the neck of the flask. The flask was allowed to cool and 50 ml of water was added to the flask slowly. Ten (10) ml of the digest was carefully transferred into another clean macro-kjeldahl flask (750 ml). And then 20ml of boric acid (H_3BO_3) indicator solution was added into a 250 ml Erlenmeyer flask which was placed under the condenser of the distillation apparatus. Then, a 750 ml kjeldahl flask was attached to the distillation apparatus and 40 ml of 40% NaOH was poured through the distillation flask by opening the funnel stop cook. Furthermore, 40 ml of the distillate was collected and the NH_4-N in the distillate was determined by titrating with 0.01 M standard HCl using a 25 ml biuret graduated at 0.1 ml interval. The colour change at the endpoint was from green to pink. The percentage of crude protein was calculated using the relation;

$$\%\ \text{Crude protein} = \frac{(\text{a} - \text{b}) \times volume\ made \times 6.25}{\text{volume of aliquot} \times \text{weight of sample}} \times 100$$

The crude lipid contents were determined using the method of AOAC (2006). Briefly, two hundred and fifty (250) ml extraction flask was dried in an oven at 105°C, allowed to cool in a desiccator and weighed. Two (2) g of the sample was weighed into the porous thimble; the mouth of the thimble was covered with white cotton wool, and 200 ml of petroleum ether was added into the dry 250 ml extraction flask. The covered porous thimble was placed into the condenser and the apparatus was assembled. Extraction was done for 5 hours. The porous thimble was removed carefully to collect the ether in the top container for re-use. Extraction flask was removed from the water bath when it was almost free of petroleum ether. The extraction flask containing the lipid was oven dried at 110°C for 1 hour, cooled in a desiccator and then

weighed after cooling. The percentage crude lipid was calculated using the relation;

$$\%\ \text{Crude lipid} = \frac{W3-W2}{W1-W0} \times 100$$

The crude fiber contents were determined using the method of AOAC (2006). Briefly, two (2) g of the sample was weighed into 1 L conical flask (W_0), and 200 ml of boiling 1.25% H_2SO_4 was added and boiled gently for 30 minutes. The solution was filtered through muslin cloth, and then rinsed well with hot distilled water. The sample was then scraped back into flask with spatula and 200 ml of boiling 1.25% NaOH was added and allowed to boil gently for 30 minutes. The solution was filtered through a muslin cloth and the residue was washed thoroughly with hot distilled water and rinsed once with 10% HCl, twice with methylated spirit and trice with petroleum ether (BP 40-60°C). It was then allowed to drain dry and then the residue was scraped into a crucible, allowed to dry overnight at 105°C in an oven, and then cooled in a desiccator. The sample was weighed (W_1), ashed at 550°C for 90 minutes in a muffle furnace, cooled in a desiccator and then weighed (W_2). The percentage crude fiber was calculated using the relation;

$$\%\ \text{crude fiber} = \frac{W2-W1}{W0} \times 100.$$

Available carbohydrate was estimated by subtracting the total of the percentages of crude protein, crude lipid, crude fiber and ash from 100% moisture free sample.

%Carbohydrate = 100% - (%Ash + %Crude protein + %Crude lipid + %Crude fiber)

Phenotypic changes

The weight of the flies was measured using a Kern analytical weighing scale (Kern and Sohn Ltd. Balingen, Germany). Thirty flies per group were anaesthetized on ice and then divided into three groups of ten flies each. Flies in each group were placed inside a pre-weighed empty microtube (GEB, USA) and re-weighed. The differences in weights were recorded in micrograms and multiplied by 1000 to convert to milligrams.

The locomotor performance of the flies was determined using the negative geotaxis method as previously described (Adedara *et al*., 2016). Ten flies were immobilized under mild ice anesthesia, placed separately in labelled vertical glass columns (length, 15 cm; diameter, 1.5 cm). After the recovery from ice exposure (about 20 minutes), the flies were gently tapped to the bottom of the column and the number of flies that climbed up to the 6 cm mark of the column in 10 seconds, as well as those that remain below the mark after this time were recorded.

Biochemical analyses

Haemolymph glucose was estimated by the glucose oxidase method using the Spinreact kit, Girona, Spain. Elisa plate reader was calibrated and the samples and reagents were pipetted into a 96 well plate with one well each as blank, test and standard. Ten µl each of hemolymph and standard glucose solution were added to the test and standard wells respectively and 100 (µl) of working reagent was added to each well. The tubes were mixed properly, incubated at 37oC for 10 minutes and the absorbance of standard and tests was read against the blank at 505 nm in an ELISA plate reader. The glucose concentration was calculated using the formula:

$$\text{Glucose (mg/dL)} = \frac{\text{Abs. of test}}{\text{Abs. of standard}} \times \text{conc. of standard}$$

Where Abs. = Absorbance, and conc. = concentration.

Trehalose level of haemolymph was quantified using the Anthrone colorimetric kit (Solarbio Life Science, Beijing, China). Working solution was prepared by adding 16mL of distilled water to each tube of reagent and followed by 64 mL of concentrated sulfuric acid slowly until fully dissolved. The assay was done by taking 0.25µL each of sample and standard solution and 100 µL of working solution into different micro centrifuge tubes and incubating in water bath at 95°C for 10 minutes. Then, the mixture was allowed to cool at room temperature, and 100 µL was dropped into a microplate and the absorbance read at 620 nm on an ELISA plate reader. Standard curve was established based on the concentration (y) and absorbance (x) of the standard. The trehalose content y (mg/g) was calculated according to the standard curve.

