Magnesium-Rich Indonesian Brown Rice 'Sintanur' Improves Insulin Sensitivity in High Fat High Fructose Diet-Induced Obesity Sprague Dawley Rats

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Abstract: This study aimed to analyze the effect of 'Sintanur' brown rice on Lee's index, fasting blood glucose levels, and HOMA-IR administered to male Sprague Dawley rats. This research was an experimental laboratory study with a post-test-only control group design. The subjects were thirty-five male Sprague Dawley rats divided into five groups. Group 1 consisted of the negative control with a standard diet. Group 2 consisted of the positive control with HFFD-induced obesity for 20 weeks, while groups 3, 4, and 5 were the treatment groups with HFFD-induced obesity for 12 weeks, which were intervened with different dosages of brown rice diet from week 13 to week 20. At week 21, the rats were sacrificed. Fasting blood glucose levels were tested using a glucometer. Fasting serum insulin levels were tested using ELISA. HOMA-IR was calculated using fasting glucose and insulin levels. Serum magnesium levels were tested using Atomic Absorption Spectrophotometry. A non-parametric test of Kruskal-Wallis was used to analyze differences in mean dietary intake, Lee index, fasting blood glucose, and HOMA-IR. As a result, there were significant differences between groups ($p<0.05$). Spearman correlation test was used to analyze the relationship between the Lee index, fasting blood glucose levels, and HOMA-IR with serum magnesium levels. As a result, there were negative correlations between parameters ($r=-0.299$; $r=-0.393$; $r=-0.257$). Group 5 had the best results in lowering insulin resistance. In conclusion, consuming local 'Sintanur' brown rice decreased the Lee index, fasting blood glucose levels, and HOMA-IR by increasing serum magnesium levels in obese rats. High magnesium intake reduces insulin resistance by correcting the disruption of glucose metabolism and insulin signaling pathways.

Key-Words: Brown Rice; Insulin Resistance; HOMA-IR; Magnesium; Obesity

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1 Introduction

Obesity is an accumulation of excessive fat in the body that can impair health, [1]. Body mass index (BMI) is used for clinical obesity screening, [2]. A BMI same as or more than 30 kg/m2 is considered obese, [1]. In Indonesia, the prevalence of obesity in the adult population reaches 23.1% , [3]. The risk factors of obesity include an energy imbalance, genetic, and socioeconomic determinants, [4], [5], [6]. Some of the comorbidities associated with obesity are type 2 diabetes, osteoarthritis, hypertension, congestive heart failure, coronary artery disease, pulmonary embolism, and cerebrovascular accident, [7]. Type II diabetes mellitus is the most common type of diabetes today and is characterized by a series of malfunctions such as insulin resistance, the inadequate secretion of insulin and excessive or inappropriate glucagon secretion. This type of diabetes affects mainly adults, [8], [9].

Obesity is related to type 2 diabetes based on its ability to induce insulin resistance. Insulin is a hormone secreted by pancreatic beta cells. It is the most important hormone in the regulation of blood glucose levels, [10].

Insulin resistance is a decrease in the ability of tissues to respond to insulin action, [11]. In obese individuals, adipocytes lose their effectiveness in responding to insulin's antilipolytic action, [12]. Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) is a method to predict the occurrence of insulin resistance by using the fasting blood glucose and fasting serum insulin level in the calculation. The cut-off values differ depending on

race, age, gender, and disease, [13]. Obese individuals have higher levels of HOMA-IR than those with average body weight, indicating higher insulin resistance, [14].

Maintaining diet, physical activity, adherence to treatment, and education may control type 2 diabetes, [15]. Dietary regulation can stabilize blood glucose and lipid levels within the normal range, [16]. Brown rice is a highly nutritious food obtained through a milling process by removing the husk from the paddy to obtain rice grain that contains a brown outer layer of bran, [17]. 'Sintanur' is one of the brown rice variants in Indonesia. A 100 grams of local 'Sintanur' brown rice contains 386.67 calories of energy, 8.39 grams of protein, 86.19 grams of carbohydrates, 0.91 grams of fat, 22.04 grams of fiber, 230 mg of magnesium, 4.41 mg of manganese, and 340 mg of potassium. The micronutrients in local 'Sintanur' brown rice are higher than those in white rice of the same variety, i.e., magnesium content is seven times higher, [18]. Local brown rice also has a higher fiber content than local white rice. Therefore, brown rice has a longer digestion time and lower glycemic index, [19], [20].

Indonesian local brown rice 'Sintanur' is a good magnesium source, [18]. Magnesium contributes to the insulin-mediated regulation of glucose absorption and increases insulin sensitivity, [20]. Numerous enzymes in the metabolic cycles need Mg2+ or MgATP as a cofactor throughout the processes. Mg2+ may immediately impact the glucokinase activity in pancreatic β-cells since the glucokinase action relies upon MgATP. Glucokinase is needed for converting glucose to glucose-6-phosphatase (G6P), [21]. Serum magnesium homeostasis is regulated by the reabsorption of magnesium in the kidneys. Serum magnesium levels were found to be inversely correlated with blood glucose levels. Renal tubular reabsorption of magnesium decreases in the presence of severe hyperglycemia, [22].

A trial on overweight, non-diabetic subjects shows significant evidence that oral magnesium supplementation (365 mg/day) for six months improves insulin sensitivity, [23]. A Japanese study reports that the consumption of brown rice may be advantageous because of its effect on lowering glycemic response and protecting postprandial endothelial function in individuals with metabolic syndrome. It also helps lower body weight and insulin resistance risk, [24]. A study in India on overweight subjects shows that consumption of brown rice can reduce blood glucose and fasting insulin responses, [25]. Research in Malaysia suggests that consumption of brown rice on female Sprague Dawley rats shows better oral glucose tolerance test, lower weights, and HOMA-IR values compared to the white rice group, [26].

2 Materials and Methods

2.1 Research Design

This study used an experimental laboratory method and a post-test-only control group design. This study was part of extensive research exploring the overall benefits of Indonesian local brown rice 'Sintanur' and received a certificate of ethical eligibility from the Health Research Ethics Commission, Faculty of Medicine, Universitas Brawijaya.

