ORGINAL ARTICLE

Modulation of Some Insulin Signaling Genes Due to Prenatal Rice Consumption

Bilyaminu Abubakar[1,2*], Norsharina Ismail[1], Md Zuki Abubakar[1], Maznah Ismail[1]

1. Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.
2. Department of Pharmacology and Toxicology, Usmanu Danfodiyo University Sokoto, Nigeria.

***Correspondence:**
Bilyaminu Abubakar, Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.
Tel: (234) 803 064 8935
Email: abu3163abu@yahoo.co.uk

Received: 27 September 2019

Accepted: 08 January 2020

Published in April 2020

Abstract

Objective: A clinically observable metabolic disorder often takes its root from modulation of transcriptional factors which in turn are responsible for perturbed protein expressions and their sequelae. Perinatal perturbations due to chronic prenatal exposure to a certain type of rice could predispose parents exposed to such 'insult' and their subsequent offsprings to metabolic diseases.

Materials and Methods: We investigated the effect of chronic prenatal exposure to different types of rice (in context of a balanced normal diet and a high-fat diet) on some insulin signaling genes using nulliparous Sprague Dawley rats. The rats were exposed to various predetermined rice diets for 90 days. After returning them to standard chow, they were mated with male rats raised on standard chow. The resulting pups (F1) and dams were sacrificed and their tissues were examined for modulation of genes related to insulin signaling.

Results: Our results show that dams fed with white rice in context of standard diet modulated *MAPK*1*, MAFA*l and *SLC2A2.* Also, germinated brown rice prevented dysregulation of *MAPK1,* and *SLC2A2* in both dams and pups exposed to this diet in the context of a high-fat diet. In general, germinated brown rice retarded dysregulations due to high-fat diet exposure while white rice enhanced the dysregulatory effects of high-fat diet.

Conclusion: We conclude that chronic prenatal exposure to a certain type of rice, could be a factor to modulation of some genes related to insulin signaling pathways and that these modulation could be inherited by at least one generation of offsprings.

Keywords: Germinated brown rice, Inheritance, Insulin signaling

Introduction

Perinatal environment could repress or induce genes that encodes relevant proteins (1,2). These modifications, which could be due to epigenetic signatures (mostly DNA methylation) could be inherited by offsprings of parents exposed to a candidate insult (3). Depending on its germination status and amylose content, consumption of rice as a staple could modify gene expression in individuals and their offsprings (4,5). These modifications usually correlate with metabolic disorders which includes obesity, insulin

[DOI: 10.18502/ijdo.v11i4.2880]

resistance and their sequelae. In recent past, some metabolic diseases are reaching pandemic proportions and have been partly linked with diet (6,7). Since rice is staple food and its consumption have been strongly correlated with several metabolic diseases (8), an understanding of the modifications in some genes that encodes relevant metabolic proteins could partly elaborate the mechanisms by which these metabolic diseases ensue. The aim of the study is to investigate the effect of chronic prenatal exposure to different types of rice (in context of a balanced normal diet and a high-fat diet) on some insulin signaling genes using nulliparous Sprague Dawley rats and their offsprings. This study could also provide insight into the theory of intergenerational inheritance of metabolic diseases.

Materials and Methods

White and brown rice from two commercially available Malaysian rice (MRQ 76 and MRQ 74 cultivars) were obtained from the Malaysian Agricultural Research and Development Institute (MARDI). As described previously (9), the brown rice form of each cultivar was germinated to obtain a corresponding germinated brown rice (GBR).

Animal handling

Forty-eight nulliparous Sprague-Dawley rats weighing 90-110 g and of related genealogy were purchased from the animal house facility of the Universiti Putra Malaysia, Serdang, Malaysia. The animals were housed in a well-ventilated room of approximate 12/12 h dark/light cycle. The surrounding temperature was maintained between 25-30 ˚C and the animals were kept in pairs. Approval for the use of the animals was sought from the Institutional Animal Care and Use Committee (IACUC) of the University Putra Malaysia (project approval number: UPM/IACUC/AUP-R017/2016); guidelines for the management of the animals was adhered to as specified in the committee guidelines. These guidelines meet the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Animals were acclimatised to the new environment for seven days on standard chow ad libitum and free access to water prior to the commencement of experiment. The standard rat chow (Gold Coin, Port Klang, Malaysia) consisted of 50% carbohydrate, 21% protein, 3% fat, 13% moisture, 8% ash and 5% fiber while the high-fat diet consisted of a mixture of 50% of the standard rat chow, 24% ghee, 20% full-cream milk and 5% starch (10). The rice cultivars' proximate analyses has been described previously (9).

