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The Effect of Aerobic Exercise and Ethanolic Extract of Rice Bran on The Expression of Acetyl-CoA Carboxylase and HMGCR Genes in the Liver Tissue of Rats Fed with a High-Fat Diet

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A B S T R A C T

Alteration in cholesterol homeostasis is a consequence of overweight and obesity induced by diet, with the liver being one of the key organs in the cholesterol synthesis pathway. Since the effect of rice bran and aerobic exercise on the hepatic cholesterol synthesis pathway is not well understood, this study aimed to investigate the effect of aerobic exercise and ethanolic extract of rice bran on the expression of Acetyl-CoA carboxylase and HMGCR genes in the liver tissue of rats fed with a high-fat diet. In a preclinical trial, 30 eight-week-old female rats were randomly divided into five groups (6 rats per group): control with normal diet, control with a high-fat diet, aerobic exercise with a high-fat diet, and aerobic exercise with rice bran and a high-fat diet. The aerobic exercise program included running on a treadmill at moderate intensity (50-60% Vo2max), 5 sessions per week for 4 weeks. The ethanolic extract of rice bran was administered at a dose of 60 mg/kg via gavage to the supplement and exercise-supplement groups. The expression of Acetyl-CoA carboxylase in the control group with a normal diet significantly increased compared to the control group with a high-fat diet ($P = 0.000$), while the expression of HMGCR significantly decreased ($P = 0.050$). Additionally, the expression of Acetyl-CoA carboxylase in the aerobic exercise group with a high-fat diet showed a significant increase compared to the control group with a high-fat diet ($P \leq 0.034$), and the expression of HMGCR showed a significant decrease ($P = 0.000$). Furthermore, intergroup comparisons revealed that the increase in the expression of Acetyl-CoA carboxylase in the rice bran diet group was significant compared to the control group with a high-fat diet ($P \leq 0.001$), while the expression of HMGCR significantly decreased ($P \leq 0.028$). Similar changes were observed in the aerobic exercise-rice bran group compared to the control group with a high-fat diet, showing a significant increase in the expression of Acetyl-CoA carboxylase ($P \leq 0.002$), while the decrease in HMGCR expression was significant ($P = 0.000$).

Keywords: *Aerobic exercise, rice bran, Acetyl-CoA carboxylase, HMGCR, high-fat diet.*

1. Introduction

Obesity is currently one of the most serious public health problems worldwide. In developing countries, this disease is rapidly becoming the most common nutritional disorder, characterized by a decrease in the prevalence of malnutrition. Obesity is strongly associated with many chronic diseases, such as dyslipidemia, hypertension, diabetes, coronary atherosclerotic heart disease, cancer, and non-alcoholic fatty liver disease (1). The occurrence of these chronic metabolic diseases is closely related to changes in dietary structure, particularly the intake ratio of the three main macronutrients (carbohydrates, fats, and proteins) (2). It has been reported that a high-fat diet can lead to obesity (3). Consuming a high-fat and high-cholesterol diet can cause steatohepatitis, inflammation, and fibrosis (4). A high-fat diet also leads to severe weight loss, abnormal serum transaminases, and cholesterol as the main fat in the liver (5). On the other hand, the occurrence of obesity is accompanied by changes in the structure and function of cholesterol levels in different tissues, such as the liver tissue (6). It has been shown that liver tissue plays a vital role in regulating cholesterol homeostasis in the body, and regulating cholesterol homeostasis is one of the most important factors in preventing liver-related and heart-related diseases; therefore, identifying the function of enzymes involved in cholesterol homeostasis is essential (7).