2.2 Subjects

The subjects of this study were 35 Sprague Dawley male rats, white fur, ages 70-90 days post-natal, and weights 200-250 grams. The sample quantity was calculated by using Federer's formula. The rats were obtained from the Animal Laboratory of Institut Pertanian Bogor, Bogor, Indonesia. The rats were treated at the Biosciences Institute, Universitas Brawijaya, Malang, Indonesia. The brown rice used in the study was Indonesia's local brown rice, 'Sintanur'. The rice was purchased from a local market. The subjects were randomly divided into five groups. Group 1 was the negative control with a standard diet AIN-93M. Group 2 was the positive control with HFFD (High Fat, High Fructose Diet)-induced obesity for 20 weeks. Groups 3, 4, and 5 were the treatment groups with HFFD-induced obesity for 12 weeks, which were intervened with different dosages of brown rice diet from week 13 to week 20. At week 21, the rats were sacrificed by ketamine injection, and the parameters were tested. During the research period, three experimental animals died (dropped out). Therefore, there were 32 rats at the end of the study, namely seven in group 1, six in group 2, five in group 3, seven in group 4, and seven in group 5.

2.3 Dietary Intake

The standard diet consisted of cornstarch, dextrinized cornstarch, sucrose, soybean, gelatin, fish flour, casein, egg white, fiber, minerals, vitamins, L-cystine, and choline bitartrate. HFFD was a modification that substituted carbohydrate and protein sources in the standard diet with animal fat, lard, and 30% fructose. Rice consumption per capita in March 2015 was 98 kilograms/year or 370-380 grams/day, [27]. The carbohydrate content of brown rice added replaced the carbohydrate source from

HFFD. Rat feed consumption was 15-30 grams/day, and an average of 20 grams/day was used. The addition of brown rice to rat feed per 20 grams was converted to 0.018 as a human-to-mouse dose conversion factor, [28]. In humans, brown rice needed per day was 125 grams for one meal, 250 grams for two meals, and 375 grams for three meals. Conversion to rat feed per day was 2.25 grams, 4.5 grams, and 6.75 grams. In making 1000 grams of feed, 112.5 grams of brown rice were added for dose I (group 3), 225 grams of brown rice was added for dose II (group 4), and 337.5 grams of brown rice were added for dose III (group 5) to substitute cornstarch, dextrinized cornstarch, sucrose, and fructose (carbohydrate source in rat feed).

2.4 Parameters Test

The blood glucose levels were examined using Nesco's glucometer. The fasting serum insulin levels were examined using Bioassay Technology Laboratory's ELISA kit, [29]. The HOMA-IR values were calculated by multiplying fasting blood glucose (mg/dL) and fasting serum insulin (μU/mL) levels, divided by 405. The Lee index was obtained by calculating the cube root of body weight (grams), divided by the length of the nasal-anal (cm), and multiplied by 1000. This study was a part of extensive research; the serum magnesium levels were already examined using Atomic Absorption Spectrophotometry, [28].

2.5 Data Analysis

Data were analyzed using IBM SPSS 26.0 for windows, with a significance level of 0.05 and a confidence level of 95% ($p<0.05$ indicates a significant difference/relationship). ANOVA (normal distribution and homogeneous data) or Kruskal-Wallis (abnormal distribution and heterogeneous data) test was used to analyze the difference in mean data of dietary intake, Lee index, fasting blood glucose levels, and HOMA-IR values between treatment groups. The Post Hoc Tukey HSD (normal distribution and homogeneous data) or Mann-Whitney (abnormal distribution and heterogeneous data) test was used to analyze further where the difference lay. The Pearson (normal distribution and homogeneous data) or Spearman correlation test (normally distributed data), or Spearman (abnormal distribution and heterogeneous data) test was used to analyze the correlation between the Lee index, fasting blood glucose levels, and HOMA-IR values with serum magnesium levels.

3 Results and Discussion

3.1 Dietary Intake

Based on the normality and homogeneity tests, the only normally distributed and homogeneous data was the fructose intake data ($p>0.05$). Therefore, the ANOVA test was used on fructose intake data. The Kruskal Wallis tests were used on average daily feed, brown rice, fiber, and total energy intake data. As a result, significant differences were found in the data on average daily feed intake ($p=0.000$), brown rice ($p=0.000$), fructose intake ($p=0.000$), fiber ($p=0.001$), and total energy ($p=0.002$). Group 2 had the highest total energy, and group 1 had the lowest total energy. Group 5 had the lowest total energy among the brown rice intervention groups. Group 1 had the highest average daily feed intake (13.60±2.78 g), while group 5 had the lowest (7.94±0.71 g).

Table 1. The Average Daily Intake during the Intervention

Parameter	Group				
	1	2	3	4	5
Average daily feed intake (g)#	13.60 ± 2.78[a]	11.32 ± 1.16[a,b]	10.09 ± 1.29[b]	8.33 ± 0.91[c]	7.94 ± 0.71[c]
Brown rice (g)#	0.00 ± 0.00	0.00 ± 0.00	1.25 ± 0.16[a]	2.07 ± 0.23[b]	2.96 ± 0.26[c]
Fructose intake (ml)*	0.00 ± 0.00	23.01 ± 3.83[a]	33.94 ± 3.59[b]	33.70 ± 4.13[b]	33.72 ± 3.31[b]
Fiber (g)#	3.86 ± 0.79[a]	2.75 ± 0.28[b]	2.86 ± 0.28[b,c]	2.89 ± 0.32[b,c]	3.17 ± 0.28[c]
Total energy (kcal)#	55.62 ± 11.38[a]	80.47 ± 7.75[b,c]	86.62 ± 7.01[b]	78.07 ± 5.25[b,c]	76.21 ± 5.03[c]

Note:
The data are mean ± standard deviation
*One Way Anova test $p<0.05$; #Kruskal Wallis test $p<0.05$
Data accompanied by different notations show significant differences
Group 1: Negative control
Group 2: Positive control
Group 3: HFFD + brown rice dose I (11.25%)
Group 4: HFFD + brown rice dose II (22.5%)
Group 5: HFFD + brown rice dose III (33.75%)

