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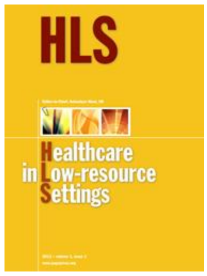

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Original Research

The effects of brown rice as functional food on Lee Index, adipose tissues and PRDM16 levels in obesity model *Rattus norvegicus*

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Running title: The effects of brown rice as functional food

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Significance of public health: Brown rice is considered a functional food that has been shown to impact the Lee index positively and may influence PRDM16 levels in cases of obesity. Research suggests that brown rice (BRG) may promote the enhancement of beige adipose tissue (BeAT), brown adipose tissue (BAT), and PRDM16 levels. However, further investigation is necessary to determine the optimal dietary ratio required to facilitate the browning process of white adipose tissue (WAT).

Abstract

Brown rice is a functional food known to improve the Lee Index, influencing PRDM16 levels in obesity conditions. Therefore, this study aims to determine the differences in Lee Index, Brown Adipose Tissue (BAT) weight, White Adipose Tissue (WAT) weight, BeAT weight, total body fat, and PRDM16 levels of obese model rats with the intervention of brown and white rice, as well as γ-oryzanol.

A true experimental method was used with a post-test-only control group in vivo design. The obesity model was constructed with male Sprague Dawley rats *(Rattus norvegicus)*, divided into five diet groups namely standard and HFHF diet control, as well as HFHF + brown rice, HFHF + white rice, and HFHF + γ-oryzanol combination diet. The experiment was carried out for 26 weeks, with details of 14 weeks to form an obese model and 12 weeks for the intervention. Before statistical correlation was tested, Lee index values, adipose tissues, and PRDM16 were analyzed. The anthropometric data collection method was carried out by weighing before and after the intervention, while adipose tissue was collected by weighing after sacrifice. The immunofluorescence method was used to collect the expression of PRDM16 and the mean of PRDM16 levels was analyzed in the ImageJ application. After the data collection process, analysis was performed using SPSS to determine possible differences in each group. Normally distributed data were analyzed using One-Way ANOVA, while those without normal distribution were assessed using the Kruskall-Walis method and the Mann Whitney-U advanced test, with a p-value of <0.05 considered significantly different.

The result showed that there were differences among several groups regarding total body fat (p=0.012), WAT (p=0.026), and BAT (p=0.025). However, no differences were found between all groups regarding the Lee index (p=0.275), BeAT (p=0.079), and PRDM16 level (p=0.292).

In conclusion, brown rice intervention did not significantly affect Lee index values, the expression of PRDM16, and adipose tissue weights at the end of the intervention.

Introduction

Obesity is a chronic metabolic disease caused by the prolonged buildup of energy in the body, leading to fat tissue accumulation. The global cases are increasing each year, with the prevalence reaching 650 million in 2016. In Indonesia, the prevalence among adults jumped from 6.4% to 21.8% between 2013 and 2018.[1] Considering that obesity plays a significant role in developing metabolic syndrome,[2] fast and appropriate interventions are needed.

Several factors can be a risk for obesity with diet being the most influential[3] followed by genetics, lifestyle, physical activity, environment, health, and psychology.[3] Obesity interventions are focused on dietary habits, physical activity improvement, and lifestyle changes. In this context, highly aggressive methods capable of causing side effects such as pharmacological and surgical are usually not recommended.[4] However, obesity with complications can be treated by using several methods consisting of diet, physical exercise, behavior modification, pharmacological therapy, and bariatric surgery.[4]

Brown rice is one of the functional foods which has antiobesity and antihyperglycemic effects. Due to the intact outer skin layer and aleurone,[5] brown rice has better potential nutritional content compared to white rice which contains dietary fiber, minerals, and γ-oryzanol, a bioactive compound useful as an antiobesity agent.[6,7] With this content, brown rice is capable of influencing parameters of obesity, including body anthropometry and PRDM16 levels.

According to a previous study, obesity is not associated with total body weight but specifically with the quantity of white adipose tissue (WAT)[8] which accumulates the excessive energy into triglyceride form. There is also brown adipose tissue (BAT) playing an important role in metabolizing fatty acids to produce heat,[9] although the amount is often lower than WAT in obese people.[8] Specific interventions such as maintaining diet and physical activity can cause white adipocytes to form beige adipocytes in browning activity. This process indirectly affects the body weight of rats and improves the anthropometric profile in animal models of obesity.