HMG-CoA reductase (HMGCR) is the rate-limiting enzyme in cholesterol biosynthesis (8). Therefore, its activity is crucial in controlling cholesterol synthesis. To maintain cholesterol homeostasis, HMGCR can be regulated by multiple mechanisms such as transcription, translation (9), enzyme degradation rate (10), phosphorylation-dephosphorylation (11), and feedback inhibition (12). Reducing HMGCR activity can effectively decrease the production of total cholesterol (13), as cholesterol is synthesized by a series of enzymes from dietary fat in the liver, and HMGCR activity leads to cholesterol production far beyond the body's natural needs (14). Under physiological conditions, cholesterol content in intracellular locations is low because most of it is transferred to the plasma membrane, with an estimated over 80% of cellular cholesterol being in the plasma membrane, in dynamic equilibrium with the endoplasmic reticulum and Golgi system. Cellular cholesterol may arise from endogenous synthesis or absorption from lipoproteins (15). The first process accounts for about 50% of the body's cholesterol and mainly occurs in the liver (16). At the cellular level, the biosynthetic pathway in the endoplasmic reticulum is carried out through the mevalonate pathway. After synthesis, cholesterol is primarily transferred through a cellular pathway to the plasma membrane or other organelles, such as endosomal compartments (17).

On the other hand, acetyl-CoA carboxylase is a key metabolite in carbon metabolism and participates in a series of vital processes, such as lipid synthesis and protein acetylation, to maintain life activities (18). Therefore, acetyl-CoA is one of the most essential metabolic intermediates (19). Acetyl-CoA is produced in various ways and derived from a wide range of sources, primarily through carbohydrates, free fatty acids, and branched-chain amino acids (20). When HMGCR activity is reduced, the amount of cell-associated cholesterol decreases, which activates SREBP-2-mediated signaling pathways (21). One of the critical results of SREBP-2 activation for cholesterol homeostasis is the upregulation of low-density lipoprotein (LDL) receptor (LDLR) and the essential precursor for cholesterol synthesis, HMGCR activity (22). HMG-CoA is formed from the condensation of acetyl-CoA and acetoacetyl-CoA, catalyzed by HMGCR synthase (23). HMGCR is an essential growth enzyme, and inhibiting its activity and the concurrent deficiency of the isoprenoids it produces can lead to defects in germ cell migration and intracerebral hemorrhage (24). Inhibiting HMGCR in patients with type 2 diabetes reduces the growth of coronary atherosclerotic plaques (25). Therefore, identifying the effects and mechanisms of physical activity on this enzyme is important.

Given the importance of reducing the rate of cholesterol synthesis and absorption in the body and controlling its pathogenic pathways, researchers have proposed pharmaceutical, dietary, and physical activity strategies to control and limit cholesterol levels (26). Increasing physical activity has become an essential part of a non-pharmacological strategy to control obesity and weight gain (27). In this regard, the guidelines of the American College of Sports Medicine and the Centers for Disease Control and Prevention specify that every sedentary individual should engage in 30 minutes or more of moderate-intensity physical

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activity on most days of the week to promote health and prevent diseases (28). Physical activity can increase LXR and ABCA1 levels to enhance the reverse cholesterol transport (RCT) process, leading to increased plasma HDL cholesterol levels (29). Similarly, de Lima et al. (2022) demonstrated in their study that following 8 weeks of continuous moderate-intensity training and high-intensity interval training, BDNF and HMGCR levels significantly improved in overweight middle-aged men (18). Kumar et al. (2012) investigated the protective effect of HMG-CoA reductase inhibitors against fatigue induced by running on a wheel in rats. Their results showed that animals subjected to 6 minutes of daily physical activity on a wheel for 21 days exhibited increased wheel rotation, reduced anxiety behaviors, and decreased HMGCR enzyme activity (30).