3.2 Lee Index

Based on the normality test, Lee index data was not normally distributed ($p<0.05$). Therefore, the Kruskal-Wallis and Post Hoc Mann-Whitney tests were used to see the relationship between variables. The average Lee index at the end of the intervention significantly differed between groups ($p=0.000$)

(Figure 1). Group 2 (positive control) had the highest average Lee index, while group 1 (negative control) had the lowest average Lee index. The Lee index values in all rats administered with Sintanur rice (groups 3, 4, and 5) were lower than in group 2. Lee index was observed throughout the intervention; the changes were 7.43±12.03 g/cm^3 in group 1, 4.50±9.57 g/cm^3 in group 2, -10.77±7.77 g/cm^3 in group 3, -16.61±7.77 g/cm^3 in group 4, and -17.29±12.39 g/cm^3 in group 5. Group 5 had the most significant decrease in the Lee index.

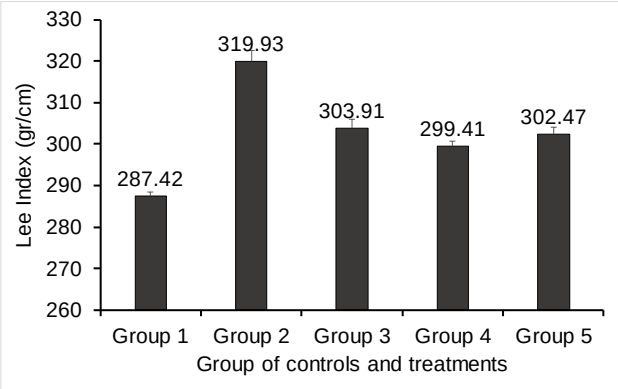

Fig. 1: Average Lee Index at the End of Intervention

Note:
The data are mean ± standard deviation
#Kruskal Wallis test p<0.05
Data accompanied by different notations show significant differences
Group 1: Negative control
Group 2: Positive control
Group 3: HFFD + brown rice dose I (11.25%)
Group 4: HFFD + brown rice dose II (22.5%)
Group 5: HFFD + brown rice dose III (33.75%)

Correlation analysis showed that the Lee index and serum magnesium levels had a fair negative correlation, but it was insignificant (r=-0.299; p=0.060) (Figure 2).

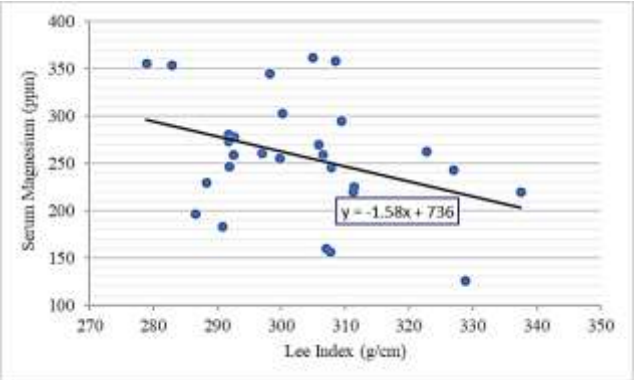

Fig. 2: The Spearman Correlation Test between the Lee Index and Serum Magnesium Levels

The Lee index negatively correlated with serum magnesium levels but was insignificant (r=-0.299; p=0.060).

3.3 Fasting Blood Glucose

Based on the normality and homogeneity tests, fasting blood glucose data were normally distributed (p>0.05) but heterogeneous (p<0.05). Therefore, the Kruskal-Wallis and Post Hoc Mann-Whitney tests were used to see the relationship between variables. The average fasting blood glucose levels significantly differed between groups (p=0.000) (Figure 3), but group 5 had no significant difference from group 1 (negative control). Group 2 had the highest average fasting blood glucose levels, while group 1 had the lowest average fasting blood glucose levels. Group 5 had the lowest average fasting blood glucose levels among the brown rice intervention groups.

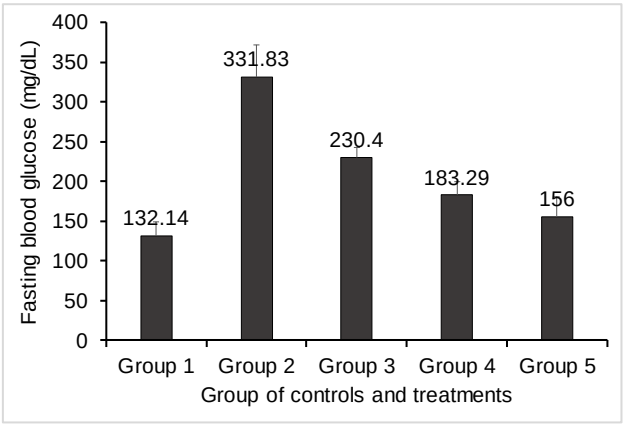

Fig. 3: Average Fasting Blood Glucose Levels at the End of Intervention

Note:
The data are mean ± standard deviation
#Kruskal Wallis test p<0.05
Data accompanied by different notations show significant differences
Group 1: Negative control
Group 2: Positive control
Group 3: HFFD + brown rice dose I (11.25%)
Group 4: HFFD + brown rice dose II (22.5%)
Group 5: HFFD + brown rice dose III (33.75%)

Correlation analysis showed that the fasting blood glucose levels and serum magnesium levels had a significantly fair negative correlation (r=-0.393; p=0.019) (Figure 4). The lower the serum magnesium levels, the higher the fasting blood glucose levels.

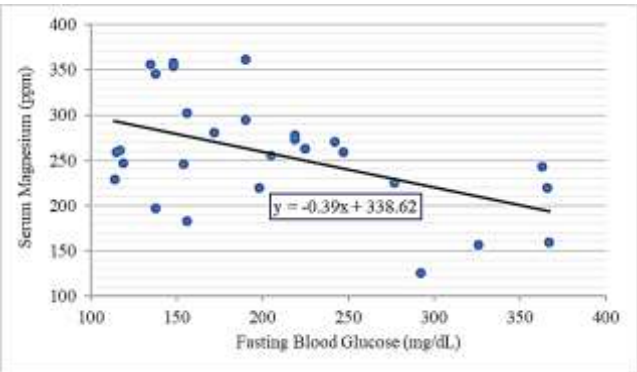

Fig. 4: The Spearman Correlation Test between Fasting Blood Glucose Levels and Serum Magnesium Levels

The fasting blood glucose levels negatively correlated with serum magnesium levels, significant (r=-0.393; p=0.019).

