UNIVERSITI PUTRA MALAYSIA

WHITE RICE-BASED CARBOHYDRATE DIETS AS DIET-INDUCED OBESITY MODEL IN RATS

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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Chair: Barakatun Nisak binti Mohd Yusof, PhD
Faculty: Medicine and Health Sciences

High fat intake is a typical dietary pattern that is commonly consumed by Western populations. This diet is widely used in diet-induced obesity studies using animal models. On the other hand, in most Asian countries including Malaysia, dietary carbohydrate particularly white rice is a staple diet. Nevertheless, study on diet-induced obesity using white rice is limited. Therefore, this study aimed to investigate whether the Experimental Diet i.e. White Rice-based Carbohydrate Diets (WRBCD) can be used as a diet-induced obesity model in rats. It was hypothesized that, WRBCD were effective as a Corn-Based High Fat Diet (CBHFD) to induce obesity in rats. This study was divided into two phases; Phase I was a pre-experimental study and Phase II was an experimental study. In Phase I, nutrient and amylose content of selected white rice; namely fragrant white (FWR) and white rice, 5% broken (WR5%) were determined. White rice with lower amylose content was used at Phase II to prepare WRBCD. In Phase II, a total of 40 rats were divided equally into (i) Control group (Normal Purified Rat Diet (PD) and High Fat Diet (HFD)) and (ii) Experimental group (Normal ($NCHO_{Rice}$) and High Carbohydrate Rice Diet ($HCHO_{Rice}$)). A total of 8 rats died due to cardiac puncture procedure after the acclimatization period. Thus, there were only 32 rats included in the 8 weeks study. Obesity in rats was measured using body weight and body composition, while metabolic parameters including fasting plasma glucose, triglyceride and insulin levels were assessed. Rats were sacrificed at the end of study to measure weight of heart, kidney, liver, abdominal fat and thigh fat. In this study, the WR5% contained comparable amount of ash, fat, protein and available carbohydrate with the FWR ($p>0.05$). FWR had comparable amylose content with WR5% ($p>0.05$). Both WR5% and FWR were categorized as low amylose and therefore, WR5% or FWR rice can be used for Phase II. At Phase II, all the baseline parameters were comparable in all groups. After 8 weeks period, HFD (n=10) had comparable body weight (466.4 ±32.3 g) with $NCHO_{Rice}$ (n=7) (457.4 ±42.9 g) and $HCHO_{Rice}$ (n=7) (450.4 ±48.7 g) ($p>0.05$). In terms of body composition of the rats, rats consumed $NCHO_{Rice}$ (78.4 ±13.0 g) had the highest while rats in PD (59.3 ±4.4 g) had the lowest fat mass; the differences were significant ($p<0.05$). Total mass (378.0 ±16.4 g), lean body mass (308.6 ±12.7 g) and bone mineral content (8.6 ±0.5 g) were significantly highest in

HFD ($p<0.05$) as compared to the other three groups. At the end of the study, weight of heart, kidney, liver and abdominal fat of the rats were comparable except for thigh fat. The thigh fat weight of the rats in $HCHO_{Rice}$ (6.8 ±2.2 g) was significantly higher than $NCHO_{Rice}$ (2.6 ±1.3 g) ($p<0.05$). For the metabolic parameters, all baseline data were comparable. The $HCHO_{Rice}$ group rats exhibited the highest fasting plasma glucose (16.9 ±2.4 mmol/L) and the highest fasting triglyceride (1.3 ±0.0 mmol/L) ($p<0.05$) than the other 3 groups at the end of the study. The insulin level of HFD (41.9 ±2.5 mU/L) was significantly higher than PD (33.9 ±1.5 mU/L) and $NCHO_{Rice}$ (36.3 ±1.1 mU/L) groups ($p<0.05$) after 8 weeks of the study. This study shows that WRBCD were effective as HFD to induce obesity in rats. Despite having comparable body weight in all groups, rats in WRBCD had produced highest blood glucose level ($HCHO_{Rice}$) and highest fat mass ($NCHO_{Rice}$) than the HFD. Thus, it can be concluded that WRBCD can be used as a diet-induced obesity model in rats.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