Another non-pharmacological strategy is to prescribe naturally derived drugs in daily nutrition. Previous research has shown that products derived from the rice milling process, which are by-products, are rich in various nutrients and bioactive compounds, beneficial for health (29). Numerous studies on the benefits of rice bran indicate that it effectively improves certain health parameters through its antioxidant, anti-inflammatory, and anti-cancer activities (25). Several studies in humans and animals have shown that rice bran consumption reduces serum cholesterol and triglycerides (17). From human and animal studies, researchers have hypothesized that the hypocholesterolemic effect of rice bran is attributed to its specific phytochemical components and antioxidant properties (21). It has been shown that γ-Oryzanol, one of the main components of rice bran, reduces plasma cholesterol in rats. The amount of γ-Oryzanol decreased serum and liver cholesterol levels in hypercholesterolemic rats caused by excessive dietary cholesterol (4). One of the cholesterol-lowering mechanisms of rice bran and γ-Oryzanol is through reduced cholesterol absorption in the intestine (25). Munkong et al. (2023) demonstrated that rice bran extract administration at various doses, especially medium and high doses (0.5 and 1 g/kg per day), significantly improved the expression levels of lipid metabolism-related genes such as adipose triglyceride lipase (ATGL), CD36, and HMGCR in liver tissue (25). Similarly, Suwannachot et al. (2023) reported the antioxidant activity of rice bran protein hydrolysates in reducing malondialdehyde (MDA) levels, plasma protein carbonyls, increasing glutathione (GSH) levels, and HMGCR (28). Additionally, Lei et al. (2018) reported that the reduction in plasma cholesterol levels using wheat bran oil and rice bran oil is associated with decreased hepatic HMGCR and fatty acid synthase (29). Cote et al. (2012) stated in their study that a high-fat diet leads to TAG accumulation in the liver, along with increased liver and plasma cholesterol without increasing peripheral fat mass, and a high-fat diet is associated with lower levels of HMG-CoA-r transcripts. However, the mechanisms by which rice bran reduces cholesterol levels under a high-fat diet are unclear (19).

Since both aerobic exercise and rice bran can affect the hepatic cholesterol synthesis signaling pathway, it seems logical to examine the effects of these two interventions on the expression of genes in the hepatic cholesterol synthesis signaling pathway. A review of the studies shows that the simultaneous effect of these two interventions has not been studied. Therefore, this study was designed and conducted to determine the effect of aerobic exercise and ethanolic extract of rice bran on the expression of acetyl-CoA carboxylase and HMGCR genes in the liver tissue of rats fed with a high-fat diet. The results of this study can provide valuable information regarding the simultaneous effects of aerobic exercise and rice bran on hepatic cholesterol synthesis and subsequent dyslipidemia caused by a high-fat diet. It can provide strategies for preventing dyslipidemia and reducing obesity or high cholesterol complications.

This study hypothesizes that rice bran and aerobic exercise are related to the hepatic cholesterol synthesis pathway and that there may be differences between the type of diet and aerobic exercise. Therefore, this study aims to answer whether a period of moderate-intensity aerobic exercise and rice bran supplementation affects the expression of acetyl-CoA carboxylase and HMGCR in the liver tissue of rats fed with a high-fat diet.

2. Methods and Materials

2.1. Study Design and Participants

In a preclinical trial, 30 eight-week-old female rats, weighing 150 to 180 grams, were randomly divided into 5 groups (6 rats per group): control with normal diet, control with a high-fat diet, aerobic exercise with a high-fat diet, aerobic exercise with rice bran, and rice bran diet. All rats

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were obtained from the Pasteur Institute of Iran and transferred to the animal house of the Islamic Azad University, Central Tehran Branch. To acclimate the animals to the animal house environment and control confounding factors, the rats were housed for 2 weeks before the start of the study. The animal house environment was controlled with the following standards: 50% ± 10% relative humidity, 12-hour light/dark cycle with a timer, 23 ± 3°C temperature, ventilation to remove odors with a silent exhaust fan, and housing 3 rats per polycarbonate cage (1526.542 cm) with free access to municipal water and standard rodent food pellets. All procedures involving laboratory animals were conducted following the guidelines of the Ministry of Health and Medical Education of the Islamic Republic of Iran.

2.2. *Induction of Obesity*

To induce obesity, a high-fat diet containing 40% fat (20% soybean oil and 20% animal fat), 13% protein, and 47% carbohydrate was used. The control groups fed with a high-fat diet and aerobic exercise-high-fat diet followed this diet for six weeks, while the normal diet control group used a standard rat diet in pellet form. After six weeks, all groups were fed a normal diet (18).