3.4 HOMA-IR

Based on the normality test, HOMA-IR data was not normally distributed ($p<0.05$). Therefore, the Kruskal-Wallis and Post Hoc Mann-Whitney tests were used to see the relationship between variables. The average HOMA-IR values significantly differed between groups (p=0.003) (Figure 5), but group 5 had no significant difference from group 1 (negative control). Group 2 had the highest average HOMA-IR, and group 1 had the lowest average HOMA-IR. Group 5 had the lowest average HOMA-IR among the brown rice intervention groups.

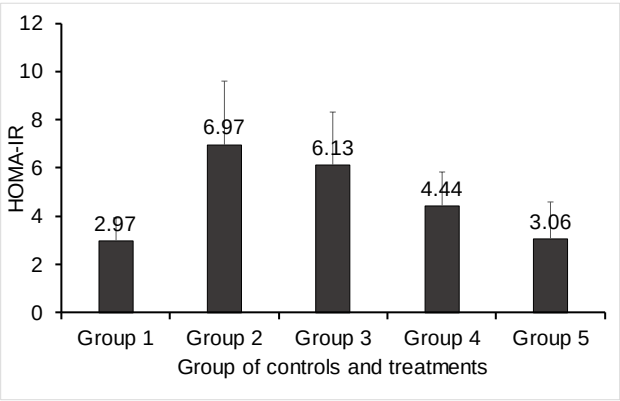

Fig. 5: Average HOMA-IR values at the End of Intervention

Note:
The data are mean ± standard deviation
#Kruskal Wallis test $p<0.05$
Data accompanied by different notations show significant differences
Group 1: Negative control
Group 2: Positive control
Group 3: HFFD + brown rice dose I (11.25%)
Group 4: HFFD + brown rice dose II (22.5%)
Group 5: HFFD + brown rice dose III (33.75%)

Correlation analysis showed that the HOMA-IR values and serum magnesium levels had a fair negative correlation but were insignificant (r=-0.257; p=0.093) (Figure 6).

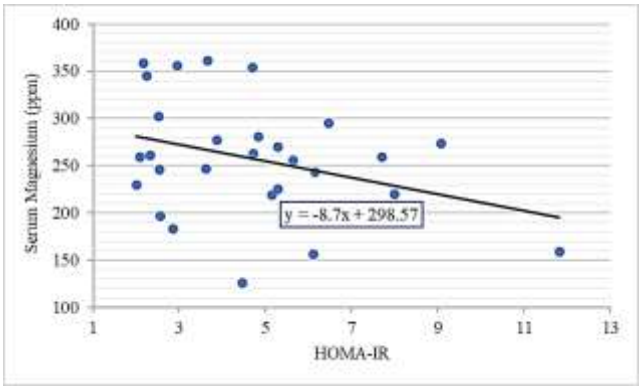

Fig. 6: The Spearman Correlation Test between HOMA-IR Values and Serum Magnesium Levels

The HOMA-IR values negatively correlated with serum magnesium levels but were insignificant (r=-0.257; p=0.093).

3.5 Discussion

Body mass index is a typical formula to describe levels of obesity in humans, the same as the Lee index in an animal experiment, [30]. Lee index is calculated by dividing the cube root of body weight (g) by the length of the nasal-anal (cm), then multiplied by 1000. Obesity is Lee index above 300, [31]. Obesity occurs due to an imbalance between energy needs and intake. A high-fat and high-fructose diet can lead to a positive energy balance and obesity, [32]. Obesity can increase the risk of type 2 diabetes, [33]. Meanwhile, a healthy diet can reduce the risk of type 2 diabetes, [34].

Rice meets the energy needs of billions of people worldwide. Rice quality is currently focused more on excellent taste and less on nutrition, mainly due to the milling process that removes the nutritious bran (white rice), [35]. Meanwhile, Indonesian local brown rice 'Sintanur' still has nutritious bran. It contains relatively higher amounts of protein, fiber, unsaturated lipids, and micronutrients (Magnesium, Manganese, and Potassium) than the same variety of local white rice, [18]. This study examines the potential of 'Sintanur' local brown rice as a diet therapy for obesity.

In this study, group 1 was given a standard diet for twenty weeks. Group 2 was given an HFFD for twelve weeks until the Lee index>300 and then an HFFD again for another eight weeks. Groups 3, 4,

and 5 were given an HFFD for twelve weeks until the Lee index>300, then an HFFD modified with different dosages of brown rice diet for another eight weeks. During the intervention, significant differences were found in dietary intake data (average daily feed intake, brown rice, fructose intake, fiber, and total energy).

Among the three treatment groups, group 5 had the highest average intake of brown rice and fiber but had the lowest average daily feed intake and total energy. Local brown rice 'Sintanur' contains more fiber and energy than local white rice of the same variety. One hundred grams of local brown rice, 'Sintanur', contains 386.67 calories of energy and 22.04 grams of fiber, whereas, in the same variety, local white rice only contains 376.43 calories of energy and 20.58 grams of fiber, [18]. Soluble fiber can form a thick liquid in the digestive tract, so it takes longer to be digested in the stomach. Fiber will also attract water and give a more prolonged feeling of fullness, thus preventing from consuming more food, [19].

Based on the study results, there was a significant difference in Lee index values at the end of the intervention, especially between the controls and the intervention groups. The results showed that all rats administered with Sintanur rice (groups 3, 4, and 5) had a lower Lee index than the positive control (group 2). Group 5 had the most significant change in the Lee index among the treatment groups.