Trehalose (mg/g sample) = Y ÷ W

Where: y = trehalose content from the standard curve, and W = weight of the flies

Glycogen level was determined using the Anthrone colorimetric kit (Solarbio Life Science, Beijing, China). Elisa plate reader was calibrated and the wavelength was adjusted to 620 nm. Ten (10) ml of distilled water was poured into reagent II and 40 ml of concentrated sulphuric acid was also added, dissolved and mixed thoroughly. Twenty-five (25 µl) each of distilled water, reagent I and hemolymph were added to wells labelled Blank (A1), standard (A2) and test (A3) while 100 µl of reagent II was added to each well. The contents were mixed well and placed in a boiling water bath for 10 minutes, cooled and the absorbance of the blank, standard and test were read at 620 nm in an ELISA plate reader and recorded as A1, A2 and A3, respectively. The amount of glycogen was calculated using the relation;

Glycogen (mg/mg) = 0.450 × (A3−A1) ÷ (A2−A1) ÷ W

Where: W= weight of the flies

Triglyceride levels were assayed using triglycerides assay

kit (Spinreact, Girona, Spain). Elisa plate reader was calibrated and the samples and reagents were pipetted into a 96 well plate. Three wells were labelled blank, test and standard. Ten (10) µl each of hemolymph and standard were added to the test and standard wells respectively while 100 µl of working reagent was added to each well. The mixture was incubated at 37°C for 5 minutes and the absorbance of the standard and tests were read at 505 nm against the blank in an ELISA plate reader. The TG levels were calculated using the relation:

$$\text{TAG (mg/dl)} = \frac{\text{Abs of Test} - \text{Abs of blank}}{\text{Abs of Standard} - \text{Abs of blank}} \times \text{Conc. of Standard}$$

Gene expression analyses

RNA extraction

Total RNA was extracted from the flies using the Total RNA extraction kit (RBC Bioscience, Seoul, Korea). Lysis buffer (300 µl) was added to the flies and homogenized, after which additional 300 µl of chloroform was added. The mixture was vortexed and centrifuged at 12000 rpm for 1 minute. Then, 300 µl of the aqueous layer was transferred to a new tube, and equal volume of ethanol was added and mixed. The mixture was then transferred to RNA binding column and centrifuged at 12000 rpm for 1 minute and the waste was discarded. Thereafter, 500 µl of wash buffer 1 was added and centrifuged at 12000 rpm for 1 minute, and the step was repeated with equal volume of wash buffer 2 twice. The column was spin-dried to remove any waste at 12000 rpm for 3 minutes. The column was transferred to a new collection tube and 100 µl of elution buffer was added and allowed to soak the RNA for 3 minutes. Finally, the column was centrifuged at 12000rpm for 1 minute. The total RNA was used for one-step qRT-PCR.

The PCR experiments

All solutions were thawed on ice, gently vortexed and centrifuged and the reaction components were prepared in a thin-walled PCR tube on ice as shown in the Table 2. One (1 µL) of each primer is diluted with 9 µL of molecular grade water. The solution was gently mixed and spun down in a microcentrifuge. The cycling conditions used are as follows and the result was analyzed using the melting curve analysis.

- Denaturation at 90^{o}C for 30seconds.
- Reverse transcription at 61^{o}C for 20 minutes.
- Pre-denaturation at 95^{o}C for 1 minute.
- 45 cycles of Denaturation at 95^{o}C 20 seconds, annealing at 60^{o}C for 15seconds and extension at 74^{o}C for 15 seconds.

Statistical analyses

All data were subjected to statistical analyses. The values were expressed as mean ± standard deviation. One-way analysis of variance was used to compare the means followed by Tukey-Kramer Multiple Comparisons Test using statistical software Instat 3 version (San Diego, USA). $P<0.05$ were considered as significant.

RESULTS

The proximate composition of the five local rice varieties

The proximate composition of the rice varieties is presented in Table 4. The protein contents ranged from 8.91 ± 0.1% to 11.14 ± 0.1% with all forms of PR significantly different from their UR counterparts except Yar-katabore variety. Carbohydrate ranged from 67.48 ± 0.0% to 72.74 ± 0.0% showing that all the rice varieties can be considered as good sources of carbohydrate. The moisture ranged from 7.51 ± 0.0% to 10.0 ± 0.5%, while ash contents range from 4.16 ± 0.03% to 7.60 ± 0.00%. Fiber ranged from 1.10 ± 0.0% to 4.16 ± 0.3% with all forms of UR varieties significantly higher than their PR counterparts and lipids ranged from 2.30 ± 0.0% to 2.83 ± 0.3% with no significant differences between the lipid contents of the different varieties.

Effects of consumption of the rice varieties on weight changes

The weight changes in flies after 7 days of consumption of the intervention diets are presented in Figure 1. As shown in the Figure 1, the flies on PR of Jamila variety had the highest weight (11.4 ± 0.0 mg) while those of UR of Yar-katabore recorded the least (4.3 ± 0.00 mg) body weight, all the groups are significantly different from the control groups ($p<0.05$) except those on UR of Kwandala variety.

Effects of the consumption of the rice varieties on negative geotaxis

The locomotor activities in flies after 7 days of consumption of the intervention diets are presented in Figure 2. The mean flies that were able to cross 6 cm marks in 10 seconds are depicted in the Figure 2. All flies on UR varieties were not significantly different from the normal diet group ($p>0.05$) except the flies on Yar-washagi and Mai bakin-carki varieties which had average of 8.00±0.00 and 7.67±0.58 flies respectively. The flies on PR varieties on the other hand did not differ significantly to the HFD group ($p<0.05$).

Table 2. Procedure for polymerase chain reaction.

Component	Volume (µL)	Final concentration
SYBR Green qRT-PCR Master Mix	10	1X
50mM Mn(OAc)2	1	2.5mM
10 µM forward primer	0.4	0.2µM
10µM Reverse primer	0.4	0.2µM
Template RNA	2	Total RNA <1µg
PCR grade water	6.2	
Total Volume	20	

Table 3. List of primer sequences.

S/N	Name	Left	Right
1	IRS(Chico)	TGGCTCCAAATCGAAGATACC	GGTAATGCTACTCTGACTCCTATTC
2	ACC	GCTGAGGAGGTTAAAGCTATGT	TAGTCCTCGGTGCTCAAGTA
3	PEPCK1	CCTCGATGGCATGAAGGATAAG	GACTCGAAGTAGGTGCGAATATC
4	RPL32	AAGTGTGCGGCTCGTATTT	GCTAGCTTCTTGGGCAGTATC

IRS=Insulin receptor substrate. ACC= acetyl-coA carboxylase. PEPCK= phosphoenolpyruvate carboxykinase. RPL= ribosomal protein.

Table 4. Proximate composition of the rice varieties (n=3).