2.3. *Aerobic Exercise Protocol*

The exercise program used in this study consisted of six weeks of moderate-intensity treadmill running, five sessions per week. The exercise intensity in the first week was 50% of maximum oxygen consumption, reaching 65% in the final week. To acclimate the rats, a one-week adaptation exercise with a speed of 9 meters per minute for 20 minutes was performed before the main exercise program. The exercise duration started at 15 minutes in the first week, increasing by 5 minutes each week. The exercise intensity began at 14 meters per minute on the first day, increasing by 3 meters per minute each week. Each session included a 5-minute warm-up at 10 meters per minute and a 5-minute cool-down at 10 meters per minute (18).

2.4. *Preparation and Administration of Ethanolic Extract of Rice Bran*

Rice bran from the plant Oryza sativa was obtained from reliable sources and verified by a botanist. After drying in the shade, it was powdered using a mill and prepared for extraction. After drying and milling, 200 grams of the plant were placed in a percolator. Extraction was performed using 1000 ml of 50% ethanol. This process was repeated three times. The collected extracts were stored in a refrigerator for further analysis. To determine the amount of dry matter in the liquid extract, a specific amount was dried in an oven. Based on this, the dry matter content in the extract was 1%. The ethanolic extract of rice bran was dissolved in distilled water and administered by gavage to the rats at a dose of 60 mg per kg (25).

2.5. *Sacrifice and Liver Tissue Sampling*

To sacrifice and sample tissues for genetic assessments, the perfusion method was used. For ethical considerations, the rats were fasted for at least 8 hours 48 hours after the last intervention, anesthetized with chloroform solution, and blood was collected from the left ventricle of the heart using a 3-cc syringe to sacrifice the rats. The liver tissue was quickly separated longitudinally with a scalpel, washed with phosphate-buffered saline (PBS), placed in a microtube, and frozen in a nitrogen tank. The samples were stored at -80°C until analysis.

2.6. *Gene Expression Analysis*

To analyze the expression of acetyl-CoA carboxylase and HMGCR genes in each group, real-time PCR was used. Primers were designed, and total RNA was extracted from the tissues and converted to cDNA. The cDNA was amplified by PCR, and the expression of the mentioned genes was analyzed. For primer design, the mRNA sequence of the cholesterol gene was obtained from the NCBI site. The primers were designed using AllelID software and evaluated with BLAST software to ensure the uniqueness of primer binding sites. The primers were synthesized by Sinagen Company. The GAPDH gene was used as an internal control in this study. The designed primer sequences are listed in Table 1.

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Table 1

Primer Sequences Used

Gene name	Forward	Reverse
acetyl-CoA carboxylase a	ACATCCCCACGCTAAACAG	TGTCCAACAGAACATCGCTG
HMG-CoA reductase	CAGGATGCAGCACAGAATGT	CTTTGCATGCTCCTTGAACA
GAPDH	AACCCATCACCATCTTCCAG	CCAGTAGACTCCACGACATAC

2.7. Data Analysis

All genetic assay data were reported as mean and standard deviation. One-way ANOVA was used to analyze the data. Tukey's test was used to determine the differences when significant differences were observed. SPSS-22 software was used for data analysis, and the significance level was set at 0.05 for all calculations.

3. Findings and Results

The values (mean ± standard deviation) of acetyl-CoA carboxylase and HMGCR in rats after the completion of the exercise intervention in the five groups (exercise, supplement, exercise + supplement, obese control, and healthy control) are reported in Table 2.

Table 2

Values (mean ± standard deviation) of the studied indicators

Variable	Aerobic Exercise - High-Fat Diet	Rice Bran Diet	Aerobic Exercise - Rice Bran	High-Fat Diet Control	Normal Diet Control
Acetyl-CoA Carboxylase	0.007626 ± 0.007	0.003068 ± 0.002	0.004096 ± 0.004	0.000147 ± 0.000	0.001642 ± 0.001
HMGCR	0.00020 ± 0.000	0.000056 ± 0.000	0.000397 ± 0.001	0.00071 ± 0.000	0.000166 ± 0.000

The results of the one-way ANOVA statistical test for acetyl-CoA carboxylase and HMGCR in the different research groups showed that the calculated F-value for comparing the mean differences was 2.767 for acetyl-CoA carboxylase and 3.175 for HMGCR, which was significant at the 5% alpha level (P = 0.05). Subsequently, the results of the Tukey test in Table 3 showed that the increase in acetyl-CoA carboxylase expression in the normal diet control group compared to the high-fat diet control group was significant (P = 0.000), while the decrease in HMGCR in the normal diet control group compared to the high-fat diet control group was also significant (P = 0.050).

