Effect of aerobic exercise and rice bran ethanol extract on Akt/mTOR activity and oxidative stress in skeletal muscles in a rat model of high-fat diet-induced obesity

Efecto del ejercicio aeróbico y el extracto etanólico de salvado de arroz sobre la actividad de Akt/mTOR y el estrés oxidativo en los músculos esqueléticos en un modelo de rata con obesidad inducida por una dieta rica en grasas

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Abstract

Introduction and Objective: Insulin resistance is a main complication associated to obesity. Rice bran and exercise seem effective for reducing obesity complications. Therefore, this study aimed to determine the effects of aerobic exercise (EX) and rice bran extract (RB) on the expression of Akt/mTOR genes and oxidative stress markers in the skeletal muscle of rats fed a high-fat diet (HFD).
Methodology: Thirty female Wistar rats were randomly divided into five groups: control normal diet (ND-Con), control HFD (HFD-Con), HFD-aerobic exercise (EX), HFD-RB extract (RB), and HFD-EX-RB. EX-groups ran on a treadmill five times a week for four weeks and RB groups received 60 mg/kg/day of RB extract. At the end of the study, rats were sacrificed, and their quadriceps muscles were removed. Akt/mTOR gene expression and oxidative stress markers were determined.
Results: HFD led to significant decrease in gene expression of Akt, mTOR, superoxide dismutase (SOD) and catalase activities, while increasing malondialdehyde (MDA) concentration. The EX-group showed significant increase in Akt/mTOR gene expression, while no noticeable effects were observed in RB. The Akt gene expression was significantly higher in EX-RB group than in HFD-CON group, with no effect in SOD. RB caused significant increase in SOD activity compared to HFD-Con group. Catalase activity in EX, RB, and EX-RB groups was significantly higher than in the HFD-Con group. The MDA concentration in the EX, RB, and EX-RB groups was significantly lower than in HFD-Con group.
Conclusions: EX and RB can be suitable treatments for reducing HFD complications in skeletal muscle tissue.

Keywords

Aerobic exercise; Akt/mTOR; oxidative stress; rice bran.

Resumen

Introducción y Objetivo: Resistencia a la insulina es una complicación clave asociada a obesidad. Salvado de arroz y ejercicio parecen métodos efectivos para reducir estas complicaciones. El objetivo del estudio fue determinar efectos del ejercicio aeróbico (EX) y extracto de salvado de arroz (RB) en la expresión de genes Akt/mTOR y marcadores de estrés oxidativo en músculo esquelético de ratas alimentadas con dieta alta en grasas (HFD).
Metodología: Treinta ratas Wistar hembras distribuidas aleatoriamente en cinco grupos: control dieta normal (ND-Con), control HFD (HFD-Con), HFD-ejercicio aeróbico (EX), HFD-extracto de RB (RB) y HFD-EX-RB. Los grupos EX corrieron en cinta cinco veces por semana durante cuatro semanas, y los grupos RB recibieron 60 mg/kg/día de extracto de RB. Finalmente, las ratas fueron eutanasiadas y se extrajeron los músculos cuádriceps, determinando expresión de Akt/mTOR y marcadores de estrés oxidativo.
Resultados: HFD produjo disminución significativa en la expresión de Akt, mTOR, y actividades de superóxido dismutasa (SOD) y catalasa, aumentando la concentración de malondialdehído (MDA). EX mostró aumento significativo en la expresión de Akt/mTOR, sin efectos en RB. La expresión de Akt fue significativamente mayor en EX-RB que en HFD-CON. EX no afectó a SOD. RB causó un aumento significativo en SOD en comparación con HFD-Con. La actividad de catalasa en EX, RB y EX-RB fue significativamente mayor que en HFD-Con. La concentración de MDA en EX, RB y EX-RB fue significativamente menor que en HFD-Con.
Conclusiones: EX y RB pueden ser tratamientos adecuados para reducir complicaciones de HFD en tejido muscular esquelético.

Palabras clave

Akt/mTOR; ejercicio aeróbico; estrés oxidativo; salvado de arroz.

