ORIGINAL ARTICLE

Effects of White Rice-Based Carbohydrates Diets on Body Weight and Metabolic Parameters in Rats

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ABSTRACT

Introduction: We clarified the extent to which white rice (WR)-based carbohydrate diets affect body weight and metabolic parameters in rats. Methods: In this experimental study, a male Sprague Dawley (n=32) rats fed with WRbased CHO diet in two different proportions of total energy intake (TEI 55% moderate-CHO (MCHO, n=8) and 65% High-CHO (HCHO, n=8)) or high-fat diet (HFD, n=8) were compared with rats maintained on standard pellet diet (SD, n=8) for eight weeks period. Carbohydrate sources in the HFD and SD were mainly based on cornstarch (25% of amylose). Outcomes measures include body weight and metabolic parameters. Results: At baseline, body weight and metabolic parameters (fasting plasma glucose, insulin, and triglyceride levels) were comparable in all rats. Despite higher daily caloric intake in rats fed with HFD (103.9±3.0) than the other diets, no significant differences in body weight between groups after 8 weeks of study. However, rats' feds with WR-based CHO diets (both moderate and high carbohydrates) had higher fasting blood glucose (MCHO=12.8±1.6, HCHO=16.9±2.4) and triglycerides level (MCHO=1.2±0.0, HCHO=1.3±0.0) than rats in cornstarch-based HFD and SD (p<0.05). Both HFD and HCHO had higher fasting insulin than MCHO and SD (p<0.05), but the homeostatic model assessment of insulin resistance (HOMA-IR) was significantly higher in WR-based CHO diets (both moderate and high carbohydrates) than the rats in cornstarch-based HFD and SD (p<0.05). Conclusion: A WR-based CHO diet exhibits higher fasting blood glucose, triglycerides, and insulin resistance state than a high-fat diet without a significant impact on body weight. These findings may explain the growing incidence of diabetes in Asia and worth studying further.

Keywords: White rice, Diabetes, Obesity, Insulin resistance, Blood glucose

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INTRODUCTION

Obesity is a considerable public health crisis worldwide, as it increases the likelihood of various chronic diseases, particularly type 2 diabetes (T2D) (1). Obesity is not solely related to the excess storage of body fat but coexists with other metabolic abnormalities such as insulin resistance (2). The sharp rises in obesity contribute to the escalating prevalence of T2D, especially in Asia, where it accounts for 60% of the world's' diabetes population (3). The dietary intake appears to be shifting universally towards a diet high in energy density from dietary fats (4). The relationship between excess fat consumption

and obesity development was confirmed by various diet-induced-obesity in animal models (5,6).

Nonetheless, the dietary pattern of Asian populations is different than Western counterparts. The diet is dominated by dietary carbohydrate, which is mainly based on polished white rice (3). Polished white rice has a high glycemic index (GI) value, which causes a rise in blood glucose and insulin responses (7). The habitual consumption of a high-carbohydrate, high GI diet may contribute to the accelerated diabetes epidemic in Asia (3). Also, white rice is low in fibre, which may reduce the feeling of fullness, leading to overeating (8). This nutritional property would affect the adult propensity to obesity (8). Findings from the Shanghai Women's Health Study demonstrated that food with high GI, mostly white rice, is associated with increased risk of T2D by 78% (RR 1.78 (95%CI 1.48-2.15, highest vs lowest quintile of white rice consumption) (9). However, the impact of white rice on obesity was inconsistent and warranted further investigation.

Studies using animal models have established the deleterious roles of high-carbohydrate diet on body weight gain and metabolic parameters, but these studies used corn syrup, fructose or sucrose as the primary source of carbohydrates in the diet regimen (10-12). Although the previous study showed that prenatal diet exposure to white rice led to worsening glucose and insulin resistance state of their offspring, chronic exposure of white rice during adulthood remains elusive (13). Therefore, this study supplement previous work by clarifying the extent to which a moderate to a high-carbohydrate (CHO), white rice (WR)-based diets affect body weight and metabolic parameters in rats in comparison to a high-fat diet. We hypothesized that the CHO, WR-based diet is as effective as a high-fat diet in increasing body weight and metabolic parameters of the rats.