Examining the effect of the aerobic exercise-high-fat diet program, intergroup analysis showed that the increase in acetyl-CoA carboxylase expression in the aerobic exercise-high-fat diet group compared to the high-fat diet control group was significant (P ≤ 0.034), and the results also showed that the reduction in HMGCR expression in the aerobic exercise-high-fat diet group compared to the high-fat diet control group was significant (P = 0.000).

Intergroup analysis also showed that the increase in acetyl-CoA carboxylase expression in the rice bran diet group compared to the high-fat diet control group was significant (P ≤ 0.001), while the decrease in HMGCR expression in the rice bran diet group compared to the high-fat diet control group was significant (P ≤ 0.028).

Similar changes were observed in the aerobic exercise-rice bran group compared to the high-fat diet control group, where the increase in acetyl-CoA carboxylase expression in the aerobic exercise-rice bran group compared to the high-fat diet control group was significant (P ≤ 0.002), while the decrease in HMGCR expression in the aerobic exercise-rice bran group compared to the high-fat diet control group was significant (P = 0.000).

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Table 3

Results of post-hoc gene expression tests in different research groups

Gene	Group 1	Group 2	Mean Difference	Standard Error	Significance Level
acetyl-CoA carboxylase	Aerobic Exercise - High-Fat Diet	Rice Bran Diet	0.0045574	0.002	0.347
		Aerobic Exercise - Rice Bran	0.0035303	0.001	0.593
		Normal Diet Control	0.005984	0.002	0.126
		High-Fat Diet Control	0.007478	0.003	0.034
	Rice Bran Diet	Aerobic Exercise - Rice Bran	-0.0010271	0.001	0.993
		Normal Diet Control	0.0014269	0.002	0.975
		High-Fat Diet Control	-0.0029211	0.003	0.001
	Aerobic Exercise - Rice Bran	Normal Diet Control	0.0039482	0.002	0.487
		High-Fat Diet Control	-0.0035303	0.001	0.002
	Normal Diet Control	High-Fat Diet Control	0.0014942	0.001	0.000
HMGCR	Aerobic Exercise - High-Fat Diet	Rice Bran Diet	0.00014417	0.0002	0.012
		Aerobic Exercise - Rice Bran	-0.0001971	0.0001	0.001
		Normal Diet Control	0.0000340	0.0003	0.081
		High-Fat Diet Control	-0.0005190	0.0001	0.000
	Rice Bran Diet	Aerobic Exercise - Rice Bran	-0.0003413	0.0002	0.002
		Normal Diet Control	-0.0001100	0.0001	0.001
		High-Fat Diet Control	-0.0006632	0.0001	0.028
	Aerobic Exercise - Rice Bran	Normal Diet Control	0.0002312	0.0003	0.002
		High-Fat Diet Control	0.0003218	0.0002	0.000
	Normal Diet Control	High-Fat Diet Control	-0.00055312	0.0001	0.050