Interventions and anthropometric measurement

The animals were divided into eight different groups of six rats per group. Experimental diets were assigned to each group (Table 1). The pre-assigned experimental diets (with or without acarbose (60 mg/kg/day) as the case may be) and water ad libitum were fed to the rats for 90 days. On completion of the 90-day

Table 1. Diet interventions

S/no	Diet	Protein (g/100 g)	Carbohydrate (g/100 g)	Fat (g/100 g)	Average Total calorie per (kcal/100 g)
1	Standard chow (SC)	21.0	62.0	3.0	359
2	SC of which 50% is substituted with HAGBR	15.8	67.2	3.0	359
3	SC of which 50% is substituted with LAWR	14.4	68.9	3.3	363
4	SC of which 50% is substituted with LAWR + AC	14.4	68.9	3.3	363
5	High fat diet (HFD)	22.5	46.6	30.6	552
6	HFD of which 50% of the pellet is substituted with LAWR	17.3	50.0	30.8	546
7	HFD of which 50% of the pellet is substituted with HAGBR	18.0	49.2	30.6	544
8	HFD of which 50% of the pellet is substituted with LAWR +AC	17.3	50.0	30.8	546

AC: acarbose (60 mg/kg); GBR: germinated brown rice; HAGBR: high amylose germinated brown rice; HAWR: high amylose white rice; HFD: high-fat diet; LAGBR: low amylose germinated brown rice; LAWR: low amylose white rice; SC: standard chow.

[DOI: 10.18502/ijdo.v11i4.2880]

treatment, the rats were reverted to standard chow and mated with male rats raised on normal pellet. On conception and delivery of their pups, each dam was housed in a separate cage. Three weeks post weaning, the pups and dams were fasted for 12 h and sacrificed under xylazine and ketamine anesthesia (10 mg/kg and 100 mg/kg, respectively). The liver, skeletal muscle and adipose tissues were harvested and rinsed with ice-cold normal saline before transferring into RCl_2 and stored in a refrigerator at -80 ˚C for further analyses.

Extraction of ribonucleic acid

Ribonucleic acid (RNA) isolation from the liver, skeletal muscle and adipose tissue of rats was accomplished using GF-TR-100 RNA Isolation Kit (RBC Bioscience Corporation, Taiwan), according to the manufacturer's protocol. During the extraction of RNA from adipose tissue, the lysis buffer of the extraction kit was substituted with TRIzol (Invitrogen, Scotland, UK) to maximise the RNA isolation process. The purified RNA was eluted with RNase-free water. RNA concentration was then determined by a Nano Drop spectrophotometer (Thermo Scientific Nanodrop, Nano Drop Technologies, Wilmington, DE, USA). Ratios of A260/230 and A260/280 were used to indicate the urity of the extracted total RNA. A minimum benchmark ratio of 2.0 for both indicators was deemed pure for the eluted RNA.

Primer design

Genome Lab eXpress Profiler software was employed in designing the primers using Rattus norvegicus sequence adopted from the National Center for Biotechnology Information Gen Bank Database (http://www.ncbi.nlm.nih.gov/nucleotide/).

The genes of interest were *SOD1, SOD2, TNF, GCK, APOB, ADIPOQ, GPX1, PRKCZ, MAPK1, MAFA, KCNJ11, SLC2A2, INSR, IRS1,IRS2*, an internal control (KanR) and housekeeping genes (Table 2). The reverse (right) and forward (left) primers had universal sequences (tags) in addition to nucleotides that are complementary to the target genes. Primers were provided by First Base Ltd. (Selangor, Malaysia) and diluted in 1× Tris-EDTA buffer to a concentration of 200 nM for forward primers and 500 nM for reverse primers.