DIET KARBOHIDRAT BERASASKAN NASI PUTIH SEBAGAI MODEL PERKEMBANGAN OBESITI PADA TIKUS

Oleh

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Diet tinggi lemak adalah cara pemakanan lazim populasi Barat. Diet ini digunakan secara meluas sebagai model obesiti haiwan. Tetapi, karbohidrat terutamanya nasi putih ialah makanan ruji kebanyakkan negara Asia termasuk Malaysia. Walau bagaimanapun, kajian mengenai model obesiti yang menggunakan nasi putih adalah terhad. Oleh itu, kajian ini adalah untuk menentukan sama ada Diet Karbohidrat berasaskan Beras Putih (WRBCD) boleh digunakan sebagai model obesiti untuk tikus. Ia telah dihipotesis bahawa, WRBCD adalah berkesan seperti diet tinggi lemak berasaskan jagung untuk menyebabkan obesiti pada tikus. Kajian ini terbahagi kepada dua fasa: Fasa I adalah kajian pra-eksperimen dan Fasa II adalah kajian eksperimen. Dalam Fasa I, kandungan nutrien dan amilosa beras putih wangi (FWR) dan beras putih hancur 5% (WR5%) telah ditentukan. Beras putih dengan kandungan amilosa yang lebih rendah telah digunakan pada Fasa II bagi membentuk diet WRBCD. Sebanyak 40 ekor tikus telah dibahagikan sama rata kepada empat kumpulan, iaitu *Normal Purified Rat Diet* (PD), *High Fat Diet* (HFD), *Normal Carbohydrate Rice Diet* ($NCHO_{Rice}$) dan *High Carbohydrate Rice Diet* ($HCHO_{Rice}$). Lapan ekor tikus telah mati semasa proses pengambilan darah dengan teknik *cardiac puncture*. Justeru, jumlah tikus untuk pengajian ini ialah 32. Tempoh masa kajian ini adalah selama 8 minggu. Obesiti pada tikus telah ditentukan dengan mengukur berat dan komposisi tubuh, manakala parameter metabolik yang diukur adalah glukosa, trigliserida dan paras insulin. Tikus telah dikorbankan pada akhir kajian untuk mengira berat organ seperti jantung, buah pinggang, hati, lemak abdominal dan lemak paha. Jumlah abu, lemak, protein dan karbohidrat tersedia beras putih hancur 5% (WR5%) adalah setara dengan beras putih wangi (FWR) ($p>0.05$). Kandungan amilosa FWR adalah setara dengan WR5% ($p>0.05$). WR5% dan FWR dikategorikan sebagai rendah amilosa dan salah satu daripadanya boleh dipilih untuk fasa seterusnya. Pada Fasa II, semua parameter asal adalah setara dalam semua kumpulan. Pada akhir kajian, tikus yang diberi makan dengan HFD (n=10) mempunyai berat badan yang setara (466.4 ±32.3 g) dengan tikus diberi makan dengan $NCHO_{Rice}$ (n=7) (457.4 ±42.9 g) dan tikus yang diberi makan dengan $HCHO_{Rice}$ (n=7) (450.4 ±48.7 g) ($p>0.05$). Bagi jisim lemak yang terenap, tikus dalam kumpulan $NCHO_{Rice}$ (78.4 ±13.0 g) mempunyai bacaan tertinggi manakala tikus dalam kumpulan PD (59.3 ±4.4 g) mempunyai lemak terenap paling rendah;