Introduction

Obesity causes insulin resistance in peripheral tissues, especially skeletal muscle, through various mechanisms (Ugwoke et al., 2022). Although the exact mechanism of obesity-induced insulin resistance is not fully understood, evidence suggests that the accumulation of triglycerides in skeletal muscle disrupts the Insulin Receptor Substrate-1 (IRS-1)/ Phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway. Consequently, the muscle's ability to uptake glucose in response to insulin stimulation decreases and leads to insulin resistance (Silveira et al., 2008).

Disruption of insulin function in skeletal muscle not only affects glucose metabolism but also impairs the process of protein synthesis in skeletal muscle. This process, activated by the insulin signaling pathway, is essential for increasing muscle mass (Guillet & Boirie, 2005). Evidence indicates that obesity and diabetes resulting from a high-fat diet disrupt the Akt/mTOR signaling pathway, reducing muscle protein synthesis (Anderson et al., 2008; Ferretti et al., 2018). On the other hand, oxidative stress in skeletal muscle increases with obesity, disturbing protein turnover and leading to muscle atrophy (Fletcher et al., 2022; Ou et al., 2022). This disruptive action of oxidative stress in skeletal muscle seems to be mediated by alterations in the Akt/mTOR pathway, resulting in a reduction of protein synthesis (Abrigo et al., 2018).

Nevertheless, the role of oxidative stress in the Akt/mTOR pathway is dual. Reactive oxygen species (ROS) in small amounts activate this pathway, whereas overproduction inhibits this pathway (Bashan et al., 2009; Papaconstantinou, 2009). Various interventions have been proposed to mitigate the harmful effects of obesity on skeletal muscle structure and function. One such intervention is physical activity, which aims to restore muscle structure and function (Heo et al., 2018). In this line, different studies have shown that aerobic exercise reduces muscle atrophy caused by different pathological conditions, including myocardial infarction (Feng et al., 2022), ovariectomy (Tang et al., 2021), cancer (Pereira et al., 2021), and obesity (Alizadeh Pahlavani, 2022). The mechanism seems to involve activation of the Insulin-like Growth Factor-1 (IGF-1)/IGF-1 Receptor R(IGF-1R)- PI3K/Akt signaling pathway. Additionally, aerobic exercise can mitigate obesity-induced oxidative stress in skeletal muscle by enhancing antioxidant capacity (Ulbricht et al., 2019; Wang et al., 2022). Moreover, evidence shows that medicinal plants, like regular exercise training, can also counteract the adverse effects of obesity on skeletal muscle (Lee et al., 2018). Certain medicinal plants, such as rice bran, contain phytochemical compounds that can reduce insulin resistance and promote protein turnover through activation of Akt/mTOR pathway in skeletal muscle (Vlavcheski et al., 2020; Zhang et al., 2014). Rice bran exhibits anti-inflammatory, anti-obesity, and antioxidant properties due to numerous nutritional compounds and phytochemicals, particularly gamma oryzanol (Mattei et al., 2021). Scientific literature shows that previous studies have investigated the impact of aerobic exercise and rice bran on oxidative stress markers and obesity independently. In the context of a high-fat diet, both aerobic exercise and rice bran may act synergistically to reduce oxidative stress in white adipose tissue. Therefore, we hypothesize that their combined effects could be more beneficial for metabolic pathophysiology than either intervention alone. Therefore, this study aimed to determine the combined effects of aerobic exercise and rice bran extract together on the Akt/mTOR pathway and skeletal muscle oxidative stress markers in rats fed with a high-fat diet.