Reverse transcription and polymerase

Table 2. Gene name, accession number and primer sequences

Gene	Primers sequence with universal tag	
	Reverse primer	Forward primer
IRS2 **[NM_001168633]**	GTACGACTCACTATAGGGAGCAG CACTTTACTCTTTCAC	AGGTGACACTATAGAATAAGGCACTGGAGCC TTAC
SLC2A2 **[NM_012879]**	GTACGACTCACTATAGGGAGACT TTCCTTTGGTTTCTG	AGGTGACACTATAGAATACAGTACATTGCGG ACTTC
KCNJ11 **[NM_031358]**	GTACGACTCACTATAGGGAGAAC TTTCCAATATTTCTTTT	AGGTGACACTATAGAATACTACTTCAGGCAA AACTCTG
INSR **[NM_017071]**	GTACGACTCACTATAGGGAAAGG GATCTTCGCTTT	AGGTGACACTATAGAATAAGCTGGAGGAGTC TTCAT
IRS1 **[NM_012969]**	GTACGACTCACTATAGGGAAGTA AACAAACTGTAAGGGATG	AGGTGACACTATAGAATACACAGGCAGAATG AAAGAC
MAFA **[XM_345846]**	GTACGACTCACTATAGGGAATAC CCGCTCATCCAGTA	AGGTGACACTATAGAATACTTCGCCCAGCTT CT
MAPK1 **[NM_053842]**	GTACGACTCACTATAGGGAAACT CTCTGGACTGAAGAAT	AGGTGACACTATAGAATACATTTTTGAAGAG ACTGCTC
SOD1 **[NM_017050]**	GTACGACTCACTATAGGGATCCA ACATGCCTCTCT	AGGTGACACTATAGAATAATATGGGGACAAT ACACAA
SOD2 **[NM_017051]**	GTACGACTCACTATAGGGAAACT CTCCTTTGGGTTCT	AGGTGACACTATAGAATACAGGTTGCTCTTC AGC
RPL13A **[NM_173340]*[a]**	GTACGACTCACTATAGGGAATTT TCTTCTCCACATTCTT	AGGTGACACTATAGAATAATGGGATCCCTCC AC
***KAN-R*[b]**		

[a]House keeping genes. [b]internal control. Normalisation gene. *Kan-R*: Kanamycin resistant, *RPL13A*: Ribosomal Protein L13a, *SOD1*: Superoxide dismutase 1, *SOD2*: Superoxide dismutase 2, *INSR*: Insulin receptor, *IRS1*: Insulin receptor substrate 1, *IRS2*: Insulin receptor substrate 2, *KCNJ11*: Potassium voltage-gated channel subfamily J member 11, *MAFA*: MAF BZIP Transcription factor A, *MAPK1*: Mitogen-activated protein kinase 1.

[DOI: 10.18502/ijdo.v11i4.2880]

chain reaction

Reverse transcription (RT) reaction and multiplex polymerase chain reaction (PCR) of RNA samples (50 ng/mL) were carried out in XP Thermal Cycler (BIOER Technology, Hangzhou, China) according to the protocol of GenomeLab™ GeXP Start Kit (Beckman Coulter, Inc., Miami, FL, USA). Briefly, RT reaction mixture was prepared using RNA sample (1 mL each), 4 mL of 5X RT buffer, 2 mL of RT reverse primers, 1 mL of KanR,1 mL of reverse transcriptase and 11 mL of DNAse/RNase-free water. cDNA was synthesized according to the reaction protocol: 48˚C for 1 min, 42˚C for 60 min, 95˚C for 5 min and 4˚C hold. Then, 9.3 mL of each RT product was mixed with 10.7 mL of PCR reaction mixture consisting of 5X PCR buffer, 25 mM $MgCl_2$, PCR Forward Primer Plex, and Thermo-Start DNA polymerase. Amplification conditions were 95˚C for 10 min followed by 34 cycles of 94˚C for 30 s, 55˚C for 30 s, 70˚C for 1 min and 4˚C hold.

Gene expression data analysis

The PCR products (1 mL each) were mixed with 38.5 mL of sample loading solution and 0.5 mL of DNA size standard 400 (Beckman Coulter, Inc., Miami, FL, USA) in a 96-well sample loading plate and analyzed in the GeXP machine (Beckman Coulter, Inc., Miami, FL, USA). The results from the machine were analyzed using the fragment analysis module of the GeXP system software and then imported into the analysis module of eXpress Profiler software. Normalisation of the expressed genes was accomplished with *RPL13A*.

Statistical analysis

Results were represented as mean ± standard deviation (SD). Statistical analysis was carried out using Minitab 17. One-way analysis of variance (ANOVA) was used. The significance level was set at P-value< 0.05. Values are represented as figures.

Ethical considerations

Approval for the use of the animals was sought from the Institutional Animal Care and Use Committee (IACUC) of the Universiti Putra Malaysia (project approval number: UPM/IACUC/AUP- R017/2016); guidelines for the management of the animals was adhered to as specified in the committee guidelines. These guidelines meet the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Results

Liver tissue

Dams fed with SC-based LAWR down-regulated *MAFA1* expressions on the one hand while they up-regulated *MAPK1* and *SLC2A2* on the other hand. Compared to their counterparts, the pups of rats fed with SC-based LAWR also up-regulated *SLC2A2* (Table 3a). The expressions of *IRS2, INSR* and *KCNJ11* were generally unperturbed by the SC-based interventions (Table 3a). Except for *PRKCZ,* the dams and pups treated with acarbose demonstrated similar mRNA expressions.