Variety	Protein (%)	Carbohydrate (%)	Moisture (%)	Ash (%)	Fiber (%)	Lipid (%)
Jamila PR	9.43 ± 0.1^{a}	70.21 ± 0.7^{a}	8.34 ± 0.1^{a}	7.33 ± 0.6^{a}	2.39 ± 0.3^{a}	2.30 ± 0.0^{a}
Jamila UR	10.97 ± 0.1^{b}	67.48 ± 0.0	7.51 ± 0.0^{ab}	7.60 ± 0.0^{a}	4.11 ± 0.0^{b}	2.33 ± 0.3^{a}
Mai Bakin-Carki PR	10.37 ± 0.2^{c}	70.52 ± 0.1^{ab}	8.83 ± 0.3^{a}	5.33 ± 0.6^{b}	2.28 ± 0.2^{a}	2.67 ± 0.3^{a}
Mai Bakin-Carki UR	11.14 ± 0.1^{b}	68.55 ± 0.0^{c}	8.66 ± 0.3^{ab}	4.66 ± 0.3^{bc}	4.16 ± 0.3^{b}	2.83 ± 0.3^{a}
Yar-Washagi PR	9.16 ± 0.1^{a}	72.74 ± 0.6^{de}	9.33 ± 0.6^{a}	5.0 ± 0.5^{b}	1.10 ± 0.0^{c}	2.67 ± 0.3^{a}
Yar-Washagi UR	10.91 ± 0.1^{bc}	68.76 ± 0.6^{abc}	9.50 ± 0.5^{a}	4.5 ± 0.5^{b}	3.5 ± 0.1	2.83 ± 0.3^{a}
Kwandala PR	8.91 ± 0.1^{a}	72.98 ± 0.0^{d}	9.50 ± 0.5^{a}	4.16 ± 0.3^{c}	1.78 ± 0.0	2.67 ± 0.3^{a}
Kwandala UR	11.02 ± 0.2^{bc}	68.50 ± 0.4^{c}	9.33 ± 0.6^{a}	4.16 ± 0.3^{c}	4.16 ± 0.3^{b}	2.83 ± 0.3^{a}
Yar-Katabore PR	10.30 ± 0.5^{c}	72.02 ± 0.1^{e}	10.00 ± 0.5	4.16 ± 0.3^{c}	1.19 ± 0.0^{c}	2.33 ± 0.3^{a}
Yar-Katabore UR	11.14 ± 0.1^{c}	68.57 ± 0.4^{c}	9.00 ± 0.5^{a}	4.66 ± 0.3^{bc}	3.80 ± 0.0^{b}	2.83 ± 0.3^{a}

Proximate composition of the rice varieties. Values are expressed as mean ± SD, mean values in the same column superscripted by the same letters are not significantly different from each other, n= number of replicates, PR= Polished rice, UR= Unpolished rice.

Effects on the biochemical parameters

Effects of the consumption of the rice varieties on haemolymph glucose level

Figure 3 shows the results of the fasting haemolymph glucose levels of the flies after 7 days of exposure to the intervention diets. The PR groups showed similar values to the HFD ($p< 0.05$) showing the hyperglycaemic effects of PR with the PR of Yar-washagi variety having 13.93 ± 0.20 mg/dL, which was significantly higher ($p<0.05$) than HFD. On the other hand, the UR varieties showed no significant difference when compared to the normal control group ($p>0.05$).

Effects of the consumption of the rice varieties on haemolymph trehalose level

The effects of the intervention diets on haemolymph trehalose level in male and female flies is shown in Figure 4. The flies on UR varieties had trehalose levels within the range of 0.09 ± 0.01 mg/g to 0.11 ± 0.01 mg/g, which were not significantly different with the normal control ($p>0.05$). Those on PR ranged from 0.12 ± 0.03 to 0.16 ± 0.02 mg/g. All the values did not significantly differ from normal control (0.11±0.00 mg/g) ($p0>0.05$) except PR of Yar-washagi variety, which showed similar (0.16 ± 0.02 mg/g) value to HFD group.

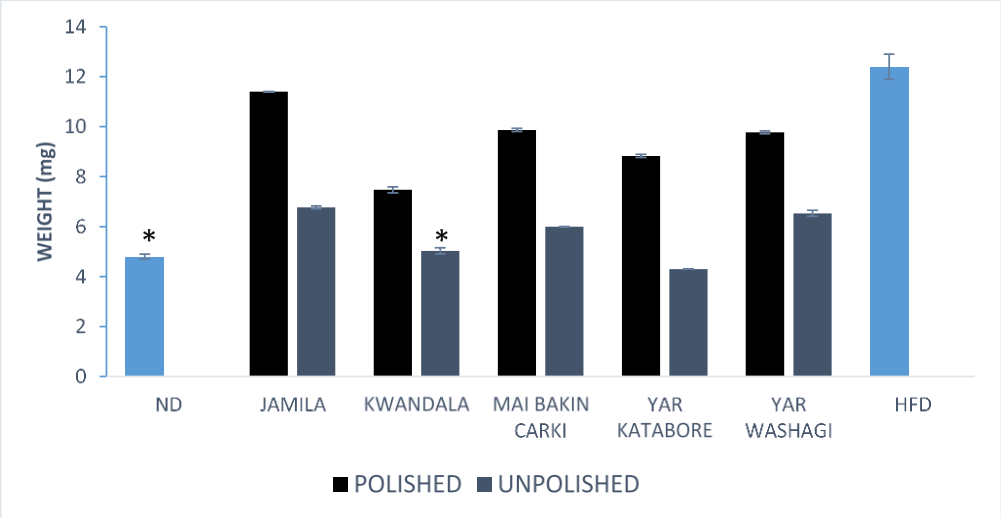

Figure 1. Weight of flies after 7 days of exposure to the intervention diet. Values are reported as mean ± SD of three replicates (n=30). One-way analysis of variance was used to compare the means followed by Tukey-Kramer Multiple Comparisons Test. Values with the same superscripts are not significantly different ($p<0.05$).