4. Discussion and Conclusion

The results of the present study showed that feeding with a high-fat diet reduces the expression of acetyl-CoA carboxylase in the liver tissue of rats fed with a high-fat diet. Nowadays, due to insufficient physical activity and unhealthy diets, the prevalence of obesity is increasing worldwide. In obesity, adipose tissue is more exposed to hypertrophy, and studies show that hypertrophy of adipose tissue and excessive caloric intake combined with physical inactivity disrupt the balance of lipogenic and lipolytic pathways in the liver, leading to the accumulation of lipids, especially triglycerides {de Lima, 2022 #31882}. Liver damage caused by a high-fat diet is characterized by the accumulation of triglycerides in hepatocytes, which form through the esterification of free fatty acids and glycerol {Eftekharzadeh, 2023 #31888}. The increase in free fatty acids in the liver originates from three separate sources: lipolysis, a high-fat diet, and de novo lipogenesis. Along with the intake of a high-fat diet, inflammation, insulin resistance, lipid metabolism disorders, and other features of metabolic syndrome occur. The results of the present study, concerning the expression of the acetyl-CoA carboxylase gene, indicate a significant reduction in the expression of this gene in the liver tissue of rats exposed to a high-fat diet {Wu, 2013 #31875}. Acetyl-CoA carboxylase is one of the important enzymes in lipid synthesis, and its dysregulation is important in the development of obesity. The acetyl-CoA carboxylase enzyme converts acetyl-CoA to malonyl-CoA {Bastida, 2019 #31891}. Consistent with our study results, Ayogut et al. showed that the expression of the acetyl-CoA carboxylase gene in adipose tissue and liver of obese individuals was significantly lower than in the healthy control group with normal weight {Ortega, 2010 #31876}. Ortega et al. also showed that the level of acetyl-CoA carboxylase in the liver tissue of obese individuals was significantly lower than that of healthy individuals {Côté, 2013 #31877}. The regulation of acetyl-CoA carboxylase is associated with lipogenic hormonal stimuli, including insulin and thyroid hormones. Insulin increases the expression of lipogenesis-regulating genes by 3 to 5 times {Yang, 2021 #31873}. Obesity is a known factor for insulin

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resistance, and when cells become insulin resistant, they lose their relative capacity to absorb glucose and free fatty acids {Galdieri, 2014 #31889}. As a result, the cell undergoes intracellular hypoglycosytopenia, which can reduce the expression of lipogenesis-regulating genes {Luo, 2020 #31870}.

The results of the present study showed that feeding with a high-fat diet increases the expression of HMGCR in the liver tissue of rats fed with a high-fat diet. HMGCR is the rate-limiting enzyme in the mevalonate pathway {Wu, 2013 #31875}. A high-fat diet is associated with chronic disorders such as obesity, diabetes, hyperlipidemia, and metabolic syndrome {de Lima, 2022 #31882}. Feeding with a high-fat diet can also modulate systemic fats and increase the risk of coronary heart disease and non-alcoholic fatty liver disease (NAFLD) {Alvarez-Jimenez, 2022 #31898}. Lipidomic evaluation of liver biopsies has shown high levels of esterified and non-esterified cholesterol in the livers of overweight patients with NAFLD {Cao, 2021 #31885}. Interestingly, increased HMGCR expression has been observed in the livers of obese individuals {Eftekharzadeh, 2023 #31888}. The liver plays a major role in regulating hepatic and systemic cholesterol levels. When cholesterol homeostasis is disrupted due to increased synthesis, cholesterol accumulation in the liver may occur, and its level in the blood (in the form of lipoproteins) may rise. New cholesterol synthesis is regulated by the rate-limiting enzyme HMGCR, which converts HMGCR to mevalonate. Under physiological conditions, HMGCR activity is regulated by multiple mechanisms {Chatterjee, 2020 #31883}. At the transcriptional level, the expression of the HMGCR gene is regulated by sterol regulatory element-binding proteins (SREBPs) {Yang, 2021 #31873}. Increased hepatic fatty acid and cholesterol biosynthesis has been observed in rats fed with a high-fat diet, while increased hepatic cholesterol biosynthesis has also been reported in perfused fatty acid-fed rats (Alipour Talesh et al., 2020). In a recent study, we observed that feeding with a high-fat diet (60% kcal from fat) causes oxidative stress and increases triglyceride and total cholesterol levels in the liver of rats {Cardoso, 2021 #31884}. Regulating HMG-CoA reductase activity is the primary approach to controlling new cholesterol synthesis, while abnormal activation can lead to hepatic cholesterol accumulation and hypercholesterolemia {Barkas, 2020 #31892}. The results clearly show that during high-fat feeding, Sp1-mediated SREBP2 expression leads to increased HMGCR regulation, resulting in increased cholesterol biosynthesis in the liver {Makhmudova, 2021 #31868}. Cholesterol is obtained from the diet, and HMGCR regulates the rate-limiting step in cholesterol biosynthesis. Regulating hepatic HMGCR activation is essential for maintaining cholesterol homeostasis in the liver and circulation {Wu, 2013 #31875}. In the study by Wu et al., significant increases in HMGCR activity in the liver were shown after 5 weeks of high-fat diet feeding, which was consistent with significant increases in HMGCR mRNA and protein levels {Chatterjee, 2020 #31883}, and these results align with the changes in HMGCR levels in the present study. Consistent with observations in the livers of high-fat diet-fed mice, HMGCR mRNA expression was also significantly increased in cells incubated with palmitic acid {Yang, 2021 #31873}. It can be stated that the induction of HMGCR expression can lead to increased cholesterol biosynthesis and subsequently its accumulation in liver cells {Ma, 2017 #31869}. The results of the Wu et al.'s (2013) study indicated that increased cholesterol synthesis may contribute to liver fat accumulation following high-fat diet consumption {Wu, 2013 #31875}. In the present study, the high-fat diet consisted of 40% fat (20% soybean oil and 20% animal fat), 13% protein, and 47% carbohydrates, which may suggest that HMGCR activation in these rats could be a metabolic response to higher fat intake {Côté, 2013 #31877}. Therefore, the results obtained from the present study cannot rule out the possibility that in the presence of excess dietary fatty acids, the stimulatory effect of fatty acids on cholesterol biosynthesis may take precedence over cholesterol feedback inhibition and/or HMGCR regulation. Cholesterol synthesis by the negative feedback mechanism (increased cellular cholesterol) may itself be disrupted in the liver due to high-fat diet feeding.