MATERIALS AND METHODS

A total of 32 male Sprague-Dawley rats at 8 weeks of age with a bodyweight of 250 ± 23.0 g were purchased from the Faculty of Veterinary Medicine, Universiti Putra Malaysia. The rats were acclimatized for two weeks to stabilize them in the new environment. The rats were housed single cage under husbandry laboratory conditions in a humidity-controlled room at 23-25 $^{\circ}\mathrm{C}$ with regular cycles of l2-hours light and 12-hours dark. The conditions were maintained throughout the study period. During this period, rats consumed the standard diet (AIN-93M purified diet) and tap water. Nutritional composition of the standard diet were cornstarch, white rice, maltodextrin, sucrose, soybean and casein (Table 1). The study obtained approval from the Animal Care and Use Committee (ACUC), Universiti Putra Malaysia (UPM/FPSK/PADS/BR-UUH/00438). Animal experiments adhered to the ARRIVE guidelines and conducted following the U.K Animal Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

Following acclimatization, the rats were randomly split into four groups. Rats were given a white rice-based carbohydrate (CHO) diets in two different proportions of total energy intake (TEI) (55% TEI moderate-carbohydrate (MCHO, n=8) or 65% TEI High-carbohydrate (HCHO, n=8) or a high-fat diet (HFD, n=8) which were then compared with rats maintained on standard pellet diet (SD, n=8). The rats consumed the assigned diets and water for 8 weeks in ad-libtum manner.

All the diets were designed to have about the same calories with consistent proportions of protein (14-15% TEI). The difference was only made to either the proportion of carbohydrates or fats (Table I). The SD

consisted of corn-based, AIN-93M Purified Rat Diet (Test Diet®, Missouri, USA) (35), and the HFD diet was prepared using fat from ghee (milk fat) and corn oil (Table I). All the ingredients in the SD were maintained to produce the WR-based CHO diets except corn that were replaced with white rice. The white rice has high GI value and low amylose content was measured on previous study (14). For the carbohydrate-based diet, it was also prepared to contain a different proportion of dietary carbohydrates. The moderate-carbohydrate WRbased diet (55% TEI, MCHO) was designed based on the proportion of carbohydrates commonly consumed by Malaysians (15,16) (Table I). The high-carbohydrates diet (HCHO) had a higher proportion of carbohydrates (65% vs. 55% TEI) but was lower in fat (20% vs. 31% TEI) than the MCHO (Table I). The rice was bought from supermarket in Klang Valley, Malaysia. The two rice samples namely White Rice with 5% broken (WR5%) and Fragrant White Rice (FWR). Rice samples for nutritional composition analysis were cooked in a rice cooker (FRC 3018SS, FABER, Malaysia Sdn. Bhd.) based on the cooking instructions given by the manufacturers. The proximate analysis (moisture, ash, protein, fat content) of the cooked rice was analyzed based on the AOAC method (2000).

Table I: Ingredients and Nutritional Composition of the diets (per 1000g)

		Groups					
Ingredients (g/kg)	Standard Diet (SD)	High-fat Diet (HFD)	White rice- based moderate- CHO (MCHO)	White rice- based, high-CHO (HCHO)			
Corn-starch	465.0	100.0	-	-			
White rice	-	-	350.0	450.0			
Maltodextrin	155.0	120.0	50.0	100.0			
Sucrose	100.0	150.0	100.0	100.0			
Soya bean oil	40.0	-	-	-			
Corn oil	-	110.0	70.0	40.0			
Pure Ghee	-	100.0	50.0	35.0			
Cholesterol	-	10.0	-	-			
Casein	140.0	140.0	130.0	140.0			
*Powdered cellulose	50.0	50.0	50.0	50.0			
*Mineral mix	35.0	35.0	35.0	35.0			
*Vitamin mix	10.0	12.0	10.0	10.0			
*L-cystine	1.8	-	1.8	1.8			
*DL-Methionine	3.54	3.54	3.54	3.54			
*Choline Bitartrate	2.5	-	2.5	2.5			
*t-butylhydroquinone	0.008	-	0.008	0.008			
*Ethoxyquin	-	0.02	-	-			
Total energy (kcal)	3800	3930	3600	3835			
Energy per gram	3.8	3.9	3.6	3.8			
Protein (% TEI)	15	14	14	15			
Carbohydrate (%TEI)	75	35	55	65			
Fat (%TEI)	10	51	31	20			