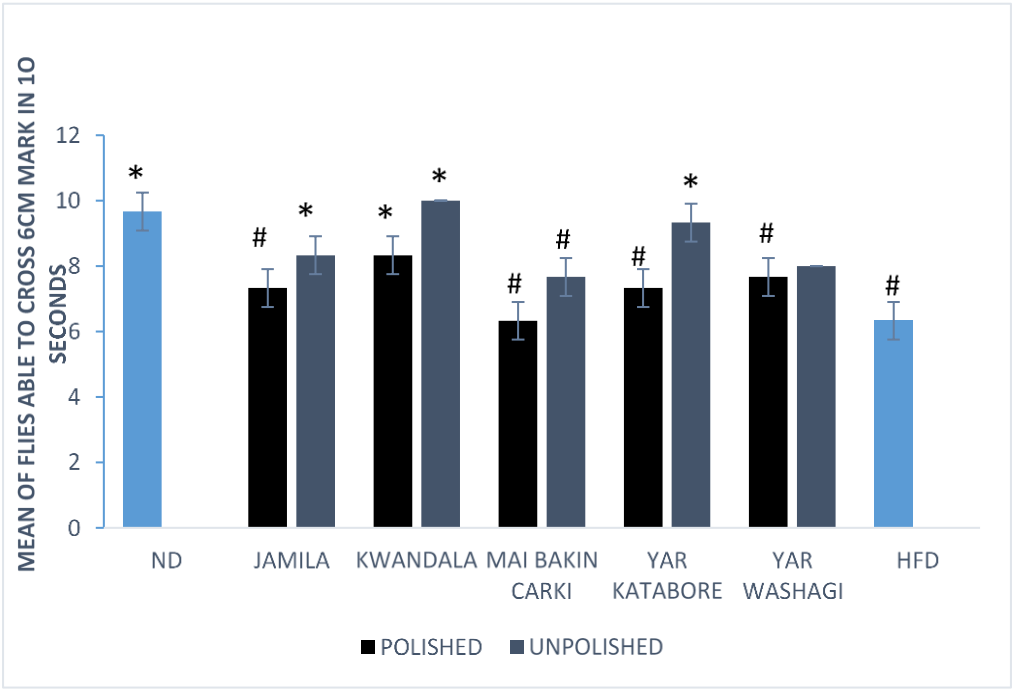

Figure 2. Negative geotaxis of flies after 7 days of exposure to the intervention diet. Values are reported as mean ± SD of three replicates (n=30), One-way analysis of variance was used to compare the means followed by Tukey-Kramer Multiple Comparisons Test. Values with the (*) and (#) are not significantly different with normal diet and HFD respectively ($p<0.05$).

Effects of the consumption of the rice varieties on haemolymph glycogen level

Figure 5 shows haemolymph glycogen levels of flies after consuming the intervention diets for 7 days. As depicted in the Figure 5, flies on UR varieties had glycogen levels that ranged from 0.17 ± 0.01 to 0.29 ± 0.02 mg/g. Flies on UR of Yar-katabore variety did not significantly differ ($p>0.05$)

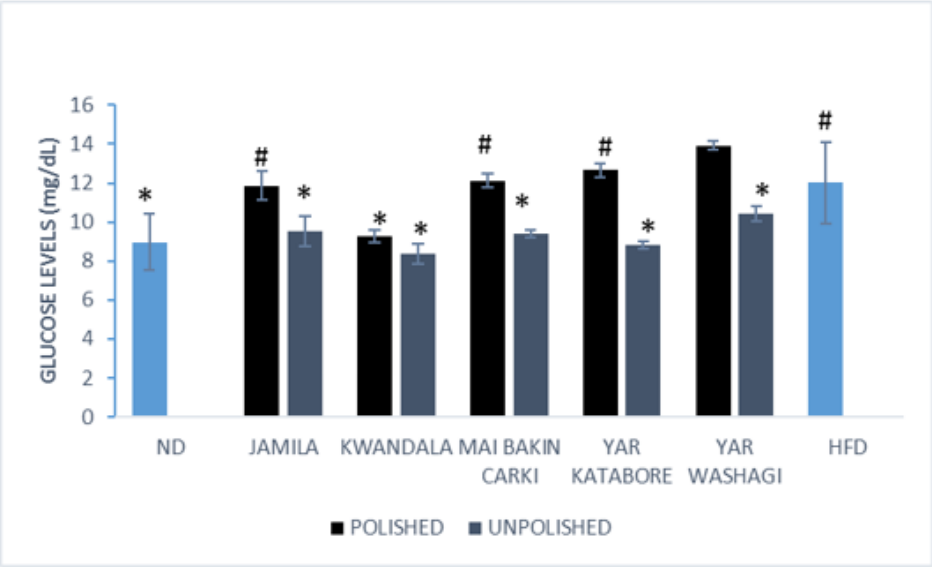

Figure 3. Haemolymph glucose level of flies after 7 days of exposure to the intervention diet. ND= normal diet, HFD= high fat diet. Values are reported as mean ± SD of three replicates. One-way analysis of variance was used to compare the means followed by Tukey-Kramer Multiple Comparisons Test. Values with the (*) and (#) are not significantly different with ND and HFD respectively ($p<0.05$).