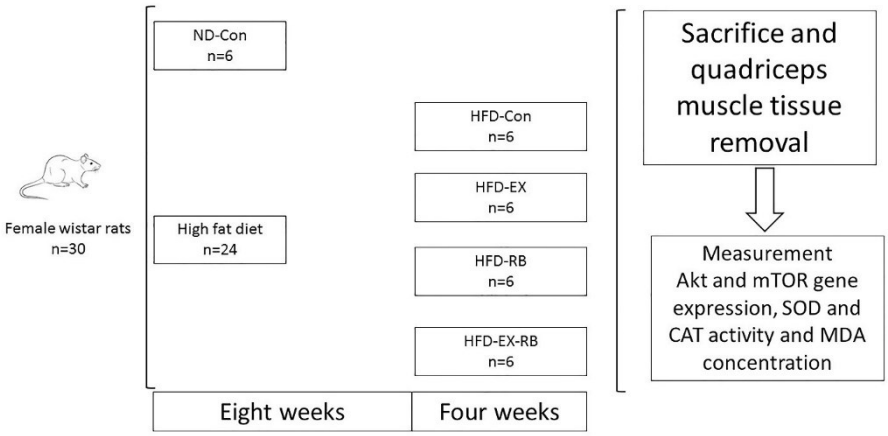
Method

Animals

Thirty adults female Wistar rats, aged 12 weeks and weighing 180 to 200 g were selected for this preclinical trial. All animals were housed under standard laboratory conditions in transparent polycarbonate cages that resist autoclave sterilization. Room temperature and relative humidity were maintained at 20-22°C and 55%, respectively. Animals had free access to sufficient water and food (provided by Behparvar Company, Iran) with a 12-hour dark/light cycle. After one week of acclimatization to the laboratory conditions, female rats were randomly divided into five groups (n=6/group): a control group fed with normal diet (ND-Con), a control group fed with high-fat diet (HFD-Con), a group fed with high-fat diet plus aerobic exercise (EX), a group fed with high-fat diet plus rice bran extract (RB), and a group

fed high-fat diet plus aerobic exercise + rice bran extract (EX-RB). A commercial pellet diet for laboratory rats (Behparvar Company, Karaj, Iran) was provide at regular intervals. The diet composition included: fat 3.5–4.5%, fiber 4–4.5%, crude protein 19.50–20.50%, calcium 0.95–1%, salt 0.5–0.55%, lysine 1.15%, phosphorus 0.65–0.7%, methionine 0.33%, tryptophan 0.25%, threonine 0.72%, energy 16.16–17 mJ kg^{-1}. The high-fat diets were prepared by mixing the normal diet with 20% palm oil, 1.5% cholesterol, and 0.25% cholic acid as previously described (Od-Ek et al., 2020). Rats were fed either a normal diet or a high-fat diet for 8 weeks. Body weight measurements were taken at baseline, after eight weeks of high-fat diet feeding, and after the completion of a four-week intervention period (Figure 1).

Figure 1. Experimental design of the study.



Abbreviations: Akt: Protein kinase B; mTOR: Mammalian target of rapamycin; SOD: Superoxide dismutase; CAT: catalase; MDA: malondialdehyde; HFD: High fat diet; RB: Rice bran; ND-Con: Control normal diet; HFD-Con: Control HFD; EX: HFD-aerobic exercise; RB: HFD- rice bran extract; HFD-EX-RB: aerobic exercise and extract-rice bran.

Inclusion and Exclusion Criteria

The rats in our study had to meet the following inclusion criteria:12-week-old female rats, weighing 180-200 g, Wistar strain, must be clinically healthy, with no signs of disease or injury, nulliparous (never having given birth) and non-pregnant female rats, since hormonal changes associated with pregnancy and lactation may affect the results of the study, and the rats must not have been subjected to prior experiments that could affect their behavior or response to treatment. Rats that did not meet the inclusion criteria were excluded.

Ethics statement

The investigation protocol and all procedures were reviewed and approved by the Regional Research Ethics Committee of the Islamic Azad University (IR.IAU.TNB.REC.1401.017). The standards described in the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guide were followed to address ethical aspects in animal research, to improve the transparency and reproducibility of our study.

Aerobic exercise protocol

The rats ran on a rodents' motorized treadmill for 20 min at a speed of 9 m/min with a 0-degree slope for one week (5 sessions/week) to habituate to exercise in this device. Following this familiarization phase, the animals underwent regular training 5 days/week for 4 weeks. During the first week, the rats ran on a treadmill at a speed of 16 m/min with a 0-degree slope for 20 min. From the second week, the running speed increased by 3 m/min each week, maintaining a 20-min duration. The 4th week, the rats reached a speed on the treadmill of 25 m/min with a 0-degree slope. The aerobic training program concluded 48 h prior to the animals' sacrifice. Each training session included a 5-min warm-up at 7 m/min, and a 5-min cool-down at 5 m/min after the main exercise.