g, gram; kg, kilogram; CHO, carbohydrate; kcal,kilocalories; TEI, Total Energy Intake, 'non-energy yielding ingredients

Measurements

Rats were weighed individually daily throughout the study, using a digital weighing scale (SK-5001WP, A&D Weighing, Milpitas, California). Metabolic parameters included fasting plasma glucose, insulin, and triglyceride levels, which were measured at baseline and the end of 8 weeks. About 0.5ml of blood from the tail vein was obtained of 12-hr fasted rats. Blood was analysed for fasting glucose using the glucose oxidase method; plasma insulin used an enzyme-linked immunosorbent assay (ELISA) with the specific kit and triglycerides used enzymatic hydrolysis method. The estimates of relative insulin resistance known as homeostasis model assessment (HOMA-IR) were determined, where plasma glucose was expressed in mmol/L, and plasma insulin was expressed in mU/L(17), Daily food intake of the rats was measured individually by subtracting the amount of leftover food from the total amount of the food kept in the cage and converted into the daily caloric intake.

Statistical Analysis

Statistical computations were performed using SPSS Statistics Version 26. Results are expressed as the Mean \pm SD unless otherwise indicated. All differences were considered significant when p< 0.05. The Shapiro-Wilk statistic confirmed the normality of the data. The differences between groups were analysed using One Way Analysis of Variance (ANOVA), and Duncan's post hoc test was conducted to explore differences in terms of body weight, fasting blood glucose, fasting triglycerides, fasting insulin and HOMA-IR between multiple groups.

(Figure I). Over the study period, all rats gained weight significantly as compared from baseline with no differences between groups (Figure 1). The food intake over 8 weeks study was lowest in HFD group and it was differed significantly than the MCHO, HCHO and SD groups (p<0.05) (Figure II). After 8-weeks, rats fed with HFD gained more weight ($36 \pm 4\%$) followed by the rats fed with SD ($34 \pm 4\%$), MCHO ($33 \pm 7\%$) and HCHO diet ($32 \pm 10\%$) but no differences between groups were observed. In comparison to SD, the incremental weight gain ranged from 3 to 6% in HFD to HCO. Despite no difference in body weight gain, rats in HFD consumed the highest energy intake than the other groups (p<0.05, Table II and Table III).

Metabolic parameters

At baseline, metabolic parameters were comparable in all rats (Figure I). After 8 weeks, rats' fed with HCHO had significantly highest fasting blood glucose (16.9 ± 2.4 mmol/L) than rats fed MCHO (12.8 ± 1.6 mmol/L), SD (6.0 ± 0.2 mmol/L) and HFD (5.3 ± 0.6 mmol/L) groups (p<0.05; Figure I). The fasting blood glucose was also significantly higher in MCHO (p<0.05) than SD and HFD groups with no difference between SD and HFD. Similarly, rats fed with white rice-based CHO diets in both MCHO (1.2 ± 0.1 mmol/L) and HCHO (1.3 ± 0.1 mmol/L) had a higher triglycerides level than rats fed with SD (1.1 ± 0.1 mmol/L) and HFD (1.1 ± 0.1 mmol/L) (p< 0.05).