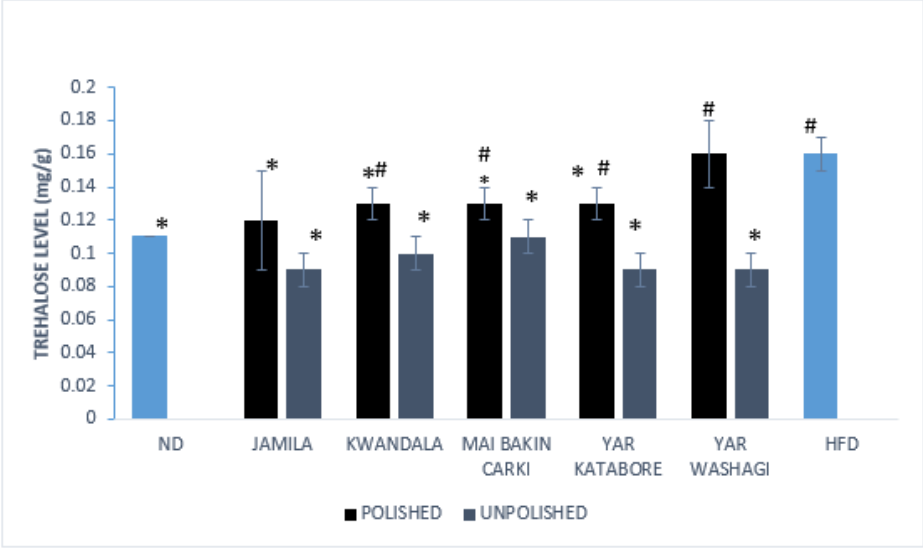

Figure 4. Trehalose level of flies after 7 days of exposure to the intervention diet. ND= normal diet, HFD= high fat diet. Values are reported as mean ± SD of three replicates (n=30). One-way analysis of variance was used to compare the means followed by Tukey-Kramer Multiple Comparisons Test. Values with the (*) and (#) are not significantly different with normal and HFD respectively ($p<0.05$).

from control group while flies on other UR varieties significantly differ from control ($p<0.05$). All flies on UR varieties showed significantly similar glycogen level to HFD group except Kwandala variety, which was significantly higher ($p<0.05$).

Effects of the consumption of the rice varieties on haemolymph triglyceride levels

The effects of the interventions on haemolymph TG levels are shown in Figure 6. All flies on the UR varieties had TG

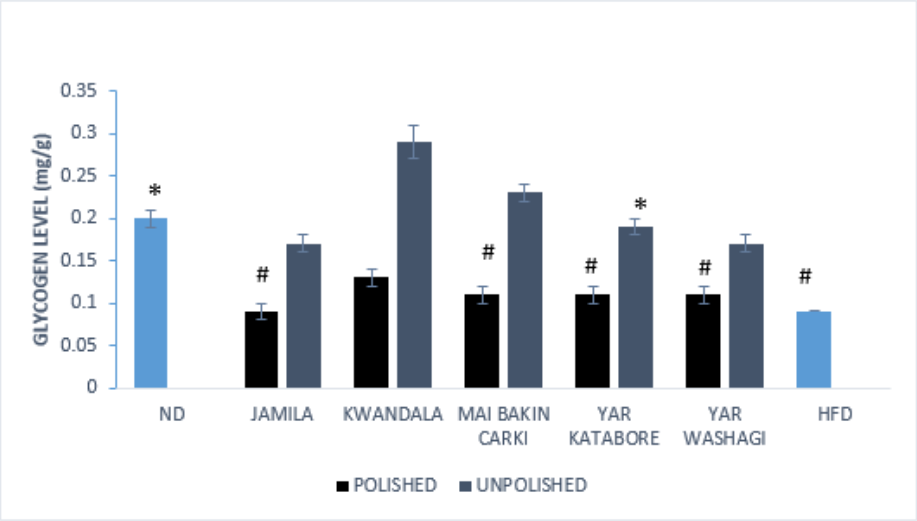

Figure 5. Glycogen level of flies after 7 days of exposure to the intervention diet. ND= normal diet, HFD= high fat diet. Values are reported as mean ± SD of three replicates (n=30). One-way analysis of variance was used to compare the means followed by Tukey-Kramer Multiple Comparisons Test. Values with the (*) and (#) are not significantly different with normal and HFD respectively ($p<0.05$).

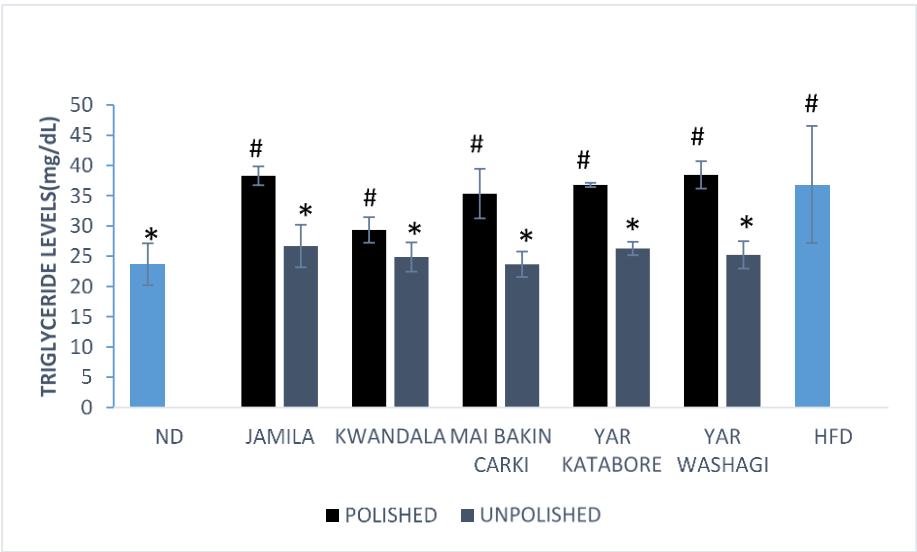

Figure 6. Triglyceride levels of flies after 7 days of exposure to the intervention diet. ND= normal diet, HFD= high fat diet. Values are reported as mean ± SD of three replicates (n=30). One-way analysis of variance was used to compare the means followed by Tukey-Kramer Multiple Comparisons Test. Values with the (*) and (#) are not significantly different with normal and HFD respectively ($p<0.05$).

levels in the range of 23.67 ± 2.12 to 26.67 ± 3.51 mg/dL showing no significant difference ($p>0.05$) with the control group (23.64±3.47 mg/dL). The PR groups on the other hand had TG levels ranging from 29.34 ± 2.09 to 38.43 ± 2.25 mg/dL showing no significant difference ($p>0.05$) from the HFD group (36.85 ± 9.66 mg/dL).