perbezaan adalah signifikan ($p < 0.05$). Jumlah jisim (378.0 ±6.4 g), jisim badan tanpa lemak (308.6 ±12.7 g) dan kandungan mineral tulang (8.6 ± 0.5g) adalah lebih tinggi dalam kumpulan HFD ($p < 0.05$). Pada akhir kajian, berat organ seperti jantung, buah pinggang, hati dan perut lemak tikus adalah setara kecuali lemak bahagian paha. Berat lemak paha bagi kumpulan $HCHO_{Rice}$ (6.8 ±2.2 g) adalah jauh lebih tinggi daripada kumpulan $NCHO_{Rice}$ (2.6 ±1.3 g) ($p < 0.05$). Pada permulaannya, semua parameter metabolik adalah sama bagi semua kumpulan. Tikus kumpulan $HCHO_{Rice}$ menunjukkan bacaan yang tertinggi bagi plasma glukosa berpuasa (16.9 ±2.4 mmol/L) dan trigliserida berpuasa (1.3 ±0.0 mmol/L) ($p < 0.05$). Tahap insulin bagi kumpulan HFD (41.9 ±2.5 mU/L) adalah lebih tinggi daripada kumpulan PD (33.9 ±1.5 mU/L) dan kumpulan $NCHO_{Rice}$ (36.3 ±1.1 mU/L) ($p < 0.05$) selepas 8 minggu kajian. Kajian ini menunjukkan WRBCD boleh menyebabkan obesiti setara dengan diet lemak tinggi pada tikus. Walaupun memiliki berat badan yang setara, tikus dalam kumpulan WRBCD mempunyai tahap glukosa darah yang tertinggi ($HCHO_{Rice}$) dan jisim lemak tertinggi ($NCHO_{RICE}$) berbanding dengan diet tinggi lemak. Kesimpulannya, WRBCD boleh digunakan sebagai model obesiti yang disebabkan oleh diet pada tikus.

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I certify that a Thesis Examination Committee has met on 20 February 2014 to conduct the final examination of Jeevetha Subramaniam on her thesis entitled "White Rice-Based Carbohydrate Diets as Diet-Induced Obesity Model in Rats" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science in Nutritional Sciences. The members of the Supervisory Committee were as follows:

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DECLARATION

Declaration by graduate student

I hereby confirm that:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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LIST OF ABBREVIATIONS

ACUC	Animal Care and Use Committee
ANOVA	Analysis of Variance
BMI	Body Mass Index
C	Control
CBHFD	Corn-Based High Fat Diet
CHO	Carbohydrate
CV	Coefficient Variation
DEXA	Dual Energy X-ray Absorptiometry
EDTA	Ethylenediaminetetraacetic acid
FWR	Fragrant White Rice
GI	Glycemic Index
HFCS	High Fructose Corn Syrup
HFD	High Fat Diet
HOMA-IR	Homeostasis Model Assessment-Estimated Insulin Resistance
HRP	Horseradish Peroxidase
HSD	High Sucrose Diet
ISO	International Organization for Standardization
kcal	kilocalories
mg	milligram
mmol/L	millimol per liter
mU/L	milliU per liter
NHLBI	The National Heart, Lung, and Blood Institute
NHMS	The National Health and Morbidity Survey

NIH	National Institutes of Health
Nm	nanometer
NR	Not Recorded
NS	Not Significant
OECD	Organization for Economic Co-operation and Development
PD	Purified Diet
RPM	Revolutions Per Minute
SD	Standard Deviation
UKMMC	Universiti Kebangsaan Malaysia Medical Center
VMH	Ventromedial Hypothalamic Nucleus
WHO	World Health Organization
WR5%	White Rice, 5% Broken
WRBCD	White Rice-Based Carbohydrate Diets

CHAPTER 1

INTRODUCTION

1.1 Background

Obesity is a substantial public health crisis around the world. It represents a state of excess storage of body fat that has an adverse effect on health. The prevalence of obesity is growing rapidly worldwide, particularly in developing countries (WHO, 2011). Malaysia has not been spared from this epidemic. The prevalence of obesity has increased rapidly by 175% over a period of 15 years between 1996 and 2011 (NHMS II, 1996; NHMS IV, 2011).

Obesity increases the likelihood of various chronic diseases particularly type 2 diabetes. This is because obesity is not solely related to the excess storage of body fat, but it also coexisted with other metabolic abnormalities including worsening of insulin resistance and elevating triglycerides level (Must et al., 1999). It comes as no surprise that the sharp increase in obesity prevalence has also contributed to the escalating prevalence of type 2 diabetes, especially in Asia, where it accounts for 60% of the world's' diabetic population (Hu et al., 2011). In Malaysia, diabetes prevalence has also increased by a staggering 83% within a period of 15 years between 1996 and 2011 (NHMS II, 1996; NHMS IV, 2011).