In the HFD-based interventions, HAGBR diet prevented HFD-induced up-regulation of *MAPK1,* and *SLC2A2* in the dams and pups fed with this diet. The HFD-based interventions did not modulate *KCNJ11*, *IRS2* and *INSR* (Table 3b).

Muscle tissue

In the muscle tissue, only dams and pups fed with SC-based LAWR up-regulated *IRS2* and *INSR*. *KCNJ11* was equally expressed among the groups fed with SC-based interventions (Table 4a). In overall, except for the group fed with SC-based LAWR, all other dams and pups within same group showed no difference in expression of the genes (Table 4a).

IRS2 down-regulation was prevented in groups fed with HAGBR diet and those treated with acarbose. The LAWR diet-fed rats did not retard HFD-induced *IRS2* down-regulation (Table 4b). The HFD interventions did not modulate *KCJN11* and *INSR*.

[DOI: 10.18502/ijdo.v11i4.2880]

Adipose tissue

The expressions of *SOD2, INSR* and *IRS1* were not modulated by rats fed with the SC-based interventions (Table 5a). However, *SOD1* was slightly down-regulated by dams fed with LAWR diet (Table 5a). HABGR diet also prevented down-regulation of *SOD1* and *SOD2*. *INSR* and *IRS1* were slightly up-regulated by the absolute HFD and LAWR interventions. In overall, the pups either expressed an equal or higher amount of the analyzed mRNA (Table 5b).

Discussion

We investigated modulations of 10 glucose-related genes in rats exposed to white and germinated brown rice. Two scenarios were depicted in this study. The standard chow diet-related interventions depicted modulation of some genes in individuals whose parents had prenatal exposure to white or germinated brown rice in the context of a standard balanced diet (standard chow diet). The high-fat diet-related interventions depicted modulation of some genes in individuals whose parents had prenatal exposure to white or germinated brown rice in the context of a diet designed to alter normal metabolism (high fat diet). Since all the varying diet interventions ended before conception (prenatal exposure), all observed modulations of gene in pups are due to prenatal perturbations from the varying diet interventions (generational inheritance).

Chronic consumption of a carbohydrate major diet (low amylose white rice) had no intergenerational effect on the pups of rats exposed to this diet. The insulin resistance

Table 3A. Regulation of expression of selected glucose handling genes in liver of dams and pups treated with standard chow-based interventions

Genes Intervention	*PRKCZ*	*MAPK1*	*MAFA*	*KCNJ11*	*SLC2A2*	*INSR*	*IRS2*
SC dams	1.00 (± 0.16)^{ae}	1.00 (± 0.12)^{a}	1.00 (±0.15)^{a}	1.00 (±0.14)^{a}	1.00 (± 0.03)^{a}	1.00 (± 0.15)^{a}	1.00 ± (0.11)^{a}
SC pups	0.26 (± 0.02)^{b}	0.94 (± 0.01)^{a}	1.29 (±0.18)^{ab}	1.26 (±0.15)^{b}	1.07 (± 0.18)^{a}	1.02 ± (0.10)^{a}	0.99 ± (0.02)^{a}
SC + HAGBR dams	0.85 (± 0.22)^{e}	1.04 (± 0.12)^{a}	1.80 (±0.39)^{b}	0.98 (±0.12)^{a}	1.13 (± 0.29)^{a}	1.14 ± (0.10)^{a}	0.89 ± (0.12)^{a}
SC + HAGBR pups	1.13 (± 0.12)^{ac}	1.07 (± 0.08)^{a}	1.28 (±0.02)^{ab}	0.82 (±0.11)^{a}	1.14 (± 0.16)^{a}	1.02 ± (0.10)^{a}	0.95 ± (0.01)^{a}
SC + LAWR dams	1.28 (± 0.05)^{c}	5.53 (± 0.74)^{b}	0.09 (±0.00)^{c}	1.51 (±0.22)^{c}	5.91 (± 1.09)^{b}	1.72 ± (0.01)^{b}	0.69 ± (0.01)^{b}
SC + LAWR pups	0.43 (±0.030^{bd}	1.00 (± 0.02)^{a}	0.67 (±0.15)^{ac}	1.10 (±0.10)^{ab}	2.17 (± 0.40)^{c}	0.38 ± (0.03)^{c}	0.70 ± (0.03)^{b}
SC + LAWR dams + acarbose	1.29 (± 0.01)^{c}	1.00 (± 0.03)^{a}	1.25 (±0.31)^{ab}	0.87 (±0.03)^{a}	0.95 (± 0.07)^{a}	0.98 ± (0.01)^{a}	0.87 ± (0.01)^{a}
SC + LAWR pups + acarbose	0.36 (± 0.00)^{bf}	0.90 (± 0.03)^{a}	1.74 (±0.83)^{b}	0.95 (±0.04)^{a}	1.19 (± 0.12)^{a}	0.93 ± (0.01)^{a}	0.87 ± (0.04)^{a}