Positive regulatory domain zinc finger region protein 16 (PRDM16), a gene number 1p36.32 on the human chromosome, can inhibit the formation of WAT while increasing the formation of brown and beige adipocytes.[10,11] It increases biogenesis in tissue mitochondria and activates several transcription factors, such as PPAR-γ, to enhance the expression of uncoupling protein 1 (UCP1), which potentially produces heat and stimulates browning in WAT. One effort to trigger browning is diet regulation through calorie restriction.[12]

Studies related to the relationship between brown rice and body fat have been carried out on both experimental animals and humans. The results showed that brown rice had a positive effect on glucose and body fat levels in 18 diabetes mellitus patients who were treated for 3 months.[5] After the intervention was given, there was a decrease in poor blood glucose control by 27.7%, high fat by 22.2%, and high visceral fat index by 22.2%.[5] In obese experimental animals, brown rice effectively improved the *Fermicutes/Bakteriodetes* microbiota ratio.[13,14] The supplementation was proven to significantly reduce fat levels in the liver and prevent the development of NAFLD in obese model rats.[15]

γ-oryzanol, one of the bioactive compounds in brown rice, plays a significant role in regulating body weight and preventing several diseases including hyperglycemia, hypertriglyceridemia, blood vessel disorders, and renal damage. It acts as an antioxidant and anti-inflammatory agent, suppressing inflammation in conditions of obesity.[16] On average, brown rice contains 10-150 mg/100 g of γ-oryzanol.[17] This presents an opportunity since the majority of Indonesians still consume white rice which potentially has a high glycemic index (79.6).[18] In general, food with a high glycemic index contributes to obesity and type 2 diabetes mellitus.

Studies regarding the effect of functional food on PRDM16 levels are limited. Therefore, this study aimed to examine differences in Lee index, BAT weight, WAT weight, BeAT weight, total body fat, and PRDM16 levels in obesity model rats given brown and white rice as well as γ-oryzanol interventions. The study novelty sets in examining the difference in PRDM16 levels and anthropometry of obesity model rats with functional food interventions of brown rice compared to white rice and γ-oryzanol. The results are expected to offer insights into brown rice as a functional food against obesity and metabolic syndrome. In addition, this study can be used as a reference in developing functional foods at the human level.

Materials and Methods

Study Design

A true experimental method was used with a post-test-only control group in vivo design. The inclusion criteria were male Sprague Dawley *(Rattus norvegicus)* with age 10-12 weeks, body weight 200-250 g, in active condition (shiny fur, no alopecia, normal extremities), and free of any treatment or chemical intake. This study was approved by the Health Research Ethics Committee, Faculty of Health Sciences, Brawijaya University No. 2020/UN10.F17.10.4/TU/2023. All procedures were carried out in collaboration with the Experimental Animal Care Laboratory, Faculty of Medicine, Universitas Brawijaya.

Study Subjects

The samples were subjected to diet intervention with brown rice (BRG), white rice (WRG), and pure γ-oryzanol (ORG) combined with high-fat high fructose (HFHF) diet. In addition, samples had both positive (PG) and negative controls (NG). Positive control was a group of rats on HFHF diet, while the negative control was on a standard diet.

Instruments Development and Data Collection

Anthropometric data were collected by measuring the body weight and length of rats before and after intervention. Subsequently, BMI and Lee index were calculated using the data obtained. When Lee index value was > 0.3 then rats were categorized as obese. An analysis was further carried out to examine possible differences in each group of rats before and after intervention. Data on the weight of adipose tissue were collected by weighing after rats were sacrificed. Adipose tissue weighed included WAT, BAT, BeAT, and Total Body Fat.

Rats were sacrificed for the assessment of WAT, BAT, and BeAT. Fat tissues were converted to slides before heating (60°C in 60 minutes) and soaking in xliol (2 x 10 minutes), absolute ethanol (2 x 10 minutes), ethanol 90% (1 x 5 minutes), ethanol 80% (1 x 5 minutes), and ethanol 70% (1 x 5 minutes). Subsequently, the slides were subjected to antigen retrieval process with citric buffer. Washing was carried out using PBS solution followed by incubation with BSA

1% for 30 minutes at room temperature before incubation with primary antibody at 4°C overnight. PBS washing was initially performed before the use of secondary antibody and DAPI incubation. Fat slides were then subjected to a staining process using the immunofluorescence method. The staining results were examined using an immunofluorescence microscope and the ImageJ to select tissue sections and analyze the average PRDM16 levels.

