Department of Community Medicine,

School of Medical Sciences

Effect of Combination of Behavioural Intervention and Nutrition Education with Brown Rice (COMBINE-BROWN) Weight Loss Program on Body Composition, Oxidative Stress and Antioxidant Level in Overweight and Obese Adults

PhD Candidate:

Ong Mei Gee

Principal investigator:

Associate Prof. Dr. Rohana Abdul Jalil (USM)

Co-Investigators:

1. Prof. Dr. Wan Rosli Wan Ishak (USM)
2. Associate Prof. Dr. Che Badariah Ab. Aziz (No. MMC 29445)
3. Dr. Noor Aman Hamid (NO MMC: 63187) (USM)
4. Prof. Frank Hu (Harvard University)
5. Prof. Walter Willet (Harvard University)
6. Dr Vasanti Malik (Harvard University)
7. Ms Wan Suriati Wan Nik (USM)
8. .Ms Nik Fadzlina Hamid (USM)

1.0 Introduction

Obesity is defined as an increase in body weight with abnormal or excessive fat accumulation that will harm health. Adults with BMI more than 30 are considered obese. WHO reported that 10% of the world population is obese, which causes high morbidity and mortality rates worldwide (WHO, 2022). Malaysia is one of the developing country in which almost half of the adult population experienced overweight and obesity (IPH, 2019).

Moderate weight loss (10 % to 15%) has significantly lowered the health risks and medical problems in 90% of obese patients. Weight loss is important in obese individuals since it improves their heart function, blood pressure, glucose tolerance and lipid profile, as well as decreased requirements for medication, decreased incidence and duration of hospitalisation, and decreased postoperative complications (Zain *et al.*, 2007). Behavioural weight loss program, which combines physical activity and diet showed effective results in long-term weight loss, compared to physical activity or dietary intervention (Johns *et al.*, 2014). Furthermore, a Specialist Lifestyle Management (SLiM) programme, a multi-component intervention combining diet, physical activity and behavioural modification, was shown to help patients achieve clinically significant weight loss and improvement in diabetic control (Brown *et al.*, 2015). In Malaysia, diet and exercise interventions in the 12-week behavioural lifestyle modification program were proven to be effective in reducing body weight, improving lipid profile and increasing antioxidant enzyme activities (Lazim *et al.*, 2012).

Whole grains appear to be one of the best options incorporated into the weight-loss regimen since the grain has a weight-loss effect due to the presence of higher dietary fibre and lower glycaemic index compared to refine grain. As a result, whole grains can increase satiety, reduce subsequent hunger, and thus reduce weight (Liu, 2003; Mazlan *et al.*, 2006). This has been demonstrated among middle-aged women (Liu, 2003). However, although whole grains have been proven by scientific studies to its advantages, many consumers are unaware of the health benefits of whole grains or the recommendations of at least three servings of whole grains daily (Slavin, 2004). Other than that, high prices, poor taste, and rough and bad texture are the barriers to whole grains consumption (Monge-Rojas *et al.*, 2014).

Rice is a staple food for nearly 50% of the world's seven billion people. It is the main food for most countries in Asia, and some countries in Africa and South America (Wuthi-Arporn). In Malaysia, rice is the staple food with 97% of the population eating rice twice daily at an average of 2 ½ plates per day (Norimah *et al.*, 2008). A previous study has shown that people usually consume white rice as a dietary habit, as compared to brown rice (Kumar *et al.*, 2011). The main barriers to the acceptance of brown rice include lack of familiarity, negative sensory perception (taste, texture, colour), high price, lack of perception as a disease-prevention food, complexity of cooking, and lack of family support and acceptance (Monge-Rojas *et al.*, 2014).

Currently, in Malaysia, the scientific evidence for substituting whole grain (brown rice) as part of a weight loss program for human have not been reported. This study will aim to determine the effect of brown rice consumption as part of the weight-loss regimen. Besides, this intervention study will focus on Malay adults since Malay ethnic is one of the Malaysian populations that has a high prevalence of overweight and obesity (Khambalia and Seen, 2010; IPH, 2019). The study will help the local community to increase health awareness, improve the quality of life, and prevent obesity-related diseases.