Effects of consumption of the rice varieties on expression of selected genes

Expression of IRS gene: Figure 7 shows the expression levels of IRS gene in flies after consuming the intervention diets for seven days. The PR and control groups showed

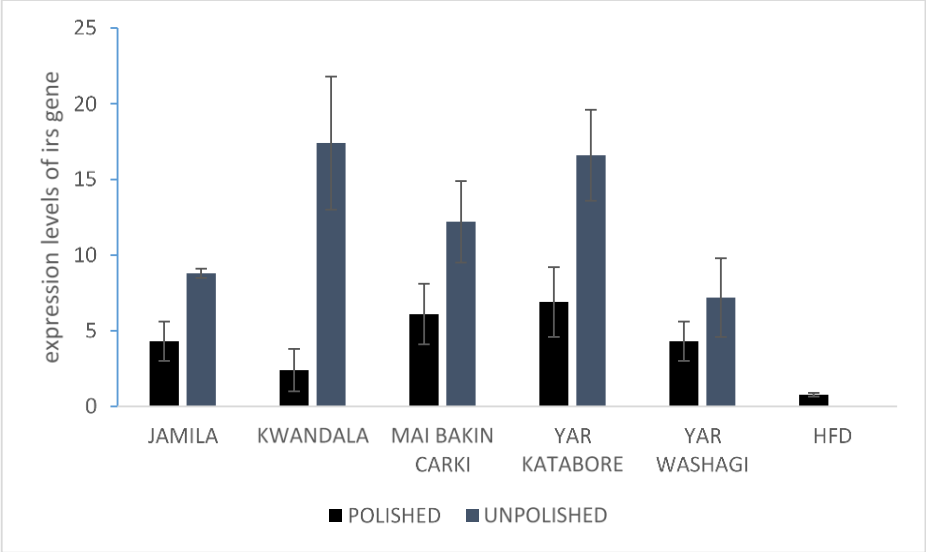

Figure 7. Expression levels of Insulin receptor substrate gene relative to RPL-32 (housekeeping gene) in flies after 7 days of exposure to the intervention diet. HFD= high fat diet. Values are reported as mean ± SD of two replicates (n=20). One-way analysis of variance was used to compare the means followed by Tukey-Kramer Multiple Comparisons Test. Values are significantly different with HFD group ($p<0.05$).

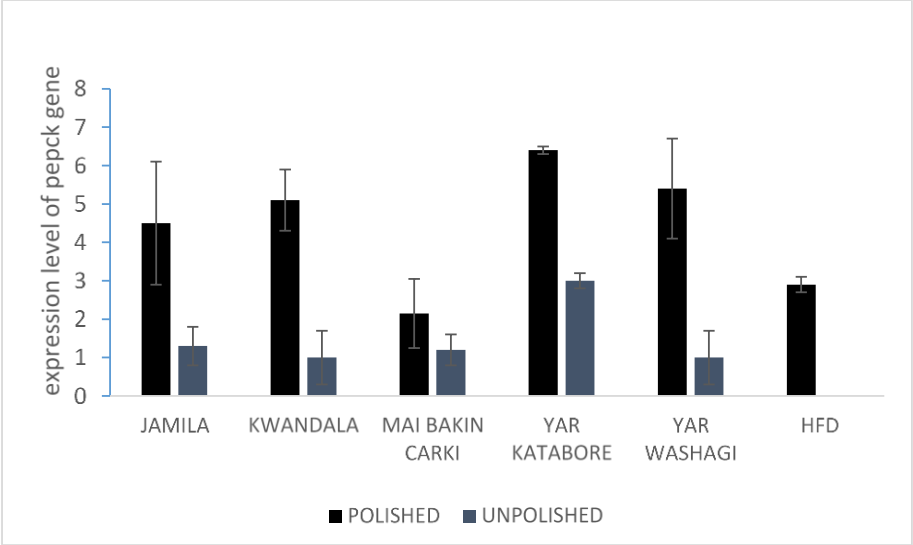

Figure 8: Expression levels of phosphoenolpyruvate carboxykinase gene relative to RPL-32 (housekeeping gene) in flies after 7 days of exposure to the intervention diet. HFD= high fat diet. Values are reported as mean ± SD of two replicates (n=20). One-way analysis of variance was used to compare the means followed by Tukey-Kramer Multiple Comparisons Test. All PR groups are significantly different with their UR counterparts and high fat diet groups ($p<0.05$).

lower expressions of the IRS gene.

Expression of PEPCK gene: The expression levels of PEPCK gene in flies after exposure to the intervention diets is depicted in Figure 8. Flies in the PR groups showed higher expression than the UR groups ($p<0.05$).

Expression of ACC gene: The expression of the *ACC* gene in male and female flies is depicted in Figure 9. Flies in the PR groups showed higher expression than those in the UR groups.

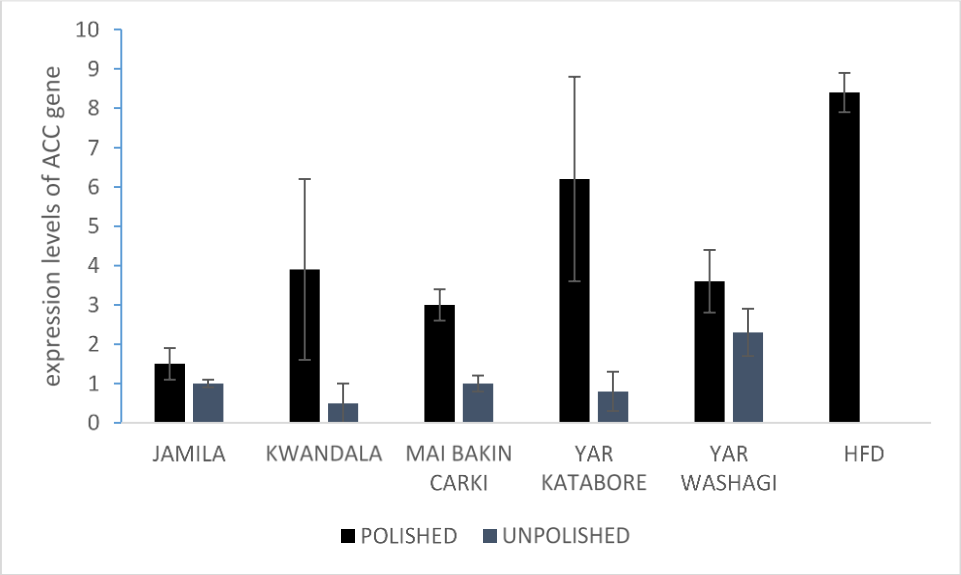

Figure 9. Expression of Acetyl CoA carboxylase gene relative to RPL-32 (housekeeping gene) in flies after 7 days of exposure to the intervention diet. HFD= high fat diet. Values are reported as mean ± SD of two replicates (n=20) One-way analysis of variance was used to compare the means followed by Tukey-Kramer Multiple Comparisons Test. All UR groups are significantly different with their PR counterparts and high fat diet groups respectively ($p<0.05$).