Data Analysis

Statistical analysis of data was conducted using IBM SPSS Statistics. The obtained data were tested for normality. For normally distributed data, a homogeneity test was initially performed, and after meeting a p-value> 0.05, One-Way ANOVA analysis was conducted to determine the differences in three or more unpaired groups. For abnormal distribution, the data was analyzed using the Kruskal-Wallis test method to determine differences in three or more unpaired groups. A significant difference between the groups was indicated by a p-value of <0.05. The analysis was continued with the Mann-Whitney U test carried out per two groups, and a p-value of < 0.05 indicated a significant difference.

Results and Discussion

Brown rice differs from other varieties due to the name which reflects the original color. This variety is subjected to only minimal processing, leaving the outer layer intact, including the rice bran.[13] According to previous studies, brown rice has a high fiber content with seven times greater magnesium and manganese content than white rice.[14] The fiber and mineral content has been proven to reduce gut microbiota dysbiosis, increase serum magnesium levels, as well as prevent rising serum magnesium levels in obese experimental animals.[6,14]

Based on the results, there was no significant difference between the control and intervention groups (Table 1). This was in contrast to a study conducted on the substitution of local brown rice varieties for anthropometry and improving blood glucose levels in patients with type 2 diabetes mellitus.[19] The result showed that brown rice intervention significantly reduced body weight, BMI, body fat percentage, abdominal circumference, fasting blood glucose, 2-hour postprandial blood glucose, and HbA1C.[19] The difference between the results could be caused by a non-significant difference between the average energy food intake of rats in each group. However, the administration of white rice and γ-oryzanol intervention showed a positive trend toward improving Lee index compared to positive control.

Body weight results of experimental animals were also influenced by total body fat. Based on the results of statistical tests, significant total body fat differences were found between positive and negative control groups (p-value=0.001), negative control and white rice intervention (p-value=0.035), as well as negative control and γ-oryzanol group (p- value=0.085). The highest average total body fat weight was found in positive control (26.6 ± 9.2 g), followed by brown rice intervention group (19.27 ± 10.8 g) (Table 1).

The result differed from a previous study where the consumption of brown rice affected the inhibition of fat accumulation, ultimately reducing the risk of central obesity in patients with type 2 diabetes mellitus.[20] The variation between the results could be caused by a non-significant difference between the average dietary intake of rats in each group. Brown rice diet intervention group had a lower average intake compared to positive control, negative control, and white rice intervention groups according to their average energy intake. Therefore, the bioactive content in brown rice did not provide significant benefits for reducing body weight and total body fat.

As shown in Table 1, there were no significant differences between PRDM16 levels in the control and the intervention group (p-value=0.292). However, the highest levels were found in brown rice intervention group (9.5 ± 8 relative mRNA levels). Brown rice has antiobesity and antidiabetic properties, attributed to aminobutyric acid (GABA), inositols, tocotrienols, ferulic acid, oryzanol, and methanol.[21,22] Compounds such as ferulic acid can block adipocyte production, while methanol potentially reduces the formation of transcription factors and adipogenic genes, including EHMT1 and CtBP1/2.[21,22,23] These results were consistent with the previous study about PRDM16 stating that PRDM16 was found to play a crucial role in adipocyte differentiation.[11] Antiobesity and antidiabetic intake of brown rice can cause adipocyte differentiation, including inhibition of production and transcription factors by increasing PRDM16 levels.

Generally, white adipocytes are stored in the body in WAT, and in conditions of obesity, the composition tends to be more significant due to the accumulation of unused energy in the form of triacylglycerol.[24] In this study, visceral and subcutaneous fat were taken as WAT samples, BAT was collected from the interscapular region, and BeAT was acquired from the testicular area. According to a previous study, visceral fat stores large circulating FFA and inflammatory markers, including IL-6, CRP, and TNF-α, which increase the risk of CVD.[25] The smallest average weight of WAT was found in white rice intervention group (8.65 ± 6.54 g), while the largest was recorded in brown rice (11.3 ± 6.08 g). This result was not in line with PRDM16 levels which were significantly high in brown rice intervention group, suggesting WAT weight obtained might be influenced by several other tissues taken during the sacrifice. A previous study about brown and beige adipose tissue explained that BeAT could appear in subcutaneous WAT storage, particularly in the anterior subcutaneous and inguinal areas.[26]