1.1 Research Questions

Are there any changes in body weight, anthropometric and body composition measurement, biochemical parameters, and quality of life between the control group and experimental group after 12 weeks of participating in a COMBINE-BROWN weight loss program among obese adults?

1.2 General objectives

To assess the effect of brown rice and white rice (*Oryza sativa* L.) consumption on body composition and oxidative stress biomarkers among overweight and obese adults in a 12-week COMBINE-BROWN weight loss program.

1.2.1 Specific objectives

1. To compare nutritional compositions and glycaemic index of brown rice and white rice (*Oryza sativa* L.) to be consumed in the weight loss intervention among overweight and obese adults.
2. To determine the weight change and body composition after participating in 12 weeks of weight loss program.
3. To determine the difference in lipid profile after participating in 12 weeks of weight loss program.
4. To determine the difference in antioxidant levels after participating in 12 weeks of weight loss program.
5. To assess the changes in quality of life after participating in 12 weeks of weight loss program.

6. To determine the weight change and body composition during follow-up for the evaluation study.

1.3Hypotheses

1.3.1 Null Hypotheses

There is no significant improvement in anthropometric and body composition measurement, biochemical parameters, and quality of life between the control group and experimental group after participating in a 12-week COMBINE-BROWN weight loss program.

1.3.2 Alternative Hypotheses

There is a significant improvement in anthropometric and body composition measurement, biochemical parameters, and quality of life between the control group and experimental group after participating in a 12-week COMBINE-BROWN weight loss program.

1.4 Rationale of the Study

A previous systematic review of weight loss intervention among overweight and obese men and women has successfully shown lower BMI, improved lipid profile as well as the increased antioxidant level (Heather *et.al*., 2006). Besides, a study showed that moderate weight loss decreased oxidative stress and increased antioxidant status in patients with metabolic syndrome. Rapid weight loss through a very low-calorie diet also reduces oxidative stress biomarkers, especially in metabolic syndrome patients (Maria et.al, 2012). In general, most studies showed that brown rice (*Oryza sativa* L.) do have health benefits as a whole grain. Meanwhile, a few studies showed that white rice contributes to the prevalence of obesity such as China (Zhang et al., 2011), India (Malik *et al.*, 2019) and other transition countries. However, many contributing factors lead to obesity apart from refined grain as a staple food. This study is trying to integrate brown rice consumption as an alternative to refined white rice in a weight loss program. This study introduces new whole grains to Malaysian overweight and obese participants in a weight loss program and measures the antioxidant level after a 3 months weight loss program. To our knowledge, there is no such study to look at these oxidative stress biomarkers after a healthy lifestyle management weight loss program and integrate with brown rice consumption among overweight and obese participants in Malaysia.

2.0 Literature Review

Whole grains are claimed to be one of the healthy food choices. They have a bark-like, protective hull, beneath, which are the endosperm, bran, and germ. Whole grains are safe for human consumption since they are processed only by removing the inedible parts (hull and husk). Whole grains are rich in nutrients with known health benefits. They have high concentrations of dietary fibre, B vitamins, minerals, rich in antioxidants including phytic acid and vitamin E. During the whole grain-refining process, the bran and germ are removed, resulting in the loss of nutrients (Slavin, 2004).

The major cereal grains include wheat, rice, and maize, whereas the minor cereal grains include oats, rye, barley, triticale, sorghum, and millet (Slavin, 2004).

Oryza Sativa L. also known as brown rice is rich in fibre content. The whole grain is produced by removing only the husk. The bran layers consist of 80 % of the minerals, whereas the germ layers consist of vitamin E, minerals, unsaturated fats, antioxidants, and phytochemicals (Jain *et al.*, 2012). White rice, which is refined grain, is formed by removing the husk, bran and germ layers in the milling and polishing process. As a result, there is a total loss of nutrients such as vitamin B, manganese, phosphorus, iron, dietary fibre, and essential fatty acid (Babu *et al.*, 2009).