Another finding of the present study was that a period of aerobic exercise resulted in increased expression of acetyl-CoA carboxylase and significantly reduced HMGCR gene expression compared to the control group fed with a high-fat diet in the liver tissue of rats fed with a high-fat diet. These results are consistent with the findings of Cuang et al. (2016). Moreover, Kumar et al. (2012) also investigated the protective effect of HMGCR inhibitors against fatigue-

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induced anxiety from wheel running activity in their study and stated that increased wheel rotations and physical activity improved oxidative protection and HMGCR enzyme activity in liver tissue. HMGCR can reduce exercise-induced proteolysis by suppressing proteolytic pathways in muscles and can also stimulate protein synthesis similar to leucine {Kumar, 2012 #31881}. In the sarcoplasm, HMGCR is metabolized to β-hydroxy-β-methylglutaryl-CoA, providing a carbon source available for cholesterol synthesis, which in turn provides materials for muscle cell growth {Wu, 2013 #31875}. Additionally, HMGCR can be polymerized and used as a structural component of the cell membrane, thereby improving its stability {Makhmudova, 2021 #31868}. Moreover, it has been suggested that HMGCR increases muscle cells' ability to oxidize fatty acids through unknown mechanisms, thereby reducing fat mass. HMGCR is considered an enzyme that limits the rate of cholesterol biosynthesis {Yang, 2021 #31873}. HMGCR consists of two domains: a region covered by 8 transmembranes that anchor the protein to the endoplasmic reticulum and a carboxy-terminal domain that extends into the cytosol and contains all catalytic activity. The enzyme's half-life depends on the concentration of cholesterol and isoprenoids in the cell {Wu, 2013 #31875}. When cellular cholesterol levels are low, the enzyme is relatively stable (half-life about 10 hours). Therefore, active HMGCR accumulates in response to slow protein degradation and increased enzyme transcription and synthesis in the endoplasmic reticulum. Since HMGCR is a rate-limiting enzyme, the result is increased cholesterol synthesis {Galdieri, 2014 #31889}. However, when cellular cholesterol levels increase, the rate of HMGCR protein degradation increases through a process that requires both a sterol and an unknown non-sterol derived from farnesyl diphosphate. Therefore, cholesterol-saturated cells have low levels of HMGCR reductase protein (and thus low rates of cholesterol synthesis) because the enzyme is rapidly degraded, and gene transcription is low as a result. Several other factors that alter HMGCR activity have also been examined for their potential role in the normal regulation of cholesterol synthesis {Wu, 2013 #31875}. The addition of cAMP to liver homogenates decreases both cholesterol synthesis rates and reductase activity. These short-term effects of cAMP may be related to the role of reversible phosphorylation in regulating HMGCR activity. Hepatocyte microsomes contain an ATP-dependent protein kinase ("reductase kinase") that inactivates HMGCR by phosphorylation, while the cytosol contains a phosphoprotein phosphatase that activates reductase by dephosphorylation {Makhmudova, 2021 #31868}. The sequence of interrelated phosphorylation cycles provides a highly sensitive mechanism for regulating HMGCR activity by amplifying such a signal as a small change in intracellular cAMP concentration to the cell. Similar and perhaps even more complex control mechanisms are responsible for modulating the activity of many enzymes that are activated or inactivated by phosphorylation. Exercise-induced increases in cAMP can reduce HMGCR activity and cause an overall decrease in the dephosphorylation rate of phosphorylated reductase (inactive form) or a decrease in the dephosphorylation rate of reductase {Luo, 2020 #31870}. The active form of cAMP reductase kinase can also act directly to activate reductase kinase and thus indirectly support the formation of the inactive form of HMGCR {Yang, 2021 #31873}. Researchers have indeed presented results that the stimulatory effect of insulin and the inhibitory effect of glucagon on HMGCR activity in liver cells are partially mediated by variability in reductase kinase activity. Therefore, it is possible that hormones that cause changes in intracellular cAMP concentration may affect HMGCR activity by altering the ratio of active to inactive forms of the enzyme, influenced by physical activity (Wu et al., 2013).