Data are in fold changes of mRNA expression and are relative to each column's respective control dams. HAGBR: high amylose germinated brown rice; HFD: high-fat diet; LAWR: low amylose white rice; SC: standard chow. Mean values within a column superscripted by the same letter are not significantly different at *P*-value < 0.050.

Table 3B. Regulation of expression of selected glucose handling genes in liver of dams and pups treated with high-fat diet-based interventions.

GENES INTERVENTION	*PRKCZ*	*MAPK1*	*MAFA*	*KCNJ11*	*SLC2A2*	*INSR*	*IRS2*
HFD dams	1.00 (± 0.02)^{a}	1.00 (± 0.13)^{a}	1.00 (± 0.20)^{a}	1.00 (± 0.03)^{a}	1.00 (± 0.18)^{a}	1.00 (± 0.05)^{a}	1.00 (± 0.29)^{a}
HFD pups	2.83 (± 0.11)^{d}	0.90 (± 0.08)^{a}	0.05 (± 0.00)^{b}	0.81 (± 0.13)^{a}	1.17 (± 0.11)^{a}	0.94 (± 0.08)^{a}	1.30 (± 0.15)^{a}
HFD + HAGBR dams	0.77 (± 0.02)^{a}	0.29 (± 0.02)^{b}	2.86 (± 0.13)^{e}	0.72 (± 0.12)^{a}	0.28 (± 0.01)^{b}	0.96 (± 0.10)^{a}	1.66 (± 0.19)^{a}
HFD + HAGBR pups	1.14 (± 0.02)^{a}	0.34 (± 0.03)^{b}	3.49 (±1.05)^{ce}	0.72 (± 0.12)^{a}	0.27 (± 0.02)^{b}	0.91 (± 0.03)^{a}	1.28 (± 0.20)^{a}
HFD + LAWR dams	1.61 (± 0.04)^{b}	1.35 (± 0.12)^{a}	1.48 (± 0.64)^{a}	0.71 (± 0.12)^{a}	0.88 (± 0.09)^{a}	1.36 (± 0.09)^{b}	1.83 (± 0.13)^{a}
HFD + LAWR pups	0.51 (± 0.23)^{c}	1.06 (± 0.09)^{a}	3.28 (± 1.03)^{c}	0.78 (± 0.09)^{a}	0.94 (± 0.08)^{a}	1.35 (± 0.07)^{b}	1.56 (± 0.15)^{a}
HFD + LAWR dams + acarbose	0.58 (± 0.23)^{c}	0.74 (± 0.08)^{c}	3.17 (± 0.94)^{c}	0.73 (± 0.15)^{a}	0.87 (± 0.07)^{a}	0.84 (± 0.11)^{a}	1.66 (± 0.22)^{a}
HFD + LAWR pups + acarbose	0.76 (± 0.13)^{a}	0.70 (± 0.09)^{c}	4.39 (±0.92)^{cd}	0.73 (± 0.14)^{a}	0.41 (± 0.29)^{ab}	0.68 (± 0.12)^{a}	1.26 (± 0.25)^{a}

Data are in fold changes of mRNA expression and are relative to each column's respective control dams. HAGBR: high amylose germinated brown rice; HFD: high-fat diet; LAWR: low amylose white rice; SC: standard chow. Mean values within a column superscripted by the same letter are not significantly different at *P*-value < 0.050.