Apart from being a staple food in the Asian population, rice is a good source of many bioactive phytochemicals including phenolic antioxidants that may have health-promoting effects. The potentially antioxidant compound is notably in the bran and germ (embryo) of the grain and has greater content in coloured varieties. A few experimental studies have reported that several classes of antioxidant compounds present in rice (tocols, oryzanols and phenolic compounds) decreased oxidative stress *in vivo* and reduced the risk of chronic diseases such as cancers (Slavin, 2004). Meanwhile, brown rice is rich in selenium, which is a cofactor of glutathione peroxidase (one of the body's most powerful antioxidant enzymes), and cooperates with vitamin E in many other important antioxidant defence mechanism (Babu *et al.*, 2009). A previous study also reported that brown rice has several precious lipid-soluble antioxidants such as γ-oryzanol, as well as a higher amount of soluble and insoluble phenolic acid contents than white rice (Britz *et al.*, 2007).

Recently, several reviews showed that white rice consumption is associated with type 2 diabetes and obesity (Hu *et al.*, 2012; Bhavadharini *et al.*, 2020; Ren *et al.*, 2021). Esmailzadeh *et al.* (2013) on the other hand found, refined grain such as rice was associated with the risk of having metabolic syndrome but not with diabetes. However, white rice consumption was not associated with cardiovascular risk among

Iranian men (Hossein *et.al*., 2013). A prospective study in Spain showed subjects who ate rice frequently had a lower risk to develop diabetes 6 years later which was opposite to the findings by Hu and colleagues (2012). Moreover, a longitudinal China Health and Nutrition Survey showed a significant negative association between rice consumption and metabolic syndrome among Chinese in both southern China and northern China. The different physical activity, dietary habits, and cooking methods might cause the different findings in this study (Huang *et al.*, 2019).

On the other hand, a pilot study showed that the short-term substitution of whole rice (brown rice) for white rice among Chinese-American with pre-diabetes was associated with a substantial improvement in their metabolic risk factors and weight loss (Wang *et al.*, 2013). However, in Shanghai, the replacement of white rice with brown rice for 16 weeks did not improve metabolic risk factors among diabetic and pre-diabetic Chinese. Therefore, it is suggested that different varieties of rice, a greater sample size, and longer durations of study are essential in determining the effect of brown rice on diabetes risk (Zhang *et al.*, 2011). In the BRAVO study, after consuming brown rice for 8 weeks, the values of weight-based parameters decreased, however, after consuming white rice for the next 8 weeks, the values returned to the original baseline value. The study also demonstrated that the consumption of brown rice has been proven to reduce total cholesterol and LDL cholesterol in this study (Shimabukuro *et al.*, 2014). Meanwhile, in India, consumption of parboiled brown rice revealed no significant changes in body weight and body fat percentage, but lead to improvement in HbA1c, a diabetes risk marker (Malik *et al.*, 2019). Brown rice has received growing research interest, due to its antioxidant properties, anti-hyperlipidaemic, and anti-diabetic effects. Brown rice could be a potential alternative to replacing refined grain or white rice to prevent obesity-related diseases.

3.0 Methodology

3.1 Background of Study

This study consists of three phases which are quantitative studies. The first phase of this study includes the determination of nutrient analysis, mineral analysis, scanning electron microscopic and texture analysis of brown rice and white rice varieties. Phase two of this study is a glycaemic index determination of rice varieties among healthy adult volunteers. Phase three is a 3-months quasi-experimental intervention of selected white rice and brown rice consumption among overweight and obese Malay adults. A few variables will be assessed in pre-intervention, post-intervention, and follow-up to observe the changes in the variables. The variables include anthropometric and body composition measurement, oxidative stress biomarkers, physical activity, nutrients intakes, and quality of life.

3.2 Study Area

This study will be conducted in Health Campus, Universiti Sains Malaysia, Kelantan. Kelantan is located in the northeast of Peninsular Malaysia.

3.3 Phase One Study

3.3.1 Research Design

Phase one study is a food science quantitative study, which involves proximate analysis, mineral analysis, texture analysis, and scanning electron microscopic of brown rice and white rice varieties.

3.3.2 Research Period

This study will be conducted for approximately 9 months.

3.3.3 Research Materials

Brown rice available in Malaysia and the most consumed white rice in Kelantan, purchased from the market in Kelantan, Malaysia will be used in the study.