Preparation of ethanolic extracts of rice bran

Rice bran (*Oryza sativa*) was obtained from reliable suppliers and approved by a botanist. After being dried in the shade, it was ground into powder using a mill, and an extraction sample was prepared. Two hundred grams of dried ground material were placed in a percolator after processing. Extraction was carried out using 1000 ml of 50% ethanol, and the process was repeated three times. The resulting extracts were collected and stored in a refrigerator for further experiments. To determine the dry matter content of the liquid extract, a known portion was heated in an oven. Based on the results, the dry matter content of the extract was 1%. The extract was dissolved in a liquid prepared with distilled water and administered by gavage at a dose of 60 mg per Kg of body weight to the rats. This treatment was carried out 5 times a week for 4 weeks.

Sacrifice and Collection of skeletal muscle

Fourty-eight hours after the last intervention, all rats were fasted for 8-10 h and weighed prior to sacrifice. Anesthesia was induced via chloroform exposure. After confirming anesthesia through a pain response test, blood samples were collected from the left ventricle of the heart. Subsequently, the quadriceps muscle tissue was removed, washed with phosphate-buffered saline (PBS), and placed in coded 2 mL microtubes that were then transferred to nitrogen tanks and stored at -80°C until biochemical analysis.

Real-Time PCR Analysis

Total RNA was extracted using a specialized kit (made in Korea) according to the manufacturer's instructions. Additionally, cDNA synthesis was conducted following the kit instructions (also made in Korea). The obtained cDNA was used for real-time PCR analysis. All primers were designed using Allele ID v7.8 Software, with the beta-actin gene selected as housekeeping gene (Table 1). In real-time processing, the RealQ 2x Master Mix kit (produced by AMPLQON, Germany) was used according to the manufacturer's instructions. Gene expression calculated using the $2^{-\Delta Ct}$ formula, with forward and reverse primer sets as shown in Table 1.

Table 1. Specific primers used in real-time PCR

Gene	Reverse (5'→3')	Forward (3'→5')
Akt	TTGATGAGGCGGTGTGATGGTGA	TGTGGGAAGATGTGTATGAGAA
mTOR	TGTCCATCAGCCTCCAATTC	ACTATAGAACCACATGCCACAC
β-actin	CAGAGGCATACAGGGACAAC	ACCGTGAAAAGATGACCCAG

Abbreviations: Akt: Protein kinase B; mTOR: Mammalian target of rapamycin; β-actin: Beta-actin.

Biochemical assays

The activities of superoxide dismutase (SOD) and catalase enzymes were measured using a commercial kit (Randox Laboratories Ltd., Crumlin, Country Antrim, UK) following homogenization of quadriceps muscle tissue, as described by Karnia et al. (2018). The Buege and Aust method (Buege & Aust, 1978) was used to measure malondialdehyde (MDA) concentrations.

Statistics Analysis

Data are presented as mean ± standard deviation. The Shapiro-Wilk and Levene tests were used to validate the assumptions of parametric tests, including the normality of data distribution and the homogeneity of variance. The results indicated that gene expression, biochemical concentrations, and enzyme activities were normally distributed. Analysis of variance (ANOVA) was used to assess the effects of the interventions (aerobic exercise/rice bran extract). This method evaluated the main effects of aerobic exercise and rice bran, the interaction between exercise and rice bran, and pairwise comparisons across groups. When a significant difference was detected, the Bonferroni post-hoc test was applied to identify the specific locations of the differences. A significance level of $p \leq 0.05$ was considered for all statistical analyses.