An ineffective diet was also implicated because the fiber, phenolic acid, and γ-oryzanol content of brown rice were not consumed to an extent capable of causing WAT browning.[27] A study comparing different laboratory feeding methods in rats found that the group given AUTO, a daily feeding method set to reduce body weight by up to 90-95%, showed better results in weight loss compared to ad libitum feeding.[28] Future studies should expand the investigation with a feeding method that has been regulated for weight loss.

According to previous reports, PRDM16 has two mechanisms in WAT differentiation process.[11] In the first mechanism, PRDM16 induces the formation of brown fat-related genes (PGC 1α, PGC-1β, PPARγ, uncoupling protein 1 (UCP1)) and forms a complex to cause thermogenesis effects on fat. This initiates the browning process, leading to the formation of BAT (Figure 1). High levels of PRDM16 in the brown rice intervention group resulted in comparable BAT weight. There was a significant difference in BAT between negative and positive control group, negative control and γ-oryzanol intervention, as well as positive control and white rice intervention group (Table 1). The highest average weight of BAT was found in the brown rice intervention group (2.81 ± 1.6 g) followed by positive control (2.97 ± 1.27 g), and white rice intervention group (1.36 ± 0.42 g).

The second mechanism is the formation of BeAT initiated by the conversion and secretion of the metabolite, β-hydroxybutyrate (BHB) from mature WAT.[11,23] This activity potentially increases beige adipogenesis and UCP-1 stimulation of adiponectin.[11,23] UCP-1 supports the formation of brown fat genes while reducing the expression of fiber precursors that cause fibrosis in fat tissue (Figure 1).[11] Statistical analysis showed that there were no significant differences between groups regarding the beige adipose tissue (p-value=0.079) (Figure 2). However, the average BeAT weight of brown rice intervention group was greater than the other groups (5.17 ± 3.58 g). This is in line with the theory stating that BeAT tissue has higher expression of UCP1 and PRDM16 gene.[29]

Further studies are needed regarding the appropriate levels of brown rice to support WAT browning process in a specific time limit. Appropriate control of feeding and environmental conditions also needs to be considered to achieve optimal intervention results. Moreover, each rat has varying metabolic abilities, which greatly determines the results of using in vivo methods.

Conclusions

In conclusion, the intervention of brown and white rice, as well as pure γ-oryzanol for 12 weeks did not show significant differences in anthropometric changes and PRDM16 levels. This study should be further conducted with a feeding method that had been adjusted for weight loss based on body weight reference of rats to obtain more significant results.