3.3.4 Research Methods

3.3.4.1 Sample Preparation for Analysis

Raw rice samples will be used in the proximate analysis, while cooked rice samples will be used in the mineral analysis, texture analysis, and scanning electron microscopic of brown rice and white rice.

3.3.4.2 Textural Properties

The samples will be measured immediately for textural properties to prevent starch retrogradation. The measurement will be performed using a Textural Analyser. The resulting force-time curves will be analysed with Exponent software for sample texture characteristics including hardness and adhesiveness (Mir *et al.*, 2013).

3.3.4.3 Scanning Electron Microscopy

Raw grains will be dehydrated and then fractured using a razor blade. Cooked rice grains will be dehydrated through 100 % ethanol and cryo-fractured in liquid nitrogen since cooked rice grains are soft and sticky. All the samples will then undergo critical point drying and mount onto round aluminium stubs with the help of double-sided adhesive tape. The samples will then be coated with approximately 100µm gold by a high vacuum evaporator and scanned. The selected region of the surfaces will be observed and photographed (Ogawa *et al.*, 2003; Deepa *et al.*, 2008).

3.3.4.4 Proximate analysis

The proximate composition will be determined using AOAC (2005) for moisture, crude protein, fibre, lipid, and ash (Horwitz, 2005).

3.3.4.5 Elemental analysis

The minerals Selenium (Se), Ferum (Fe), Manganese (Mn), Zinc (Zn), and Calcium (Ca) will be determined using Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES).

3.3.4.6 Statistical analysis

SPSS software will be used to analyse the data and results will be expressed as means ± standard deviation (SD). All the analyses will be performed in triplicates. One-way analysis of variance (ANOVA) followed by Tukey’s HSD post hoc test will be used to analyse the statistical significance of the obtained data (Thomas *et al.*, 2013). The significance level will be considered at $p \leq 0.05$ (Thomas *et al.*, 2013)

3.4 Phase Two Study

3.4.1 Research Design

Phase two study is a quantitative study, which involved the determination of the glycaemic index of brown rice and white rice varieties as well as control among healthy adults.

3.4.2 Research Period

This study will be conducted for approximately three months.

3.4.3 Study Population

3.4.3.1 Reference Population

The reference population of the Phase Two study will be healthy adults in Kelantan.

3.4.3.2 Source Population

The source population in this phase will be healthy adults in Kelantan, who age between 18 to 60 years old and fulfil inclusion and exclusion criteria.

3.4.3.3 Target population

The healthy adult population will be selected through inclusion and exclusion criteria.

The inclusion criteria are:

1. Healthy adults who have BMI ≤ 25 kg/m^2, no history of transmitted disease such as AIDS or chronic diseases such as diabetes mellitus, hypertension, and cardiovascular diseases and no history of an acute medical or surgical event within the last 6 months.

2. Subjects will be males or non-pregnant or non-lactating females aged 18 to 60 years.

The exclusion criteria

1. Individuals will be excluded from the study if they are using medication.

2. Individuals will be excluded from participation if they cannot or will not comply with the experimental procedure, including not overnight fast or if they have characteristics and behaviours that will endanger themselves or others.

3.4.4 Research Method

The subjects will be required to go through the study protocol before starting the GI test. The GI value will be determined in 10-14 healthy human volunteers. The subjects will be given 25 g of test foods in the early morning after an overnight fast (10-12 h). The test on the test foods will be repeated three times to reduce the variability within the subjects. The subjects will be asked to consume the test foods or reference foods with 250 mL of plain water after the baseline fasting blood samples are taken. The blood samples will be taken from each volunteer every 15 min during the first hour and every 30 min during the second hour. The whole blood samples will be obtained by finger prick with a lancet (HemocueSafetyLancet@USA) and will be collected into a capillary tube (Selzer® Reflotron, Germany). Blood glucose will be assayed using the glucose oxidase method by an automatic glucose analyser (Hemocue, Roche, Germany). The method involves human subjects will be approved by the Human Ethics Committee of Universiti Sains Malaysia, while voluntary consent forms will be obtained by the subjects before conducting the GI test.