Brown rice intake is associated with weight loss and reduced adipocytes. Brown rice contains high dietary fiber. High dietary fiber is correlated with a low glycemic index because glucose digestion and absorption into the circulation occurs more slowly, [36]. Consumption of brown rice has been reported to reduce hunger and increase satiety, leading to lower energy intake, [24]. This study's result is consistent with a previous study in Malaysia, which showed that consumption of brown rice (30 kcal/100 grams body weight/day) in female Sprague Dawley rats for eight weeks reduced body weight and Lee index compared to white rice, [26].

Obesity is related to type 2 diabetes based on its ability to induce insulin resistance. Insulin resistance is a decrease in the ability of tissues to respond to insulin action, [11]. In obese individuals, adipocytes are ineffective in responding to the antilipolytic action of insulin, [12]. HOMA-IR is a method for predicting the occurrence of insulin resistance, by using fasting glucose levels and fasting insulin levels in the calculations, [13]. Individuals with obesity have higher HOMA-IR levels than those with average weight, indicating a higher risk of developing insulin resistance, [14].

This study showed significant differences in fasting blood glucose levels and HOMA-IR values at the end of the intervention. Among the three treatment groups, group 5 had the most significant difference from group 2 (positive control). This study's result is consistent with a previous study that found that after ten weeks of intervention, fasting blood glucose levels in the Sprague Dawley rat group fed a high-fat diet modified with brown rice (20%) were significantly lower ($p<0.05$) than positive controls, [37]. In addition, research stated that by consuming brown rice (30 kcal/100 grams body weight/day) for eight weeks in Sprague Dawley rats, fasting insulin levels and HOMA-IR are lower than white rice, [26].

Brown rice has high fiber content, [25]. Dietary fiber has consistently been associated with increased insulin sensitivity and reduced risk of type 2 diabetes, [38]. The fiber in food is negatively correlated with the glycemic index. High fiber can cause glucose absorption into circulation to occur more slowly, [19], [39].

The fiber in food can absorb water and bind glucose, thereby reducing glucose availability. A high-fiber diet can also cause the formation of complex carbohydrates and fiber, which reduces the digestibility of carbohydrates. This situation can reduce the increase in blood glucose levels and insulin demand, [39]. Foods with high fiber content and low glycemic index can act on digestion and absorption of nutrients by reducing glucose/insulin levels, chylomicron production and secretion, and de novo lipogenesis, [40]. Fiber can act through the fermentation of indigestible carbohydrates in the large intestine by improving hepatic glucose regulation. Fiber may also increase satiety signals in the hypothalamus, [41].

One hundred grams of Indonesian local brown rice, 'Sintanur', contains 230 mg of magnesium, whereas, in the same variety, local white rice only contains 30 mg of magnesium. The intake of local brown rice strongly correlates with serum magnesium levels, [18]. Based on the results of the Spearman correlation test, showed that the Lee index (insignificant)/ fasting blood glucose levels (significant)/ HOMA-IR values (insignificant) had negative correlations with magnesium levels.

Local brown rice, 'Sintanur', is a good magnesium source, [18]. Magnesium is a cofactor required in many enzymatic reactions and is involved in the metabolism of glucose and insulin homeostasis, [42]. Glucose enters the pancreatic β-cells by passing through glucose transporter type 2

(GLUT2). After that, glucokinase transforms glucose into glucose-6-phosphate (G6P), [21].

GLUT2 and glucokinase activity are glucose sensors that control blood glucose levels, [43]. Mg^{2+} can directly affect glucokinase by acting as a cofactor for adenine nucleotides. G6P, the enzyme reaction's product, is processed to form ATP. The KATP channel's opening depends on the binding of MgATP and SUR1 subunits. Meanwhile, the KATP channel's closure depends on the binding of ATP and Kir6.2 subunits, which causes depolarization of the membrane. It then triggers the Ca^{2+} influx through the L-type Ca^{2+} channels to initiate the release of insulin vesicles, [21].

Homeostasis of magnesium regulated by magnesium reabsorption in the kidneys is found to be inversely correlated with blood glucose levels. Hyperglycemia condition can decrease renal reabsorption of magnesium, [22]. Magnesium deficiency can disrupt glucokinase function, G6P formation, and ATP accumulation in the pancreatic β-cells. It can interfere with the closure of KATP channels and delay the initial and late phases of insulin responses to glucose. In magnesium deficiency, MgATP intracellular levels decrease. It can interfere with the opening of KATP channels and prolong the depolarization of the β-cells plasma membrane, which causes more insulin release. Thus, magnesium deficiency can lead to β-cells dysfunction in type 2 diabetes, [44].

Two major signaling pathways activate most insulin actions. The first signaling pathway is the Ras/mitogen-activated protein kinases (Ras/MAPK). It modulates the expression of genes and insulin-associated mitogenic reactions. The second signaling pathway is the phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt). It manages most insulin metabolic activities and significant functions in insulin signaling. Its activation leads to the phosphorylation of many substrates that play essential roles in biological processes, such as stimulation of glucose transport, synthesis of glycogen and protein, and lipogenesis. Akt has a crucial role in insulin metabolic actions, including glucose uptake in muscle and adipose tissue through glucose transporter type 4 (GLUT4) translocation from the intracellular compartments to the cell membrane, [45]. Magnesium deficiency can lead to insulin resistance associated with decreased PI3K/Akt pathway activity and impaired expression and function of GLUT4. These can reduce glucose uptake in muscle and adipose tissue and trigger changes in the metabolic level, [44].

Several international studies have provided relevant associations between magnesium and insulin resistance. Magnesium supplementation (365 mg/day) can reduce fasting blood glucose levels and insulin resistance in obese subjects, [23]. In one study, serum magnesium levels in diabetic subjects were significantly lower than in healthy controls ($p<0.001$), [46]. Animal studies have shown that giving magnesium supplements (50 mg/mL in drinking water) for six weeks lowers blood glucose levels, improves mitochondrial function and reduces oxidative stress in diabetic rats, [47]. In one study, it was found that there were significant negative correlations between serum magnesium levels with fasting insulin levels ($r=-0.396$, $p<0.01$) and the HOMA-IR ($r=-0.518$, $p<0.001$), [48]. Hence, correcting hypomagnesemia is expected to deliver better management of type 2 diabetes.