DISCUSSION

Protein is the second major component of rice after starch and forms the basic building blocks for cells and tissues in the body (Mbatchou and Dawda, 2013). It is a major determinant of the nutritional quality of rice (FAO, 2020). Rice protein has a lot of potential as a nutritious, affordable, and widely available protein source. It is considered a novel food ingredient and an effective replacement for existing cereal and animal-based protein sources (Jayaprakash *et al*., 2022). The protein contents of the varieties studied in this work shows that all forms of PR are having lower content than their UR counterparts. The present study showed higher protein contents than that reported by Oko and Ugwu (2011) in a study of different local rice varieties and higher than the values reported previously in some foreign rice varieties (Zubair *et al*., 2015). The protein values for rice recorded in this work compare closely to those reported by Abubakar *et al.* (2017). The higher protein content in UR may be attributed to the presence of bran layer.

All the rice varieties studied had relatively high carbohydrate contents suggesting that all the rice varieties can be considered as good sources of energy. The values obtained are not surprising as rice is one of the well-known dietary sources of carbohydrate. The result of the study showed lower values than the values reported by Edeogu *et al*. (2007) in their work on staple food crops in Ebonyi State (Southern Nigeria), but they fall within the range of values reported from another study on local rice varieties (Oko *et al*., 2012).

The moisture content is an important parameter which plays significant role in determining the shelf life of grains. All the rice varieties have a moisture content within the acceptable range. Grains have the potential to be stored for long term use when they have a moisture content less than 14% which is the optimal value for bag storage of grains (Simonelli *et al.,* 2017). The UR of Jamila variety with the lowest moisture had the highest storage potential than other varieties. This result is in close agreement with the range of values reported previously (Nirmala *et al*., 2015) and lower than the result of Thomas *et al.* (2013) where the moisture was recorded to vary between 10.04 and 12.88%.

The ash content of a food sample gives an idea of the composition of inorganic constituents in the food. Ash contents showed varying results among the different varieties studied. Although the mineral elements are known to be removed during polishing, there were no much differences in the observed result possibly due to several factors like environmental factors and mineral resources in the production area. The rate of fertilizer application and the native fertility of paddy fields have been shown to affect the mineral element levels of rice (Tsujimoto *et al*., 2019). Similarly, the inorganic matter might also be as a result of inorganic materials known to be high due to the abundance of mineral resources in the study area. The presence of fiber in diet aids in digestion, increases the bulk of feces, and improves glucose tolerance. The values of fiber in this study showed that all

forms of UR varieties are significantly higher than their PR counterparts. The values indicated that the varieties studied are a very good sources of fiber. The values reported in this work are higher than those obtained by Rathna Priya *et al*. (2019) although their values fall in the range obtained. Similar range of values were reported by Edeogu *et al*. (2007).

The percentage lipid content obtained in this study falls within similar range with no significant difference ($p>0.05$) across the groups. Thus, the present result falls within the range of values reported previously (Oko *et al*., 2012). The lipid content can also be important in ensuring shelf life of food stuffs because lipid containing foods contain unsaturated fatty acids which make them potential targets of oxidative rancidity (Iwe *et al.,* 2016). Contrary to rice protein, rice fat is found mainly within the aleurone layer of the bran and consists mainly of unsaturated fatty acids (Abubakar *et al*., 2017). Though all the varieties showed interesting results, Mai bakin-carki and Kwandala varieties should get more attentions in terms of proximate compositions because of their superior nutritional values.

To examine whether chronic consumption of the local rice varieties in their polished or unpolished form can induce or prevent phenotypes that were consistent with IR and obesity, the fly experimentation was started by feeding them with the various intervention diets as previously stated in the methodology. Accordingly, weight and locomotor activities were examined, as the hall marks of obesity that strongly correlate with IR. The flies on the PR of Jamila variety had the highest weight while those of the UR of Yar-katabore recorded the least body weight. The observed differences in weight among the PR and UR varieties showed the advantage of UR over PR which is lacking in bioactive compounds. The fiber in UR varieties is known to reduce caloric density, slow ingestion of food nutrients and help in satiety. Thus, the flies in the PR groups might have different appetite than those in the UR groups. The increased weight gain due to consumption of PR is consistent with a previous report that high carbohydrate diets were associated with increased fat content at middle ages (Skorupa *et al*., 2008). There is usually observed different response to diets on weight gain of male and female subjects which is believed to be due to hormonal actions. The sex hormones estrogen, progesterone and androgens are important modulators of food intake and energy balance in mammals. They act by interacting with gastrointestinal peptides and neurotransmitters to achieve central control of appetite and energy expenditure, while also exerting direct peripheral action on adipocytes (Asarian and Geary, 2006). Interactions between glucocorticoids and elevated levels of androgens play a role in the pathophysiology of abdominal obesity and IR in women. In contrast, abdominal obesity in men is associated with lower testosterone levels (Fui *et al*., 2014). Estrogen is known to inhibit food intake, whereas progesterone and testosterone may stimulate appetite. This may explain the increased desire to eat, hunger and appetite scores in males than in females.

The normal movements for drosophila are usually against earth's gravity. The locomotor activity of the flies showed significant differences between the PR and UR groups. However, despite that many flies in the PR groups had a fair number of flies that crossed the 6 cm mark, suggesting superior locomotor ability, the flies in the UR groups reached the target distance more rapidly (4-6 seconds) than their PR group counterparts. The sluggish nature seen in the PR groups may be attributed to their higher weight and symptoms of diabetes like increase in muscle weakness and loss of reflex (Omale *et al*., 2020).

Having seen the effects of PR and UR on weight and locomotion, the study went further to investigate the effects on some biochemical parameters related to carbohydrate and lipid metabolism.