Obesity is a result from an imbalance between energy intake and energy expenditure (Johnson et al., 2008). In general, relative excessive intake of energy particularly dietary fat has been a major cause of dietary obesity (Swinburn et al., 2004). The relationship between excess fat consumption and obesity development has been supported by diet-induced-obesity in animal studies (Bray et al., 2004; Buettner et al., 2007). In addition, a high fat diet has an adverse effect on fat deposition especially at visceral areas and promoting insulin resistance (Wilcox et al., 2005).

Since diets high in fat are usually energy dense and palatable, it usually leads to an increase in energy intake (Rolls, 2009). In addition, it has been proposed that a high fat diet has appeared more efficiently to be stored as body fat resulting in a greater increase in adipose mass than iso-energetic portions of high carbohydrate or high protein diets (Brand-Miller et al., 2002).

High fat intake is a typical dietary pattern that is commonly consumed by Western populations. It is absolutely an undeniable fact that dietary fat consumption has increased over the years in Asian countries which could possibly explain the tremendous obesity rate increment. A similar trend has also been observed in Malaysia, where the total fat consumption of Malaysian adults derived from energy has increased by 4% from 23 to 27% within a decade (Kandiah et al., 2008). Nonetheless, the fat consumption pattern is not as high as compared to Western countries and it was still below the 30% limit recommended by the American Heart Association (Wylie-Rosett, 2002).

The low fat intake in Asian diets including in Malaysia will relatively increase dietary carbohydrates (Kandiah et al., 2008). In addition, Asian populations have a different dietary pattern which is mainly based on carbohydrate foods, especially rice. Rice, particularly in the form of white rice remains the major staple food and is unlikely to be displaced by any other staples in Asian countries. Recently, there has been considerable renewed interest on the significant positive association between rice consumption and risk of obesity (Kim et al., 2012) and type 2 diabetes (Nanri et al., 2010; Zhang et al., 2010; Hu et al., 2012) especially among Asian populations. This could be due to the high glycemic index of the polished white rice than those of the brown and whole grain rice (Foster-Powell, 2002).

The glycemic index (GI) is used to classify dietary carbohydrates based on their impact on the blood glucose response 2-hours after meals (Wolever, 2006). Foods that are high in GI such as white rice may result in a rapid rate of digestion and absorption, hence inducing rapid elevation of postprandial hyperglycemia as well as insulin concentration (Radulian et al., 2009). These characters of high GI foods can increase appetite and promote over consumption along with imbalance in energy partitioning, relatively increasing the fat storage over the long term (Brand-Miller et al., 2002; Hao et al., 2012). Thus, habitual consumption of high carbohydrate-high GI foods may initiate sequence of metabolic events that promote fat deposition and deteriorated metabolic parameters (Mc Neel and Mersmann, 2005; Claessens et al., 2009).

1.2 Problem statements

In Malaysia, white rice is consumed by almost 97% of the population with approximately 2 ½ plates (~ 450 g) daily (Norimah et al., 2008). The consumption of ~ 450 g white rice in a day is considered excessive in relation to diabetes risk as indicated in the Shanghai Women's Health Study, where women who consumed more than 300 g white rice/day had a 78% greater risk of developing type 2 diabetes mellitus compared to women who consumed less than 200 g white rice/day (Villegas et al., 2007). A traditional Japanese diet, with a high intake of white rice, has also shown to be significantly associated with an increased risk of obesity among Japanese women aged 18 to 20 years (Okubo et al., 2008). Similarly, in a traditional Korean diet, which includes white rice and kimchi was independently also associated

with increasing risk of obesity among Korean adults (Kim et al., 2012). There was also a significant relationship between rice intake and total and central obesity among Hispanic population (Lin et al., 2003).

Nevertheless, data relating the intake of white rice and its association with obesity and diabetes risk among Malaysian adults is scarce. As dietary compliance has always been an important bias in human studies and is it beyond the control of the investigator, the use of animal models is highly sought to investigate specific diet-induced obesity that primarily mimics the Asian-based human diet. Although studies using high carbohydrate diet in inducing obesity have been well conducted in animal models, most studies used either corn or sucrose as the primary source of carbohydrate (McNeel et al., 2005; Zhang et al., 2007; Claessens et al., 2009; Bocarsly et al., 2010).