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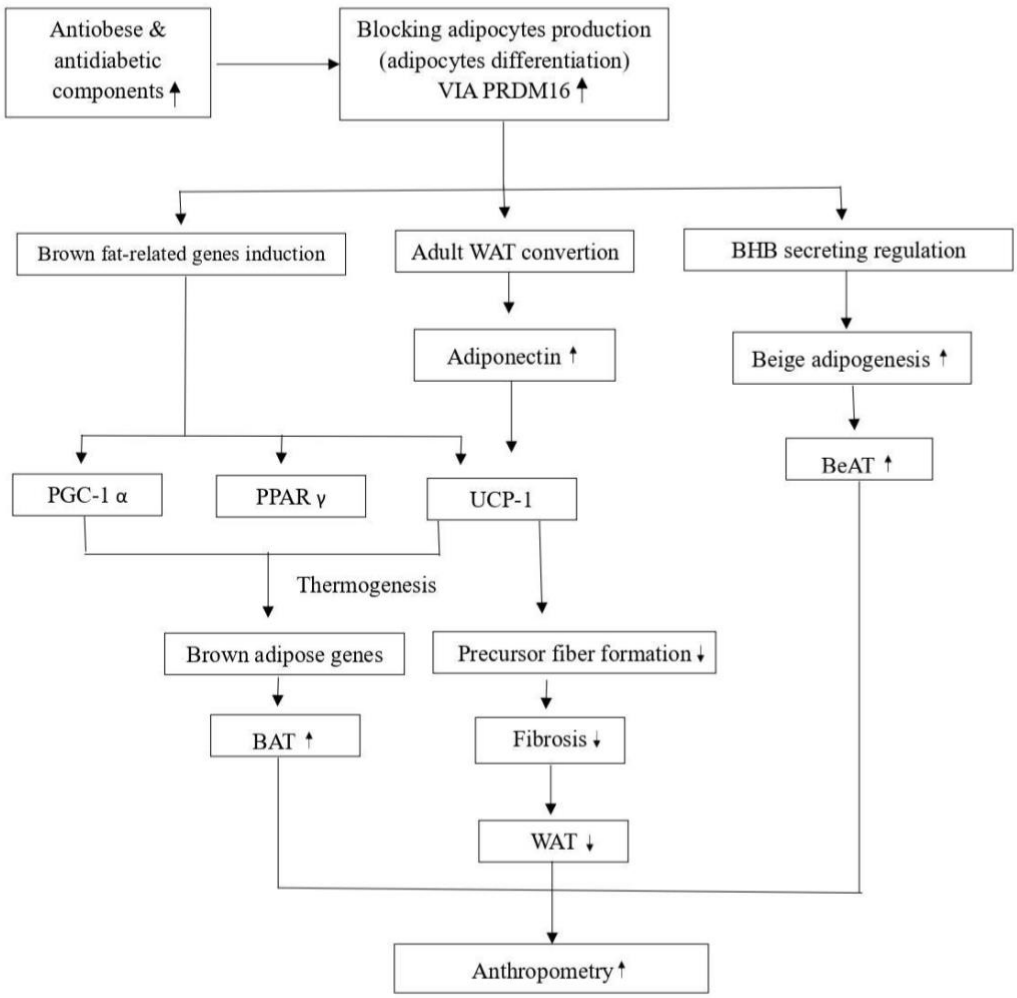
Table 1. Data of Diet, Anthropometric, Body Fat, and PRDM16 Average

Parameter	NG	PG	K1 (BRG)	K2 (WRG)	K3 (ORG)	p-value
			Diet			
Dietary Intake (kcal)	65.94 ± 8.98	66.58 ± 18.73	65.11 ± 11.03	68.85 ± 26.65	49.30 ± 5.54	0.163*
			Anthropometric			
Initial Body Weight (g)	224.07 ± 29.3	232.65 ± 15.7	217.26 ± 15.01	215.37 ± 8.81	223.41 ± 10.75	0.405**
Final Body Weight (g)	283.74 ± 37.66	384.99 ± 13.96	325.73 ± 61.2	280.6 ± 46.22	312.18 ± 32.88	0.001**
Lee Index (g/cm)	297.52 ± 13	309.76 ± 9.36	310.66 ± 16.56	301.07 ± 18.7	308.14 ± 8.5	0.275*
			Body Fat			
Total Body Fat (g)	11.57 ± 3.35^{a}	26.6 ± 9.2^{b}	19.27 ± 10.8^{ab}	14.2 ± 8.37^{a}	16.01 ± 5.83^{a}	0.012*
WAT (g)	6.98 ± 2.35^{a}	17.03 ± 6.19^{b}	11.3 ± 6.08^{ab}	8.65 ± 6.54^{a}	9.01 ± 3.44^{a}	0.026**
BAT (g)	1.36 ± 0.42^{a}	2.97 ± 1.27^{c}	2.81 ± 1.6^{abc}	1.92 ± 1.4^{ab}	2.45 ± 0.8^{bc}	0.025**
BeAT (g)	3.23 ± 0.79	6.6 ± 2.33	5.17 ± 3.58	3.63 ± 1.67	4.55 ± 1.8	0.079**
PRDM16 Mean (relative mRNA levels)	4.8 ± 5.74	8.65 ± 7.16	9.5 ± 8	5.77 ± 5.01	3.91 ± 1.97	0.292**

Notes: *One Way ANOVA Test. **Kruskal Wallis Test. A significant difference if p value<0.05 showed between groups marked by different annotations (a,b,c,d,e). NG: Negative control. PG: Positive control. K1: HFHF + brown rice (BRG) diet. K2: HFHF + white rice (WRG) diet. K3: HFHF + pure gamma-oryzanol (ORG) diet.



(Modified from Chi, 2021; Jiang et al, 2022)

Figure 1. **Mechanism of Functional Food Affecting the Components of Adipose Tissue**