The glucose levels showed that flies on PR varieties had worsened hyperglycaemia similar to those of the HFD group, which is a known model of inducing type 2 diabetes (Heydemann, 2016). The UR varieties on the other hand showed better glucose levels. It can be recalled that the UR varieties are rich in dietary fiber which make them to release sugars slowly thus helping to maintain sugar homeostasis in a sustained manner (Chen *et al*., 2016). Whereas, the PR varieties lacks fiber which make their glucose to be instantly absorbed and transported to circulation making postprandial glucose level to be high. Prolonged hyperglycaemia promote diabetic complications like retinopathy, neuropathy, nephropathy and oxidative stress (OS) and may even promote the development of diabetes (Chang *et al*., 2010). Imam *et al.* (2014) reported PR to worsen glucose tolerance test thus causing hyperglycaemia on the long term. Hyperglycaemia is shown to play a critical role in insulin signaling via the down regulation of INR, IRS-1, PI3K, GLUT-4, AMPK, and GCK, and increasing the expression of GSK and serine/threonine kinase in muscle and liver (Shen et al., 2015). Similarly, PR was shown to modulate MAPK1, MAFA1 and SLC2A2 (Abubakar *et al*., 2020). These changes in gene expression collectively lead to IR.

The effect of the intervention diets on haemolymph trehalose level was also assayed for (Figure 4). All the flies on varieties had trehalose levels that were not significantly different from the normal control ($p>0.05$) except PR of Yar-washagi variety which showed similar values to the HFD group. The PR varieties of Jamila and Kwandala also did not significantly differ from the control groups while other PR varieties had an insignificantly different trehalose level with HFD group ($p>0.05$). This result agreed with Musselman *et al*. (2011) and Ecker et al. (2017) who reported elevation of trehalose in high sugar diet induced IR in drosophila. However, the variation in trehalose level may not necessarily be as a result of IR because trehalose levels responds to a number of environmental conditions like changes in temperature, developmental stage, shock and salinity (Thompson, 2003). Hence, the variation of

trehalose levels may be adaptive and a way of responding to other external stimuli.

The haemolymph glycogen levels revealed that flies on the UR varieties had glycogen levels that are higher than the PR groups. Flies on the UR of Yar-katabore variety did not significantly differ from the control group ($p>0.05$) while flies on other UR varieties significantly differed from the control group ($p<0.05$). All flies on PR varieties showed significantly similar glycogen level to HFD group except the Kwandala variety, which was significantly higher ($p<0.05$). The low glycogen levels in the PR groups suggests depleted energy stores, which is a hall mark of IR. It is known that insulin resistant cells cannot take up glucose and therefore get their source of energy via glycogenolysis which leads to depletion of glycogen store (Honka *et al.,* 2018).

This study also checked for the difference in expression of some genes that are crucial to glucose metabolism viz; PEPCK and IRS-2. It was found that IRS-2 gene is expressed higher in the UR groups than their PR counterpart. IRS is a protein that acts as a scaffold joining INR and downstream processes by binding and activating PI3K which in turn stimulates glucose uptake by translocation of GLUT-4 transporter through a cascaded pathway (Ho et al., 2016). This higher expression of IRS gene suggests that there was insulin signaling defect in the PR groups with resultant accumulation of glucose and trehalose as well as depletion of glycogen stores. This is further supported by the findings on the expression levels of PEPCK gene. PEPCK is an enzyme catalyzing the committed step in gluconeogenesis, a pathway used by organisms to get glucose from non-carbohydrate precursors. From this finding, flies in the PR groups showed higher expression of PEPCK than the UR varieties groups. This may have caused increased glucose synthesis in the PR groups leading to persistent hyperglycaemia. The findings of this work concur with a previous report that UR treatment can significantly downregulate the gene expression level of different proteins including PEPCK gene (Gao et al., 2019). UR also showed inhibition of glucose-6-phosphatase and PEPCK enzyme activities, thus, inhibiting hepatic gluconeogenesis in mice (Gao *et al*., 2019). Although the bioactive properties of individual components of rice are often reported singly to confirm their contribution to the overall bioactivity of rice, it is suggested that phytochemicals such as phenolics and flavonoids, together with vitamins, minerals, and dietary fiber, in whole grains may act synergistically to exert optimum protective effects (Meng *et al*., 2013).

In addition to hyperglycaemia, several other factors such as dyslipidaemia are involved in the development of diabetes-related complication. Thus, this study went further to check the effects of the rice varieties on dyslipidaemia. Accordingly, the study checked for the levels of haemolymph triglyceride and the mRNA expression levels of ACC, a gene that encodes for acetyl-coA carboxylase, a regulatory enzyme for fatty acid synthesis that transforms acetyl-CoA into malonyl-CoA (a precursor for lipogenesis). The results showed that PR groups had higher levels of TG than the UR groups. High TG levels is a hallmark of obesity and IR. TGs are normally acted upon by lipoprotein lipase to allow fatty acids to be taken from circulating TGs for storage in adipocytes. However, lipoprotein lipase requires insulin, and therefore in the settings of IR, hypertriglyceridaemia results which is a great risk factor for the development of obesity, a condition associated with lots of other disorders including type2 diabetes and OS. The observed high TG levels in this study due to PR consumption may be the reason for the higher weights observed in those groups. Such increase in TG levels was also reported in flies fed with high sugar diet in another study (Musselman *et al*., 2011). In line with this finding, an increased ACC expression in flies fed with the PR varieties was observed. Moreover, the expression of this gene will lead to transcription of the enzyme with resultant increase in synthesis of fatty acids without β-oxidation (Kim, 1997). Suppression of ACC2 activity in mice induced β-oxidation and was shown to reverse hepatic IR (Abu-Elheiga *et al*., 2003).

Conclusion

In aggregate, the findings of this study showed that consumption of PR increases hyperglycaemia and dyslipidaemia. The study also showed that these effects can be ameliorated by the consumption of UR. Therefore, it can be concluded from this study that substitution of the widely consumed foreign PR with locally produced UR may reduce the risk of obesity, type 2 diabetes and their related complications.

CONFLICT OF INTEREST

The authors declare that they have not conflict of interest.

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