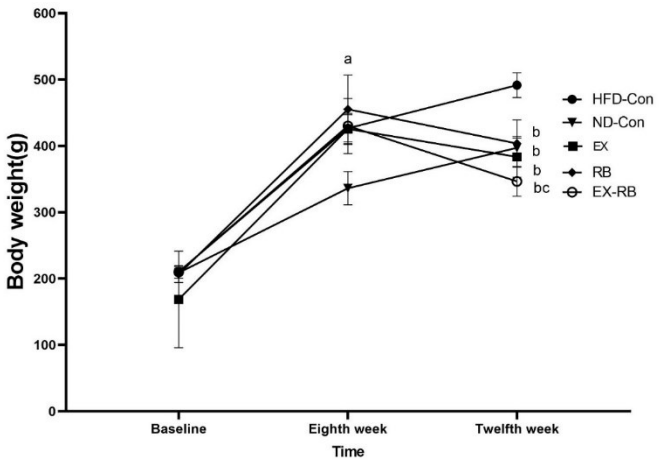
Sample size

In our study, a posteriori power calculation was used instead of an a priori sample size estimate using the GPower3 power analysis program from Düsseldorf, Germany. The a posteriori power analysis revealed that the statistical power of the effect of exercise, Rice bran, and exercise*Rice bran on Protein kinase B (Akt) and Mammalian target of rapamycin (mTOR) was between 0.7 and 0.9. Therefore, the sample size did not negatively affect statistical power.

Results

Eight weeks of feeding a high-fat diet resulted in a significant increase in the weight of rats in the HFD-Con, EX, RB, and EX-RB groups compared to the ND-Con group (p= 0.004). However, at the end of the eighth week, no significant difference in the weight was observed among the groups fed the high-fat diet (p= 0.814). After four weeks of interventions (EX, RB, and EX-RB), a significant difference in weight was observed between the HFD-Con, EX, RB, and EX-RB groups (p= 0.001). The rats in the EX-RB (p= 0.001), EX (p= 0.001), and RB (p= 0.001) groups were significantly lighter than those in the HFD-CON group. No significant difference in weight was observed between the EX-RB and the EX group (p= 0.185). However, the weight of rats in EX-RB group was significantly lower than in the RB group (p= 0.011). Additionally, no significant difference was observed between EX and RB groups (p= 1.000) (Figure 2).

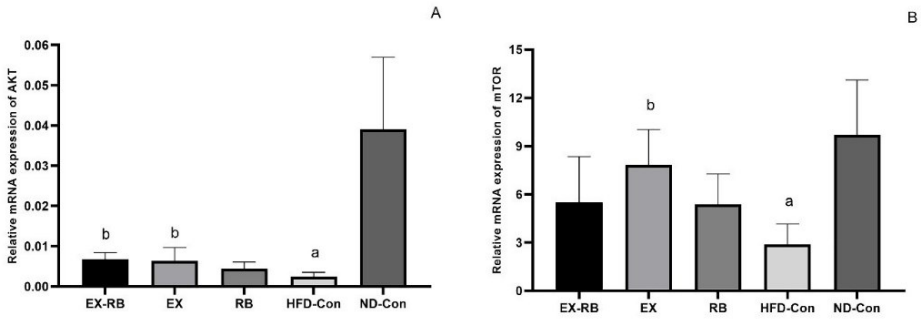
Figure 2. Effect of aerobic exercise and rice bran extract on body weight of Wistar rats.



Abbreviations: EX: HFD-aerobic exercise; g: grams; HFD: High fat diet; HFD-Con: Control HFD; HFD-EX-RB: aerobic exercise and extract-rice bran; ND-Con: Control normal diet; RB: HFD-rice bran extract (n = 6 for each group). Data are expressed as mean ± standard deviation (SD); Two-way ANOVA with repeated measure, followed by the post hoc test of Bonferroni. [a]Significant difference to baseline. [b]Significant difference compared to the group fed with HFD. [c]Significant difference compared to the RB group.

A comparison of the normal and high-fat diets revealed that the high-fat diet caused a significant decrease in the expression of Akt (p= 0.004) and mTOR (p= 0.001) genes, as well as in SOD (p= 0.001) and catalase (p= 0.002) activities. Meanwhile, the concentration of MDA increased significantly (p= 0.001). Aerobic exercise significantly increased Akt gene expression (p= 0.019) compared to HFD-Con group, whereas RB had no significant effect on the expression of this gene (p= 0.378). The expression of Akt gene in the EX-RB group was significantly higher than in the HFD-Con group (p= 0.008) (Figure 3 Pannel A). The mTOR gene expression also increased significantly with EX (p=0.004), but neither RB (p=0.219) nor the combined EX-RB intervention (p= 0.182) had a significant effect on the expression of this gene compared to the HFD-Con group. (Figure 3 Pannel B).