A magnesium deficiency can trigger beta-cell dysfunction in conditions of hyperglycemia and cause disruption of the main insulin signaling pathway and glucose uptake in muscle and adipose tissue, which triggers insulin resistance. Group 5 had the best results in reducing insulin resistance because it significantly reduced the Lee index, fasting blood glucose levels, and HOMA-IR values. The results of research using magnesium-rich Indonesian brown rice 'Sintanur' in experimental animals showed excellent benefits in reducing the risk of insulin resistance. The brown rice intervention in this study took eight weeks, the same as the brown rice intervention time in humans in studies in Japan, [24].

In other clinical medicine examples, a study involving 417 people with prediabetes or type 2 diabetes shows that a brown rice diet can lower body weight. It is discovered that brown rice may be used as a substitute for white rice in such patients, [49]. Research involving 60 people with impaired glucose tolerance demonstrates that replacing white rice with brown rice as a staple food may help control body weight and blood glucose, [50]. A study involving 58 Chinese Americans with prediabetes also verifies decreased HOMA in the brown rice group compared to the white rice group, [51]. Whole-grain intake has a protective association with type 2 diabetes risk by decreasing energy intake, preventing weight gain and increasing insulin sensitivity, [52].

4 Conclusion

Magnesium deficiency that happens in hyperglycemia conditions can lead to β-cells dysfunction. It also induces insulin resistance by disrupting the main insulin signaling pathways and impairing GLUT4 expression and function. The

intervention of Indonesian local brown rice 'Sintanur', which contains high magnesium content, can improve serum magnesium levels. The serum magnesium levels correlate negatively with the Lee index, fasting blood glucose levels, and HOMA-IR values in HFFD-induced obesity Sprague Dawley rats. The study's results using Indonesian local brown rice, 'Sintanur', shows a tremendous advantage in reducing the risk of insulin resistance, especially in group 5. Furthermore, it is necessary to carry out further research and its implementation in humans using local brown rice 'Sintanur' to test the benefits of local rice against obesity and insulin resistance it causes in humans. It is also necessary to research other local brown rice varieties.

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References

[1] WHO, "Obesity and overweight," *who.int*, Apr. 01, 2020. https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight (accessed Oct. 29, 2020).

[2] C. Arroyo-Johnson and K. D. Mincey, "Obesity epidemiology trends by race/ethnicity, gender, and education: National Health Interview Survey, 1997–2012," *Gastroenterology Clinics of North America*, vol. 45, no. 4, pp. 571–9, 2016, doi: 10.1016/j.gtc.2016.07.012.Obesity.

[3] D. S. Harbuwono, L. A. Pramono, E. Yunir, and I. Subekti, "Obesity and central obesity in indonesia: Evidence from a national health survey," *Medical Journal of Indonesia*, vol. 27, no. 2, pp. 53–59, Jun. 2018, doi: 10.13181/mji.v27i2.1512.

[4] K. D. Hall *et al.*, "Quantification of the effect of energy imbalance on bodyweight," *The Lancet*, vol. 378, no. 9793. pp. 826–837, 2011. doi: 10.1016/S0140-6736(11)60812-X.

[5] L. V. Campbell, "Genetics of obesity," *Australian Family Physician*, vol. 46, no. 7, pp. 456–459, Jul. 2017, doi: 10.5339/qfarf.2011.bmps6.

[6] B. Loring and A. Robertson, "Obesity and inequities: Guidance for addressing inequities in overweight and obesity," *World Health Organization, Europe*, pp. 1–6, 2014, Accessed: Oct. 29, 2020. [Online]. Available: http://www.euro.who.int/pubrequest

[7] S. Djalalinia, M. Qorbani, N. Peykari, and R. Kelishadi, "Health impacts of obesity," *Pakistan Journal of Medical Sciences*, vol. 31, no. 1, pp. 239–242, Jan. 2015, doi: 10.12669/pjms.311.7033.

[8] C. Platis, A. Spanou, P. Messaropoulos, C. Kastanioti and E. A. Zoulias. "Patients' Quality of Life and Their Relationship in Compliance with Antidiabetic Treatment. Case Study of Patients in a Public Hospital in Greece," *WSEAS Transactions on Biology and Biomedicine*, vol. 17, pp. 32-38, 2020.

[9] G. E. Umpierrez, "Diabetic Ketoacidosis," in The Diabetes Textbook, Cham: Springer International Publishing, 2019, pp. 619–627.

[10] L. Kardar, A. Fallah, S. Gharibzadeh, and F. "Application of fuzzy logic controller for intensive insulin therapy in type 1 diabetic mellitus patients by subcutaneous route." WSEAS Transactions on Systems and Control, 3(9), 712-721, 2008.

[11] U. J. Jung and M. S. Choi, "Obesity and its metabolic complications: The role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease," *International Journal of Molecular Sciences*, vol. 15, no. 4. MDPI AG, pp. 6184–6223, Apr. 11, 2014. doi: 10.3390/ijms15046184.

[12] A. S. Al-Goblan, M. A. Al-Alfi, and M. Z. Khan, "Mechanism linking diabetes mellitus and obesity," *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, vol. 7, pp. 587–591, Dec. 2014, doi: 10.2147/DMSO.S67400.

[13] Q. Tang, X. Li, P. Song, and L. Xu, "Optimal cut-off values for the homeostasis model assessment of insulin resistance (HOMA-IR) and pre-diabetes screening: Developments in research and prospects for the future," *Drug discoveries & therapeutics*, vol. 9, no. 6. pp. 380–385, Dec. 01, 2015. doi: 10.5582/ddt.2015.01207.

[14] B. Shashaj *et al.*, "Reference ranges of HOMA-IR in normal-weight and obese young Caucasians," *Acta Diabetologica*, vol. 53, no. 2, pp. 251–260, 2016, doi: 10.1007/s00592-015-0782-4.