3.4.5 Statistical Analysis

The incremental AUC will be calculated based on the trapezoidal (Simpson's) rule using the area above the baseline (fasting glucose). The IAUC calculation will be used as described by the Food and Agriculture Organisation of the United Nations. Subjects who have GI exceeded 2 Standard Deviation (2SD) will be excluded from the group. The IAUC calculation The GI will be calculated as follows:

$$\text{GI} = \frac{\text{IAUC of the test food}}{\text{IAUC of the reference food}} \text{ x } 100$$

SPSS software will be used to analyse the data. Results will be expressed as mean ± SEM. ANOVA will be used to measure blood glucose response at a different time intervals, the IAUC and GI values. The differences are considered significant if $p \leq 0.05$ (Yusof *et al.*, 2005).

3.5 Phase Three Study

3.5.1 Research Design

Phase three study is a quasi-experimental study, a non-randomised intervention study The Quasi-experimental design is used since it is not logistically feasible or ethical to conduct a randomised controlled trial (Harris *et al.*, 2006).

3.5.2 Research Period

This study will be conducted for approximately one year and nine months.

3.5.3 Study Population

3.5.3.1 Reference Population

The reference population of this study will be overweight and obese adults in Kelantan.

3.5.3.2 Source Population

The source population of this study will be the overweight and obese adults who attend Hospital Universiti Sains Malaysia.

3.5.3.3 Target Population

The target population of this study will be overweight and obese adults who attend Obesity Clinic at Hospital Universiti Sains Malaysia.

3.5.3.4 Sampling Frame

The sampling frame of this study is the attendance list of overweight and obese adults who attend the Obesity Clinic at Hospital Universiti Sains Malaysia.

3.5.3.5 Study criteria

The healthy adult population will be selected through inclusion and exclusion criteria.

The inclusion criteria include:

1. Healthy adults (18 -60 years)

2. BMI ≥ 27 kg/m^2

3. Do not pregnant and lactating

4. No intention to get pregnant throughout the study period

5. Able to do exercise/ walking without aid

6. Undergo a medical examination

7. Willing to consume brown rice (*Oryza sativa* L.) one meal per day for 3 months.

8. Consume white rice as a staple diet.

The exclusion criteria include:

1. Suffered from mental illness

2. Pregnant or plan to be pregnant throughout the study

3. Without a diagnosis of diabetes.

4. Consume brown rice, parboiled rice and any other commercial rice that is claimed to reduce blood sugar levels as a staple diet.

3.5.4 Research Method

A combination of behavioural intervention and nutrition education with brown rice (COMBINE-BROWN) weight loss program will be conducted among patients who attended Obesity Clinic seeking weight management. Announcement of this weight loss program will be made in the city of Kota Bharu and other health clinics in Kelantan state. For those who are interested to join voluntarily, participants will register with our program which is based at Obesity Clinic. As this will be a pilot project to introduce a brown rice consumption plan for weight loss, 66 subjects will be recruited in each arm for the control group and experimental group.

All the participants will be explained about the program in detail and they will be given two weeks of probation period. The probation period is purposely for them to rethink their participation and compliance with 3 months program as well as to coordinate with their employers for those who are working. At the baseline, the participant's data (control and experimental group) will be recorded such as sociodemographic (age, income, education, gender), dietary intake, anthropometric data, body composition, metabolic parameters (fasting lipid profile, blood glucose) and oxidative stress biomarkers. In addition, the level of physical activity will be assessed by using the Malay version of the International Physical Activity Questionnaire. Besides, quality of life will be assessed using the Impact Weight Quality of Life Questionnaire. The participants in this program come to Obesity Clinic purposely to participate weight loss program, so it is hard to do a true experiment where the interventionist of the program will randomly assign the participants who will enrol for the next weight loss program. To fulfil the principle of beneficence, the control group will attend a weekly meeting, receive 12 weekly behavioural weight loss programs, and maintain white rice as a daily diet regimen.

Meanwhile, the participants in the experimental group will be receiving 12 modules of the intervention package plus brown rice (*Oryza sativa* L.) meal plan throughout the weight loss program. A package of interventions will be delivered at weekly meeting sessions which comprised of nutrition education, behavioural modification and physical activity. During the weekly meeting, group counselling or individual counselling will be implemented to set their behavioural goals. The method involving human subjects will be approved by the Human Ethics Committee of Universiti Sains Malaysia.