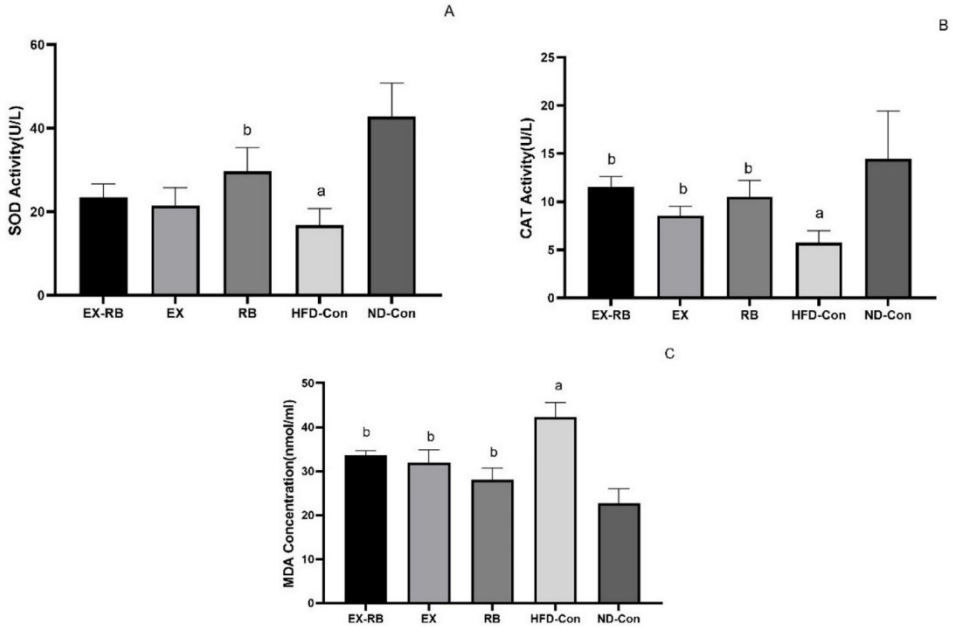
Figure 3. Effect of aerobic exercise and rice bran extract on mRNA expression of Akt (Pannel A) and mTOR (Pannel B) in quadriceps muscle of Wistar rats.



Abbreviations: AKT: Protein Kinase B; EX: HFD-aerobic exercise; HFD: High fat diet; HFD-Con: Control HFD; HFD-EX-RB: aerobic exercise and extract-rice bran; mTOR: Mammalian target of rapamycin; ND-Con: Control normal diet; RB: HFD-rice bran extract (n = 6 for each group). Data are expressed as mean ± standard deviation (SD); One- way ANOVA, followed by the post hoc test of Bonferroni. [a]Significant difference compared to the group fed with normal diet. [b]Significant difference compared to the group fed with HFD.

EX had no significant effects on SOD activity compared to HFD-Con group (p= 0.279), whereas RB caused a significant increase in SOD activity (p= 0.001). SOD activity in the EX-RB group showed non-significant increase compared to the HFD-Con group (p= 0.072) (Figure 4 Pannel A). Catalase activity was significantly higher in the EX (p= 0.006), RB (p= 0.001), and EX-RB (p= 0.001) groups compared to the HFD-Con group. (Figure 4 Pannel B). MDA concentration was significantly lower in the EX (p= 0.001), RB (p= 0.001), and EX-RB (p= 0.001) groups compared to the HFD-Con group (Figure 4 Pannel C).

Figure 4. Effect of aerobic exercise and rice bran extract on SOD (Pannel A), CAT (Pannel B) enzyme activity and MDA (Pannel C) concentration in quadriceps muscle of Wistar rats.



Abbreviations: CAT: catalase; EX: HFD-aerobic exercise; HFD: High fat diet; HFD-Con: Control HFD; HFD-EX-RB: aerobic exercise and extract-rice bran; MDA: malondialdehyde; ND-Con: Control normal diet; RB: HFD-rice bran extract; SOD: Superoxide dismutase (n = 6 for each group). Data are expressed as mean ± standard deviation (SD); One- way ANOVA, followed by the post hoc test of Bonferroni. [a]Significant difference compared to the group fed with normal diet. [b]Significant difference compared to the group fed with HFD.