[15] S. Soelistijo *et al.*, *Konsesus Pengelolaan Dan Pencegahan Diabetes Melitus Tipe2 Di Indonesia 2015*. 2015. [Online]. Available: https://www.google.com/url?sa=t&source=web&rct=j&url=https://pbperkeni.or.id/wp-content/uploads/2019/01/4.-Konsensus-Pengelolaan-dan-Pencegahan-Diabetes-melitus-tipe-2-di-Indonesia-PERKENI-2015.pdf&ved=2ahUKEwjy8KOs8cfoAhXCb30KHQb1Ck0QFjADegQIBhAB&usg=AOv

[16] N. Putri and M. Isfandiari, "Hubungan Empat Pilar Pengendalian Dm Tipe 2 dengan Rerata Kadar Gula Darah," *Jurnal Berkala Epidemiologi*, vol. 1, no. 2, pp. 234–243, 2013.

[17] A. S. M. Saleh, P. Wang, N. Wang, L. Yang, and Z. Xiao, "Brown Rice Versus White Rice: Nutritional Quality, Potential Health Benefits, Development of Food Products, and Preservation Technologies," *Comprehensive Reviews in Food Science and Food Safety*. Blackwell Publishing Inc., 2019. doi: 10.1111/1541-4337.12449.

[18] E. Sulistyowati, A. Rudijanto, S. Soeharto, and D. Handayani, "The Identification of Characteristic Macro- and Micronutrients and the Bioactive Components of Indonesian Local Brown Rice as a Functional Feed in Obesity Nutrition Therapy," *Current Nutrition & Food Science*, vol. 16, no. 4, pp. 494–500, 2019, doi: 10.2174/1573401315666190328223626.

[19] S. Pirasath, K. Thayaananthan, S. Balakumar, and V. Arasaratnam, "Effect of soluble fiber on glycaemic index," *Galle Medical Journal*, vol. 17, no. 1, p. 23, May 2012, doi: 10.4038/gmj.v17i1.4357.

[20] A. Fardet, "New hypotheses for the health-protective mechanisms of whole-grain cereals: What is beyond fibre?," *Nutrition Research Reviews*, vol. 23, no. 1, pp. 65–134, 2010, doi: 10.1017/S0954422410000041.

[21] L. M. M. Gommers, J. G. J. Hoenderop, R. J. M. Bindels, and J. H. F. De Baaij, "Hypomagnesemia in type 2 diabetes: A vicious circle?," *Diabetes*, vol. 65, no. 1, pp. 3–13, Jan. 2016, doi: 10.2337/db15-1028.

[22] M. Barbagallo and L. J. Dominguez, "Magnesium and Type 2 Diabetes: An Update," *International Journal of Diabetes and Clinical Research*, vol. 2, no. 1, pp. 1–5, 2015, doi: 10.23937/2377-3634/1410019.

[23] F. C. Mooren, K. Krüger, K. Völker, S. W. Golf, M. Wadepuhl, and A. Kraus, "Oral magnesium supplementation reduces insulin resistance in non-diabetic subjects - a double-blind, placebo-controlled, randomized trial," *Diabetes, Obesity and Metabolism*, vol. 13, no. 3, pp. 281–284, 2011, doi: 10.1111/j.1463-1326.2010.01332.x.

[24] M. Shimabukuro *et al.*, "Effects of the brown rice diet on visceral obesity and endothelial function: The BRAVO study," *British Journal of Nutrition*, vol. 111, no. 2, pp. 310–320, 2014, doi: 10.1017/S0007114513002432.

[25] V. Mohan *et al.*, "Effect of brown rice, white rice, and brown rice with legumes on blood glucose and insulin responses in overweight Asian Indians: A randomized controlled trial," *Diabetes Technology and Therapeutics*, vol. 16, no. 5, pp. 317–325, May 2014, doi: 10.1089/dia.2013.0259.

[26] M. U. Imam, M. Ismail, D. J. Ooi, N. Sarega, and A. Ishaka, "Increased risk of insulin resistance in rat offsprings exposed prenatally to white rice," *Molecular Nutrition and Food Research*, vol. 59, no. 1, pp. 180–184, 2015, doi: 10.1002/mnfr.201400396.

[27] BPS, "Konsumsi Beras Perkapita Indonesia 98 Kg/Tahun," *Databoks*, 2016. https://databoks.katadata.co.id/datapublish/2016/08/29/konsumsi-beras-perkapita-indonesia-98-kgtahun (accessed Nov. 03, 2020).

[28] E. Sulistyowati, "Beras Coklat Varietas Sinta Nur sebagai Makanan Fungsional Anti Obesitas melalui Perbaikan Disbiosis Mikrobiota Usus [Dissertation]," Malang: Univesitas Brawijaya, 2020.

[29] G. L. Kiwari, "Pengaruh Pemberian Diet Beras Coklat Lokal 'Sintanur' Terhadap Kadar Insulin Puasa dan Indeks HOMA-IR pada Tikus Model Obesitas [Thesis].," Malang: Univesitas Brawijaya, 2020.

[30] X. L. Fan, M. L. Yu, S. P. Fu, Y. Zhuang, and S. F. Lu, "Effectiveness of Acupuncture in Treatment of Simple Obesity in Animal Models: A Systematic Review and Meta-Analysis," *Evidence-based Complementary and Alternative Medicine*, vol. 2019. Hindawi Limited, 2019. doi: 10.1155/2019/5459326.

[31] A. B. Malafaia, P. A. N. Nassif, C. A. P. M. Ribas, B. L. Ariede, K. N. Sue, and M. A. Cruz, "Obesity induction with high fat sucrose in rats.," *Arquivos brasileiros de cirurgia digestiva : ABCD = Brazilian archives of digestive surgery*, vol. 26 Suppl 1, no. Suplemento 1, pp. 17–21, 2013.

[32] M. Basaranoglu, G. Basaranoglu, T. Sabuncu, and H. Sentürk, "Fructose as a key player in the development of fatty liver disease," *World Journal of Gastroenterology*, vol. 19, no. 8, pp. 1166–1172, 2013, doi: 10.3748/wjg.v19.i8.1166.