RESULTS

Body Weight and Energy Intake

At baseline, body weight was comparable in all rats

The fasting insulin was the highest in HFD (41.9 \pm 2.5 mU/L) and HCHO (39.7 \pm 3.1 mU/L) than in MCHO (36.3 \pm 1.2 mU/L) and SD (33.9 \pm 1.6 mU/L). However, HCHO had the highest HOMA-IR (537.7 \pm 96) than MCHO (373.4 \pm 50), HFD (178.5 \pm 25) and SD (161.6 \pm 8) groups (p<0.05) (Figure I). The HOMA-IR was also

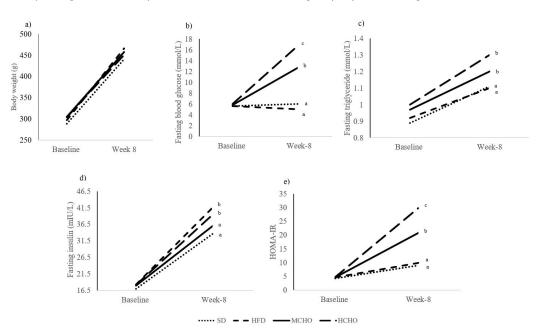


Fig. 1. Body weight and metabolic parameters of the rats (n=32) a) Body weight b) Fasting blood glucose c) Fasting triglycerides d) Fasting insulin e) HOMA-IR. Baseline weight was the weight after 2-weeks acclimatization period. a, b, c: Mean values with different superscript letters within the column show significant differences among groups, p<0.05, Duncan's Multiple Range Test)

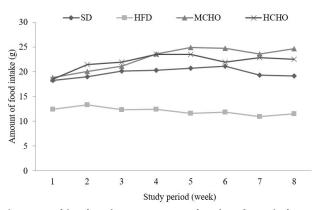


Fig. 2. Food intake of rats over 8 weeks of study period

significantly higher in MCHO (p<0.05) than rats in SD and HFD. The differences between SD and HFD were not significant.

DISCUSSION

This study clarified the extent to which a moderate to high-carbohydrate, white rice-based diets affect body weight and metabolic parameters when compared with a high-fat diet in male Sprague Dawley rats. Despite a higher daily caloric intake of a high-fat diet than the white rice-based carbohydrate diet, body weight was comparable between groups. Nonetheless, WR-based carbohydrate diets aggravate metabolic parameters further even at the common intakes of carbohydrates (15,16).

Most of the previous studies documented that a highfat diet, when compared with a high-carbohydrate diet, was superior in inducing weight gain (4–6). It could be due to the large storage capacities of adipose tissue than the glycogen stores (5). Besides, fat exerts the low satiating effects than carbohydrates and protein, suggesting that fat may lead to passive overconsumption leading to body weight gain (18). Therefore, the low-fat, low-calorie diet at 45-65% TEI as carbohydrates have been applied in managing obesity (19). Nonetheless, we observed comparable body weight and weight gain throughout the 8-weeks study.

Most studies analysed the differential effects of highcarbohydrate diets on metabolic parameters (10–12), but only a few studies have focused on white rice-based diets (13,20). Typical diets utilizing simple carbohydrates like sucrose are compared with diets containing digestible starch like cornstarch (11). The effect of carbohydrate, white rice-based diet on obesity development is controversial. In Korean adults, a specific dietary pattern that includes white rice was associated with obesity

Table II: Average daily caloric intake (kcal/day) of the rats throughout the study period (n=32)

Groups	Time-points								
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	
SD n=8	72.7 ± 1.6^{a}	76.1 ± 2.2^{a}	80.2 ± 0.9^{a}	81.3 ± 1.3^{a}	82.9 ± 1.1^{a}	84.5 ± 1.0^{a}	77.2± 1.0	76.5± 0.8ª	
HFD n=8	111.2 ± 2.1^{b}	119.8 ± 3.3^{b}	111.1 ± 1.8^{b}	$111.8 \pm 2.1^{\rm b}$	$104.8 \pm 2.3^{\rm b}$	105.9± 3.3 ^b	97.8±1.4	$103.9 \pm 3.0^{\rm b}$	
MCHO n=8	75.1 ± 2.2^{a}	80.2 ± 1.4^{ab}	84.5 ± 1.0^{ab}	94.4 ± 1.5^{ab}	99.9 ± 0.6^{ab}	98.7 ± 1.3^{ab}	94.4± 1.5	98.2± 1.2ª	
HCHO n=8	74.0 ± 1.7^{a}	85.7 ± 1.1^{ab}	87.9 ± 1.8^{ab}	94.1 ± 1.0^{ab}	93.8 ± 0.8^{ab}	87.6± 2.3 ^{ab}	91.2±1.6	$90.0 \pm 1.7^{\circ}$	