[DOI: 10.18502/ijdo.v11i4.2880]

observed at biochemical levels in our previous study (5) are probably due to *MAPK*1 and *MAF*A modulations in this study. The inability of insulin to provoke glucose uptake and utilization when available at target tissues is a hallmark of insulin resistance. The proteins responsible for insulin receptor substrate 2 synthesis, insulin receptor synthesis and synthesis of ATP-sensitive potassium channels in the pancreatic beta cells, are partly regulated by *IRS2*, *INSR* and *KCJN11* respectively (11,12). All of the aforementioned genes were not regulated by any of the HFD-based or SC-based interventions (Table 3). This gives an insight of possible locations where insulin transduction defection could have occurred. This is at the post-insulin receptor substrate level since the unmodulated genes in question (*IRS2*, *INSR* and *KCJN11*) influence insulin signaling either before or at the insulin receptor substrate level. The patterns of expression of these genes are in tandem with our previously obtained metabolomics (insulin resistance status, adipocytokines and lipid profile) results (5). This gives further credence to the culpability of at least an additional effect between HFD and carbohydrate major diet (low amylose white rice) in the progression of individuals to insulin resistance. Cytoplasmic and mitochondrial free radical production are cellular effects of chronic postprandial glucose overload. Oxidative stress sets in when cellular compensatory mechanisms are outweighed. This is usually evident as over expression of mRNAs associated with the proteins in question (13). In adipose tissue (Table 5), prevention of *SOD1* and *SOD2* down-regulation by HFD-based HAGBR diet indicates the absence of oxidative stress– a precursor to insulin resistance - in adipose tissue. *INSR* and *IRS1* were unmodulated in all the tissues analyzed. This further supports our assertion that the defection in insulin signaling transduction could have occurred post insulin receptor substrate level. This study further corroborates several previous studies that have demonstrated that white rice as against germinated brown rice predisposes individuals to metabolic diseases (14,15).

In overall, prenatal perturbations (in forms of dysregulation of glucose related genes) due to exposure to varying rice interventions in the context of a standard balanced diet and a high fat diet could be inherited by the next generation of offsprings. Also, the combined effects of daily postprandial glucose overload and a high fat diet in predisposition to dysregulation of some glucose-related genes is greater than their individual effects. Finally, acarbose retarded dysregulation of some of the studied genes probably through prevention of daily postprandial glucose overload due to daily white rice consumption.

This phenomenon further reiterates the assertion that there is an addition effect between high-fat diet and chronic postprandial glucose overload in predisposition to insulin resistance as demonstrated in metabolomics terms in our previous study (5).

Conclusions

We conclude from the above results that chronic prenatal exposure to a type of rice, could be a factor to modulation of genes related to insulin signaling pathways and that these modulations could be inherited by at least one generation of offsprings.

Acknowledgements

The authours sincerely acknowledge the contributions of Nor Asma Abdulrazak and Kim Wei Chan in the completion of this work. The authors wish to also express their gratitude towards Padi Beras Nasional Berhad (BERNAS) and Universiti Putra Malaysia (UPM) for sponsoring this research. The authors have no conflicts of interest to declare.

[DOI: 10.18502/ijdo.v11i4.2880]

Table 4A. Regulation of expression of irs2, kcjn11 and insr genes in muscle tissues of dams and pups treated with standard chow-based interventions

GENES INTERVENTION	IRS2	KCJN11	INSR
SC dams	1.00 (± 0.09)a	1.00 (± 0.06)a	1.00 (± 0.06)a
SC pups	0.95 (± 0.10)a	1.03 (± 0.04)a	0.87 (± 0.22)ac
SC + HAGBR dams	0.99 (± 0.08)a	0.94 (± 0.16)a	0.93 (± 0.11)a
SC + HAGBR pups	1.02 (± 0.14)a	1.05 (± 0.03)a	1.10 (± 0.02)a
SC + LAWR dams	2.62 (± 0.25)b	1.01 (± 0.15)a	1.69 (± 0.22)b
SC + LAWR pups	1.75 (± 0.02)c	1.21 (± 0.13)a	1.51 (± 0.43)b
SC + LAWR dams + acarbose	0.93 (± 0.04)a	1.26 (± 0.17)a	1.02 (± 0.06)a
SC + LAWR pups + acarbose	1.00 (± 0.08)a	1.29 (± 0.09)a	1.26 (± 0.08)c

Data are in fold changes of mRNA expression and are relative to each column's respective control dams. HAGBR: high amylose germinated brown rice; HFD: high-fat diet; LAWR: low amylose white rice; SC: standard chow. Mean values within a column superscripted by the same letter are not significantly different at *P*-value < 0.050.