The present study was conducted to identify whether the white rice-based carbohydrate diets can be used as a diet-induced obesity model in rats. The white rice-based carbohydrate diets were designed to mimic Malaysian food consumption pattern in terms of energy proportion from carbohydrates.

1.3 Significance of the study

The findings from this study can provide fundamental data in developing an appropriate dietary obesity induction model for animal study that represents a Malaysian-style diet using white rice-based diets. The effect induced by white rice to promote obesity and diabetes in animals can be translated to the human model in preventing obesity and diabetes development. Essentially, the diets are planned to resemble Malaysian trends in food intake and the results of the study would benefit in terms of improvised public health system.

1.4 Study objectives

The general objective of this study was to investigate whether the White Rice-based Carbohydrate Diets (WRBCD) can be used as a diet-induced obesity model in rats.

The specific objectives of the study were as follows:

1. To determine the nutritional composition, amylose content and amylose: amylopectin ratio of the two types of white rice.

2. To determine the effects of white rice-based carbohydrate diet on body weight and composition (body composition measured by DEXA and weight of organs) and metabolic parameters (fasting glucose, triglycerides, and insulin) of the rats after 8 weeks of study.

3. To compare the effects of the white rice-based carbohydrate diets against corn-based control diets, High Fat Diet and the Normal Purified Rat Diet on body weight gain and metabolic parameter in the rats after 8 weeks of study.

1.5 Study hypothesis

It was hypothesized that the White Rice-based Carbohydrate Diets (WRBCD) can induce obesity as effective as High Fat Diet.

1.6 Conceptual framework

The etiology of obesity is based on increased energy intake and reduced physical activity. The increase in energy due to macronutrients, specifically fat and carbohydrate can lead to obesity; and carbohydrate can alter metabolic parameters. Those inside the dotted lines of conceptual frame work, Figure 1.1 are interest of current study.

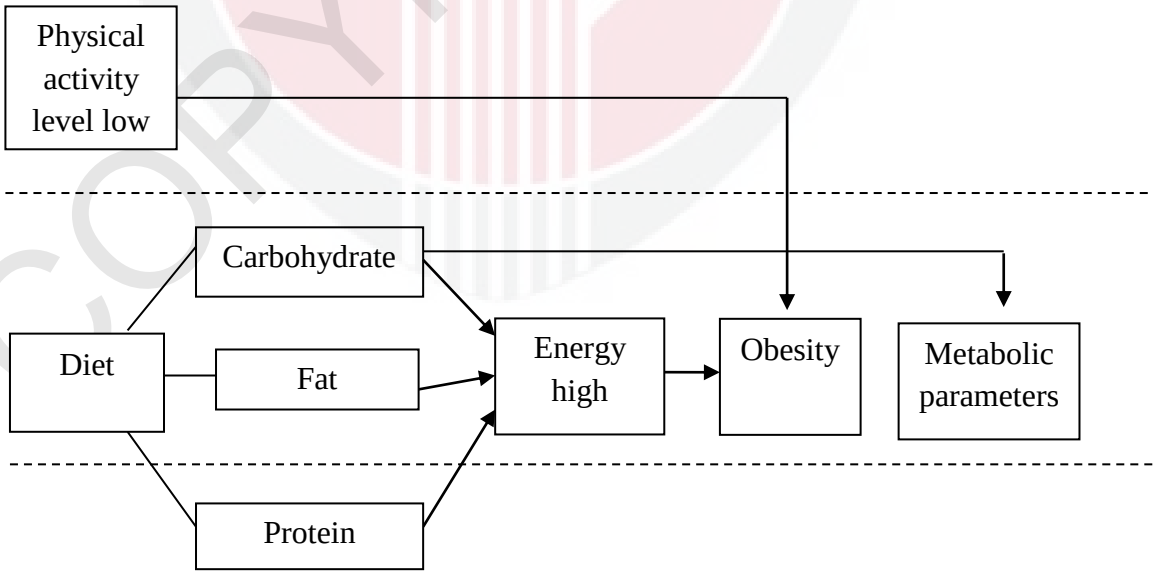

Figure 1.1: Conceptual framework of study
Boxes inside the dotted lines are interest of current study

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