Discussion

High-fat diets were found to decrease Akt and mTOR gene expression and increase oxidative stress markers in this study. A high-fat diet promotes obesity, insulin resistance, and adverse effects on energy metabolism and protein synthesis in skeletal muscle (Roy et al., 2016). Skeletal muscle function is impaired in obese and diabetic individuals. Insulin resistance disrupts muscle protein synthesis by interfering with the insulin/IRS-1/PI3K/Akt signaling pathway (Tremblay & Marette, 2001). Glucose uptake and metabolism in skeletal muscle rely on the normal functioning of the Akt signaling pathway. Therefore, any disturbance in this pathway results in decreased glucose uptake and metabolism. Many metabolic disorders arise from reduced insulin effectiveness in skeletal muscle.

Insulin plays a key role in protein synthesis in skeletal muscle, and alterations in the transduction pathway decrease muscle mass and strength (Balage et al., 2001). Consistent with previous studies, this study demonstrated that feeding on a high-fat diet resulted in reduced Akt/mTOR mRNA expression, a significant component in insulin signal transduction in skeletal muscle. Furthermore, this reduction was associated with reduced antioxidant defense capacity and significant increase lipid peroxide levels. Obesity-induced oxidative stress is one of the mechanisms responsible for disruption of the Akt/mTOR signaling pathway (Ábrigo et al., 2018). ROS, prevalent in obesity and diabetes, interfere with insulin signal transduction. This contributes to insulin resistance and altered protein turnover in skeletal muscle. Nevertheless, in the present study, aerobic exercise demonstrated the potential to mitigate the negative effects of a high-fat diet on Akt/mTOR gene expression. This finding agrees with previous research, indicating that regular aerobic exercise regulates the insulin signaling pathway by increasing PI3K phosphorylation (Bae et al., 2016; Kirwan et al., 2000). Activation of PI3K stimulates the Akt/mTOR pathway. It has been reported that even one session of physical activity lasting several hours, activates mTOR (Kirwan et al., 2000). Additionally, one hour of aerobic activity increases Akt and mTOR phosphorylation (Mascher et al., 2007). In the current study, rice bran supplementation showed no significant effects on Akt and mTOR mRNA. Since the interaction between aerobic exercise and rice bran was modest, the observed increase in Akt and mTOR mRNA expression in EX-RB group is likely due to the effects of aerobic exercise rather than rice bran. Although the direct effects of rice bran on the Akt/mTOR pathway remain underexplored in current literature, it has been shown to improve cardiometabolic risk factors and endothelial function, which may indirectly modulate cellular signaling pathways such as Akt/mTOR (Justo et al., 2013). Additionally, rice bran combined with aerobic exercise likely contributed to weight reduction in rats, which fed a high-fat diet and helped preserve muscle mass by downregulating the expression of muscle-wasting markers, including atrogin-1 and MuRF1. Furthermore, due to its active components (ferulic acid, γ-oryzanol, and high dietary fiber) rice bran may exert enhanced effects on glucose-related biomarkers (Huang et al., 2023). Therefore, future studies are recommended to include direct measurements of these biomarkers.

In the present study, aerobic exercise led to a no significant increase in SOD activity, a significant increase in catalase activity, and a decrease in MDA concentration. The reduction of MDA indicates that aerobic physical activity can reduce lipid peroxidation in skeletal muscle when fed a high-fat diet. These findings are consistent with previous studies confirming the positive effects of aerobic exercise on oxidative stress biomarkers (Li et al., 2015). However, the impact of aerobic exercise on antioxidant enzyme activity, particularly skeletal muscle SOD, remains somewhat contradictory. Research suggests that the effect of aerobic exercise on SOD activity in skeletal muscle varies based on training program characteristics. Some studies have reported that endurance training does not increase skeletal muscle SOD activity (Lambertucci et al., 2007), while others indicate a significant increase in enzyme activity (Lawler et al., 2006). These discrepancies may be explained by methodological differences in measuring antioxidant enzyme activity, such as SOD and catalase, or variations in the fiber types studied. Nevertheless, ROS are instrumental agents in the transduction pathway that culminates in the activation of genes coding for antioxidant enzymes, resulting in an optimal adaptation to endurance exercise. This ultimately leads to an increase in SOD and catalase content and activity (Vasilaki et al., 2006). Another potential mechanism for the reduction in oxidative stress observed in this study involves muscle contractions favoring glucose uptake and oxidation. High-fat diets cause obesity that leads to insulin resistance in peripheral tissues, particularly skeletal muscle. Hyperglycemia combined with mitochondrial dysfunction and endoplasmic reticulum stress results in ROS accumulation, exacerbating cell damage (Fiorentino et al., 2013). In this line, regular aerobic exercise has been shown to decrease blood