3.5.5 Sample size estimation

The sample size is calculated using Power and Sample Size (PS) Software version 3.1.2 for the independent t-test. The type 1 error (α) and the power of the study (1-β) were 5% and 80%, respectively. A total of 49 subjects are allowed to recognise statistical differences between initial and final measurements based on the true difference in the mean response of glutathione peroxidase (Kim *et al.*, 2008). Sixty-six subjects are recruited for each arm in this study to allow an expected 35% dropout rate.

3.5.6 Sampling Method and Subject Recruitment

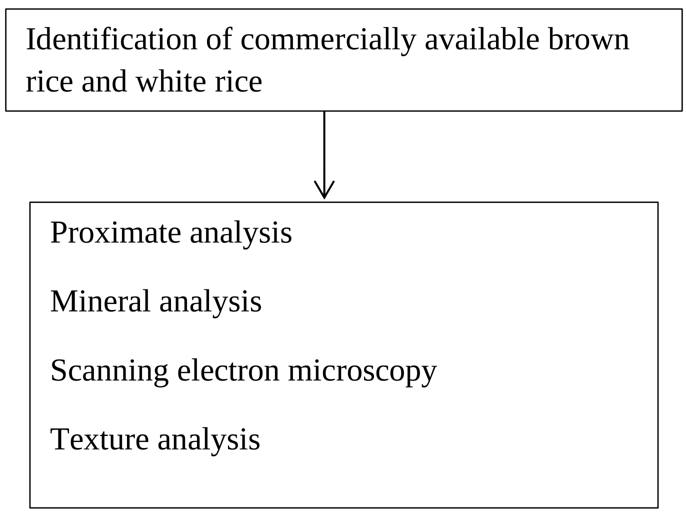
Non-probability sampling is used since random sampling is not possible. This is because the subjects are selected based on the availability of suitable diet and program design as well as their willingness to take part in the study such as willingness to consume brown rice as part of the weight-loss regimen. The interventionists will select the sample based on their judgement concerning some appropriate characteristic required (Lee, 2010).

3.5.7 Statistical Analysis

Data will be analysed using SPSS version 22. Pair-t-test will be used to analyse pre- and post-intervention variables. General linear model and ANOVA repeated measure will be used to analyse within groups and interaction times. *p*-values of <0.05 will be considered statistically significant.

Flow chart of the methodology

PHASE I



PHASE II

Recruitment of respondents

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Glycaemic index determination of brown rice and white rice according to FAO/WHO (1998) protocol, n=10-14

Inclusion Criteria

-Age between 18 to 60 years

-Male and non-pregnant or non-lactating women

Exclusion Criteria

-Individuals using medication

-Individuals cannot or will not comply with the experimental procedures

PHASE III

3 months quasi-experimental intervention of white rice and brown rice consumption

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Metabolite intermediates measurement:

1. Anthropometric and body composition
2. Lipid profile
3. Oxidative stress biomarkers
4. Nutrients intakes
5. Impact of Weight on Quality of Life (IWQOL)

Inclusion Criteria

-BMI ≥27 kg/m^2

-Age between 18 to 60 years

-Male and non-pregnant or non-lactating

Exclusion Criteria

-Individuals using medication

-Individuals cannot or will not comply with the experimental procedures

3.6 Ethical consideration:

1. Subject vulnerability: The subject is a patient under our care as a researcher. The subjects will be given full freedom to participate or not without affecting their medical condition management and care. The rights of patients will be respected with no discrimination to further management.

2. Declaration of absence of conflict of interest: There is no competing interest.

3. Privacy and confidentiality: All questionnaires were anonymous and will be entered into SPSS software. Only research team members can access the data. Data will be presented as grouped data and will not identify the responders individually. Blood samples obtained will be used for this study purpose and dealt with as per standard laboratory protocol.

4. Community sensitivities: Not applicable

5. Community benefits: The subjects can receive feedback about the status of body weight, total fat and blood profile in their body. Besides, this study can provide information about diet, lifestyle changes and a form of exercise that can improve public health and quality of life, and be used for future research.

6. Honorarium and incentives: There are no honorariums and incentives provided to the participants.

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