Mean values with different superscript letters within the column show significant differences among groups p<0.05, Duncans Multiple Range Test

Table III: Food and	Energy	intake	of rats	(n=32)
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Diets	0	, Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
PD (n = 8)	Food intake ¹ (gram)	18.2±0.4 ^{a,*}	19.0±0.5 ^{a,*}	20.1±0.2 ^{a,*}	20.3±0.3 ^{a,*}	20.7±0.3 ^{a, *}	21.1±0.2 a,*	19.3±0.2 ª,*	19.1±0.2 ^{a, *}
	Energy intake¹ (kcal)	72.7±1.6 ^{a,*}	76.1± 2.2 ^{a,*}	80.2±0.9 ^{a,*}	81.3±1.3 ^{a,*}	82.9±1.1 ^{a, *}	84.5±1.0 ^{a,*}	77.2±1.0 ^{a,*}	76.5±0.8 ^{a,*}
HF (n =10)	Food intake ¹ (gram)	12.4±0.2 ^{b,*}	13.3±0.4 ^{b,*}	12.3±0.2 ^{b,*}	12.4±0.2 ^{b,*}	11.6±0.3 ^{b,*}	11.8±0.4 ^{b,*}	10.9±0.2 ^{b,*}	11.5±0.3 ^{b,*}
	Energy intake¹ (kcal)	111.2± 2.1 ^{b,*}	119.8± 3.3 ^{b,*}	111.1± 1.8 ^{b,*}	111.8± 2.1 ^{b,*}	104.8± 2.3 ^{b,*}	105.9± 3.3 ^{b,*}	97.8±1.4 ^{b,*}	103.9± 3.0 ^{b,*}
N_{CHO} (n = 7)	Food intake¹ (gram)	18.8±0.5 ^{a,*}	20.0±0.4 ^{c,*}	21.1±0.2 ^{c,*}	23.6±0.4 ^{c,*}	24.9±0.1 ^{c,*}	24.7±0.3 ^{c,*}	23.6±0.4 ^{c,*}	24.6±0.3 ^{c,*}
	Energy intake¹ (kcal)	75.1±2.2 ^{a,*}	80.2± 1.4 ^{c,*}	84.5±1.0 ^{c,*}	94.4±1.5 ^{c,*}	99.9±0.6 ^{c,*}	98.7±1.3 ^{c,*}	94.4±1.5 ^{с,*}	98.2±1.2 ^{c,*}
H_{CHO} (n = 7)	Food intake ¹ (gram)	18.5±0.4 ", *	21.4±0.3 ^{d,*}	21.9±0.5 ^{d,*}	23.5±0.3 ^{c,*}	23.5±0.2 ^{d,*}	21.9±0.6 ^{d,*}	22.8 ± 0.4 d, *	22.5 ± 0.4 ^{d,*}
	Energy intake ¹ (kcal)	$74.0 \pm 1.7^{a,*}$	85.7± 1.1 ^{d,*}	87.9±1.8 ^{d, *}	94.1±1.0 ^{c,*}	93.8±0.8 ^{d,*}	87.6±2.3 ^{a,*}	91.2±1.6 ^{d, *}	90.0 ± 1.7 ^{d,*}

¹ Values are expressed as mean±SD. ² One-way repeated measures ANOVA.

a, b, c, d: Mean values within a column with unlike superscript letters were significantly different, p<0.05.

*: Mean values within a row with same superscript letters were significantly different, p<0.05.