glucose levels and oxidative stress markers (Nojima et al., 2008; Wycherley et al., 2008). On the other hand, rice bran has been shown to suppress the activation of inflammatory pathways like ERK1/2, p38 MAPK, and NF-κB. Rice bran is rich in antioxidants like tocotrienols, phytic acid, γ-oryzanol, ferulic acid, phytosterols, and flavonoids (Saji et al., 2020). These compounds can scavenge free radicals and reduce oxidative stress, which is implicated in various diseases. Therefore, rice bran can reduce the production of ROS and pro-inflammatory cytokines, further contributing to the reduction of oxidative stress (Law et al, 2017).

In the present study, a high-fat diet disrupted insulin signaling pathway by reducing Akt/mTOR gene expression (indicative of insulin resistance) and increasing MDA levels (sign of lipid peroxidation). Aerobic exercise seems to counteract these effects by reducing insulin resistance, enhancing glucose uptake and metabolism in skeletal muscle. This is achieved through an increase in the expression of insulin signaling pathway genes. These changes contribute to a reduction in oxidative stress in skeletal muscle tissue. On the other hand, rice bran extract was shown to increase SOD and catalase activities while decreasing MDA levels in this study. It is well known that rice bran extract contains many phytochemicals, including polyphenols and flavonoid compounds, γ-oryzanol, with antioxidant effects (Ahmed et al., 2018). It has been reported that rice bran extract reduces ROS production at the cellular level and stimulate antioxidant enzyme production (by increasing gene expression) and activity under oxidative stress conditions (Liu et al., 2021; Saji et al., 2020). Rich in antioxidant compounds such as γ-oryzanol, alpha tocopherol, and phytosterol, rice bran can neutralize free radicals generated in obesity by a high-fat diet and alleviate oxidative stress under such conditions (Huang et al., 2023). Aerobic exercise and rice bran may synergistically modulate Akt/mTOR signaling and reduce oxidative stress, particularly under high-fat dietary conditions. Aerobic exercise has been shown to activate the Akt/mTOR pathway, which plays a critical role in promoting muscle protein synthesis and preventing muscle atrophy. Rice bran, by contrast, has demonstrated potential in mitigating oxidative stress and enhancing cardiometabolic health. Given that both interventions reduce oxidative stress in adipose tissue, their combined application may serve as a viable strategy to counteract the adverse effects associated with high-fat diets.

Translating findings from animal models to humans presents several challenges. Although animal studies, particularly those using rodents, offer valuable insights into biological mechanisms, physiological differences between species can significantly affect how interventions work. For instance, metabolism, immune response, hormonal regulation, and even gene expression can differ markedly between animals and humans. Moreover, controlled laboratory settings rarely reflect the complex environmental and lifestyle factors that influence human health outcomes. As a result, promising results in animals may not always translate into clinical effectiveness in people. Therefore, caution is essential when interpreting such findings, and further validation through human trials is necessary to ensure applicability and safety.

The authors acknowledge several limitations. The study was conducted exclusively on adult female Wistar rats, and direct measurements of insulin sensitivity were not performed. Nonetheless, within the context of evaluating the effects of aerobic exercise combined with rice bran in animal models fed a high-fat diet, this research offers a novel perspective on improving obesity-related pathophysiology through physical activity and the potential benefits of rice bran.

Conclusions

In conclusion, aerobic exercise and rice bran extract improve insulin resistance by regulating Akt/mTOR genes and reducing oxidative stress. Therefore, despite the negative effect of continuous high-fat diet consumption, these two interventions have a positive effect on insulin signaling pathway proteins and oxidative stress. It is recommended that aerobic exercise combined with rice bran can be a suitable treatment for reducing complications caused by eating HFD in skeletal muscle tissue based on the findings of this study.

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