Table 4B. Regulation of expression of irs2, kcjn11 and insr genes in muscle tissues of dams and pups treated with high-fat diet-based interventions

GENES INTERVENTION	*IRS2*	*KCJN11*	*INSR*
HFD dams	1.00 (± 0.12)^{a}	1.00 (± 0.26)^{a}	1.00 (± 0.09)^{a}
HFD pups	0.81 (± 0.09)^{ac}	0.83 (± 0.12)^{a}	1.01 (± 0.09)^{a}
HFD + HAGBR dams	1.85 (± 0.11)^{be}	0.82 (± 0.13)^{a}	1.01 (± 0.06)^{a}
HFD + HAGBR pups	1.70 (± 0.08)^{b}	0.74 (± 0.23)^{a}	1.02 (± 0.08)^{a}
HFD + LAWR dams	0.58 (± 0.06)^{c}	0.80 (± 0.11)^{a}	0.96 (± 0.04)^{a}
HFD + LAWR pups	0.49 (± 0.23)^{c}	0.81 (± 0.10)^{a}	0.95 (± 0.040^{a}
HFD + LAWR dams + acarbose	2.49 (± 0.52)^{d}	0.57 (± 0.20)^{a}	0.91 (± 0.12)^{a}
HFD + LAWR pups + acarbose	1.45 (± 0.25)^{e}	0.77 (± 0.25)^{a}	1.07 (± 0.06)^{a}

Data are in fold changes of mRNA expression and are relative to each column's respective control dams. HAGBR: high amylose germinated brown rice; HFD: high-fat diet; LAWR: low amylose white rice; SC: standard chow. Mean values within a column superscripted by the same letter are not significantly different at *P*-value < 0.050.

Table 5A: Regulation of expression of sod1, sod2, insr and irs1 genes in adipose tissues of dams and pups treated with standard chow-based interventions

GENES INTERVENTION	*SOD1*	*SOD2*	*INSR*	*IRS1*
SC dams	1.00 (± 0.06)^{a}	1.00 (± 0.18)^{a}	1.00 (± 0.12)^{ab}	1.00 (± 0.12)^{a}
SC pups	0.99 (± 0.08)^{a}	1.04 (± 0.15)^{a}	0.86 (± 0.15)^{a}	0.88 (± 0.13)^{a}
SC + HAGBR dams	0.95 (± 0.08)^{ac}	1.01 (± 0.17)^{a}	0.83 (± 0.15)^{a}	0.98 (± 0.12)^{a}
SC + HAGBR pups	0.80 (± 0.11)^{ad}	0.99 (± 0.18)^{a}	0.89 (± 0.14)^{ab}	1.01 (± 0.18)^{a}
SC + LAWR dams	0.65 (± 0.03)^{b}	0.90 (± 0.16)^{a}	1.16 (± 0.18)^{bc}	1.20 (± 0.14)^{a}
SC + LAWR pups	0.73 (± 0.27)^{bcd}	1.11 (± 0.19)^{a}	0.94 (± 0.18)^{ac}	1.14 (± 0.14)^{a}
SC + LAWR dams + acarbose	0.87 (± 0.04)^{ad}	1.04 (± 0.14)^{a}	0.88 (± 0.17)^{ac}	1.01 (± 0.21)^{a}
SC + LAWR pups + acarbose	0.90 (± 0.03)^{ab}	1.16 (± 0.17)^{a}	0.87 (± 0.11)^{a}	1.03 (± 0.16)^{a}

Data are in fold changes of mRNA expression and are relative to each column's respective control dams. HAGBR: high amylose germinated brown rice; HFD: high-fat diet; LAWR: low amylose white rice; SC: standard chow. Mean values within a column superscripted by the same letter are not significantly different at *P*-value < 0.050.

Table 5B: Regulation of expression of sod1, sod2, insr and irs1 genes in adipose tissues of dams and pups treated with high-fat diet-based interventions