(21). However, a recent unpublished study identified that eating rice protects against obesity (22). Yet, it is noteworthy that the amount of rice was relatively less (50g/day) and related to the fibre, nutrient, and plant compound found in whole grains rice (22). Similarly, when male Sprague Dawley rats fed with white rice-flour, they also had higher body weight than those rats fed with wild rice (23). Wild rice has a lower GI and more fibre than white rice, which could increase the feeling of fullness, thus preventing overeating (24).

In this study, rats fed with WR-based carbohydrate diets with the proportion ranging between 55% and 65% of TEI exhibited hyperglycemia, hypertriglyceridemia and hyperinsulinemia. The diet induces a more severe insulin resistance state than the rats' in cornstarch-based HFD and SD. The findings corroborated with a study by Iman et al., who has compared the effect of locally cultivated white rice against brown and germinated brown rice on diabetes outcomes in diabetic induced rats (20). The diabetic rats who fed with white rice had higher fasting plasma glucose, than rats in brown rice and germinated brown rice groups after 28 days (20). Similarly, prenatal diet exposure to white rice in comparison to brown rice led to worsening glucose and insulin resistance state of their offspring (13). The higher glucose and insulin could also explain the lack of differences in body weight. Those rats fed with white rice, the carbohydratebased diet, may experience ongoing gluconeogenesis that utilized the fat and protein deposit to make more glucose leading to weight loss, as commonly seen in untreated diabetic rats (20).

Low amylose white rice has a higher GI value than the high amylose rice, which causes a rise in blood glucose levels (14,25). In male Wistar rats fed with low amylose white rice, their blood glucose level increased significantly than the rats fed with intermediate and high amylose rice (26). The increase in glucose responses following low amylose rice consumption could be due to the tendency of amylose to retain its crystalline structure after cooking (25). The crystalline structure reduces enzyme accessibility and results in a higher proportion of slowly digestible starch and resistant starch (27). It is noteworthy that the standard diet contained the highest proportion of carbohydrates (~75% TEI). However, it did not increase the glucose and insulin as high as after the consumption of white rice-based carbohydrate diets. Corn contains higher amylose (~25% amylose) than white rice (10-20% amylose) (28). Despite a high GI value of cornstarch, its high amylose content and viscosity produced favorable response in blood glucose levels.

A higher carbohydrate, white rice-based diets have consistently demonstrated to increase triglyceride levels in rats (20,26,29). A similar pattern was also observed when using sucrose as the source of carbohydrates (30,31). Sucrose consumption would also lead to hyperinsulinemia, which may explain the increase in fasting insulin levels among rats fed with a high-fat diet (31). The high-fat diet in this study contains a significant amount of sucrose than the other diets.

HOMA-IR is used as a surrogate marker of insulin resistance and, this has been validated (32). High and moderate carbohydrate diet shows a high insulin resistance compared to standard and high-fat diet. Insulin resistance can contribute to increased glucose production in the liver and decreased glucose uptake in the muscle, liver, and adipose tissue, resulting in high blood glucose (33).

This study using a male rat due to low variability for blood measures when compared to female rats. However, in terms of body weight, endocrine measure and cardiac measures, using a female rats can produce similar results as this study. Female rats have a greater variability because of estrous cycle. Estrous cycle has an impact on hormonal balance; hence a such control requires greater cost of the study (36).

The study has several limitations. First, body weight alone may not be a sole indicator of obesity in rats. Future studies should include body composition and adiposity measurements to delineate further the roles of high fat vs. high carbohydrate, white rice-based diets on obesity. Besides, the use of fasting plasma glucose in the study was not sufficient to be used as a diagnostic tool for diabetes in rats. However, it still provides valuable information for the initial screening measures to elucidate white rice's effect on blood glucose levels (34). Future studies should utilize the glucose tolerance test to confirm a diagnosis of diabetes in rats fed with a white rice-based diet.

CONCLUSION

In comparison to a high-fat diet, a white rice-based carbohydrate diet increases fasting glucose, insulin, and triglycerides leading to a severe insulin resistance state without significant effects on body weight gain. The phenomenon may explain the growing incidence of diabetes in Asia, which warrants further investigation.

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