[33] S. Predawan, K. Sinprajakpol, T. Ayamuang, S. Predawan, K. Sinprajakpol, and T. Ayamuang, "Rule Discovery for Diabetes Mellitus Diagnosis using Ant-Miner Algorithm," *WSEAS Transactions on Biology and Biomedicine*, vol. 16, pp. 61–68, Accessed: Nov. 13, 2022. [Online]. Available: https://www.wseas.com/journals/articles.php?id=1800

[34] C. Platis *et al.*, "Diabetic Patients' Quality of Life and Their Relationship in Compliance with Antidiabetic Treatment. Case Study of Patients in a Public Hospital in Greece," *WSEAS Transactions on Biology and Biomedicine*, vol. 17, pp. 32–38, Mar. 2020, doi: 10.37394/23208.2020.17.5.

[35] J. S. Lee, N. Sreenivasulu, R. S. Hamilton, and A. Kohli, "Brown rice, a diet rich in health promoting properties," *Journal of Nutritional Science and Vitaminology*, vol. 65. Center for Academic Publications Japan, pp. S26–S28, 2019. doi: 10.3177/jnsv.65.S26.

[36] K. Ravichanthiran *et al.*, "Phytochemical profile of brown rice and its nutrigenomic implications," *Antioxidants*, vol. 7, no. 6. MDPI AG, Jun. 01, 2018. doi: 10.3390/antiox7060071.

[37] Z. M. Mosa, Y. A. El Badry, H. S. Fattah, and E. G. Mohamed, "Comparative study between the effects of some dietary sources and metformin drug on weight reduction in obese rats," *Annals of Agricultural Sciences*, vol. 60, no. 2, pp. 381–388, Dec. 2015, doi: 10.1016/j.aoas.2015.11.001.

[38] A. Kuijsten *et al.*, "Dietary fibre and incidence of type 2 diabetes in eight European countries: the EPIC-InterAct Study and a meta-analysis of prospective studies," *Diabetologia*, vol. 58, no. 7, pp. 1394–1408, Jul. 2015, doi: 10.1007/s00125-015-3585-9.

[39] S. N. Bhupathiraju *et al.*, "Glycemic index, glycemic load, and risk of type 2 diabetes: Results from 3 large US cohorts and an updated meta-analysis," *American Journal of Clinical Nutrition*, vol. 100, no. 1, pp. 218–232, Jul. 2014, doi: 10.3945/ajcn.113.079533.

[40] A. Santoso, "Serat Pangan (Dietary Fiber) dan Manfaatnya Bagi Kesehatan," *Magistra*, 2011.

[41] A. A. Rivellese, R. Giacco, and G. Costabile, "Dietary carbohydrates for diabetics," *Current Atherosclerosis Reports*, vol. 14, no. 6, pp. 563–569, 2012, doi: 10.1007/s11883-012-0278-4.

[42] F. Cahill *et al.*, "High Dietary Magnesium Intake Is Associated with Low Insulin Resistance in the Newfoundland Population," *PLoS ONE*, vol. 8, no. 3, Mar. 2013, doi: 10.1371/journal.pone.0058278.

[43] V. L. Tokarz, P. E. MacDonald, and A. Klip, "The cell biology of systemic insulin function," *Journal of Cell Biology*, vol. 217, no. 7. Rockefeller University Press, pp. 1–17, Jul. 01, 2018. doi: 10.1083/jcb.201802095.

[44] K. Kostov, "Effects of magnesium deficiency on mechanisms of insulin resistance in type 2 diabetes: Focusing on the processes of insulin secretion and signaling," *International Journal of Molecular Sciences*, vol. 20, no. 6. MDPI AG, Mar. 02, 2019. doi: 10.3390/ijms20061351.

[45] S. Schinner, W. A. Scherbaum, S. R. Bornstein, and A. Barthel, "Molecular mechanisms of insulin resistance," *Diabetic Medicine*, vol. 22, no. 6, pp. 674–682, 2017, doi: 10.1111/j.1464-5491.2005.01566.x.

[46] A. Badyal, K. S. Sodhi, R. Pandey, and J. Singh, "Serum magnesium levels: A key issue for diabetes mellitus," *JK Science*, vol. 13, no. 3, pp. 132–134, 2011.

[47] P. Dubey, V. Thakur, and M. Chattopadhyay, "Role of minerals and trace elements in diabetes and insulin resistance," *Nutrients*, vol. 12, no. 6, pp. 1–17, 2020, doi: 10.3390/nu12061864.

[48] H. Chutia and K. G. Lynrah, "Association of Serum Magnesium Deficiency with Insulin Resistance in Type 2 Diabetes Mellitus," *Journal of Laboratory Physicians*, vol. 7, no. 02, pp. 075–078, Jul. 2015, doi: 10.4103/0974-2727.163131.

[49] A. F. Abdul Rahim, M. N. Norhayati, and A. M. Zainudin, "The effect of a brown-rice diets on glycemic control and metabolic parameters in prediabetes and type 2 diabetes mellitus: A meta-analysis of randomized controlled trials and controlled clinical trials," *PeerJ*, vol. 9, p. e11291, May 2021, doi: 10.7717/PEERJ.11291/SUPP-10.

[50] T. N. Bui *et al.*, "Pre-Germinated Brown Rice Reduced Both Blood Glucose Concentration and Body Weight in Vietnamese Women with Impaired Glucose Tolerance," *Journal of Nutritional Science and Vitaminology*, vol. 60, no. 3, pp. 183–187, 2014, doi: 10.3177/JNSV.60.183.

[51] B. Wang *et al.*, "Effects of a whole rice diet on metabolic parameters and inflammatory markers in prediabetes," *e-SPEN Journal*, vol. 8, no. 1, pp. e15–e20, Feb. 2013, doi: 10.1016/J.CLNME.2012.11.001.

[52] D. Aune, T. Norat, P. Romundstad, and L. J. Vatten, "Whole grain and refined grain consumption and the risk of type 2 diabetes: a systematic review and dose–response meta-analysis of cohort studies," *European Journal of Epidemiology 2013 28:11*, vol. 28, no. 11, pp. 845–858, Oct. 2013, doi: 10.1007/S10654-013-9852-5.