GENES INTERVENTION	*SOD1*	*SOD2*	*INSR*	*IRS1*
HFD dams	1.00 (± 0.19)^{a}	1.00 (± 0.30)^{a}	1.00 (± 0.08)^{a}	1.00 (± 0.08)^{a}
HFD pups	3.29 (± 0.40)^{b}	2.09 (± 0.21)^{b}	0.78 (± 0.19)^{b}	0.85 (± 0.07)^{b}
HFD + HAGBR dams	7.33 (± 0.26)^{c}	4.53 (± 0.13)^{c}	0.49 (± 0.01)^{c}	0.37 (± 0.05)^{c}
HFD + HAGBR pups	7.33 (± 0.30)^{c}	4.59 (± 0.21)^{cd}	0.45 (± 0.00)^{c}	0.44 (± 0.08)^{cd}
HFD + LAWR dams	2.93 (± 0.67)^{b}	2.57 (± 0.77)^{be}	0.87 (± 0.07)^{ab}	0.81 (± 0.05)^{b}
HFD + LAWR pups	2.85 (± 1.13)^{b}	3.22 (± 0.22)^{e}	0.91 (± 0.03)^{ab}	0.65 (± 0.09)^{e}
HFD + LAWR dams + acarbose	4.62 (± 1.26)^{d}	3.26 (± 0.26)^{e}	0.51 (± 0.07)^{c}	0.45 (± 0.12)^{c}
HFD + LAWR pups + acarbose	7.16 (± 0.37)^{c}	4.09 (± 0.53)^{d}	0.42 (± 0.01)^{c}	0.37 (± 0.05)^{c}

Data are in fold changes of mRNA expression and are relative to each column's respective control dams. HAGBR: high amylose germinated brown rice; HFD: high-fat diet; LAWR: low amylose white rice; SC: standard chow. Mean values within a column superscripted by the same letter are not significantly different at *P*-value < 0.050.

[DOI: 10.18502/ijdo.v11i4.2880]

Funding

The authors wish to express their gratitude towards Padi Beras Nasional Berhad (BERNAS) and Universiti Putra Malaysia (UPM) for sponsoring this research (vote no. 63536). Both funding bodies had no roles in the preparation of data and manuscript.

References

1. Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, et al. Genomic expression programs in the response of yeast cells to environmental changes. Molecular biology of the cell. 2000;11(12):4241-57.
2. Nguyen-Huu TD, Gupta C, Ma B, Ott W, Josić K, Bennett MR: Timing and variability of galactose metabolic gene activation depend on the rate of environmental change. PLoS Comput Biol 2015;11(7).
3. Ladd-Acosta C. Epigenetic signatures as biomarkers of exposure. Current environmental health reports. 2015;2(2):117-25.
4. Abubakar B, Ismail N, Omar AR, Ismail M. Rice consumption and predisposition to metabolic diseases: The role of PPARγ and GLUT4 dysregulation. Journal of Nutrition & Intermediary Metabolism. 2017;10:8-18.
5. Abubakar B, Zawawi N, Omar AR, Ismail M. Predisposition to insulin resistance and obesity due to staple consumption of rice: Amylose content versus germination status. PloS one. 2017;12(7).
6. Poretsky L. Looking beyond overnutrition for causes of epidemic metabolic disease. Proceedings of the National Academy of Sciences. 2012;109(39):15537-8.
7. Ford ES, Giles WH, Mokdad AH. Increasing prevalence of the metabolic syndrome among US adults. Diabetes care. 2004;27(10):2444-9.
8. Akhoundan M, Shadman Z, Jandaghi P, Aboeerad M, Larijani B, Jamshidi Z, et al. The association of bread and rice with metabolic factors in type 2 diabetic patients. PloS one. 2016;11(12).
9. Abubakar B, Yakasai HM, Zawawi N, Ismail M. Compositional analyses of white, brown and germinated forms of popular Malaysian rice to offer insight into the growing diet-related diseases. Journal of food and drug analysis. 2018;26(2):706-15.
10. Lim SM, Goh YM, Mohtarrudin N, Loh SP. Germinated brown rice ameliorates obesity in high-fat diet induced obese rats. BMC complementary and alternative medicine. 2016;16(1):140.
11. Bennett K, James C, Hussain K. Pancreatic β-cell K ATP channels: Hypoglycaemia and hyperglycaemia. Reviews in endocrine and metabolic disorders. 2010;11(3):157-63.
12. MacLaren R, Cui W, Simard S, Cianflone K. Influence of obesity and insulin sensitivity on insulin signaling genes in human omental and subcutaneous adipose tissue. Journal of lipid research. 2008;49(2):308-23.
13. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI. Markers of oxidative stress during diabetes mellitus. Journal of biomarkers. 2013;2013.
14. Imam MU, Ismail M, Ooi DJ, Sarega N, Ishaka A. Increased risk of insulin resistance in rat offsprings exposed prenatally to white rice. Molecular nutrition & food research. 2015;59(1):180-4.
15. Imam MU, Azmi NH, Bhanger MI, Ismail N, Ismail M. Antidiabetic properties of germinated brown rice: a systematic review. Evidence-based complementary and alternative medicine. 2012;2012.

[DOI: 10.18502/ijdo.v11i4.2880]