Another finding of the present study showed that the consumption of ethanolic extract of rice bran led to an increase in acetyl-CoA carboxylase expression in rats fed with a high-fat diet in liver tissue. Consistent with the present study, Yang et al. (2014) reported that rice bran extract could significantly reduce plasma TC and LDL-c concentrations in C57BL/KsJ-db/db mice {Yang, 2021 #31873}. The results of our study align with the findings of recent studies that reported significant changes in acetyl-CoA carboxylase levels following rice bran consumption. Only a few studies have examined the antioxidant properties of rice bran in vivo {Ma, 2017 #31869}. Research has shown that rice bran extract reduces serum levels of aspartate aminotransferase, active hepatic lipid hydroperoxide, alanine aminotransferase, and thiobarbituric acid {Makhmudova, 2021 #31868}. Laboratory studies have

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shown that rice bran extract can scavenge lipid-soluble organic radicals and prevent the formation of new free radicals. The antioxidant activity of rice bran is indeed supported by previous data {de Lima, 2022 #31882}.

Another finding of this study showed that aerobic exercise combined with the consumption of ethanolic extract of rice bran led to a decrease in HMGCR gene expression in rats fed with a high-fat diet in liver tissue. Similarly, Munkong et al. (2023) in their research stated that rice bran extract altered the expression levels of genes related to lipid metabolism (adipose triglyceride lipase (ATGL), CD36, lipoprotein lipase, liver X receptor alpha (LXRα)) and significantly improved HMGCR in liver or adipose tissue and hepatic high-density lipoprotein expression {Munkong, 2023 #31880}. Similarly, the findings of Lei et al. (2018) indicated that the cholesterol-lowering activity of dietary wheat bran oil and rice bran oil was associated with improved hepatic HMGCR and fatty acid synthase {Lei, 2018 #31872}. Synthetic LXRa agonists, by regulating the transcription of target genes CYP7A1 and SREBP-1c, have hypocholesterolemic and hypotriglyceridemic effects. Due to this dual role of synthetic LXR agonists, selective natural LXRa agonists can be developed for treating metabolic syndromes. According to the results obtained from the research by Liu et al. (2020), treatment with rice bran supplements significantly increased the expression levels of mRNA LXRα and CYP7A1 and bile acid synthesis, but decreased the expression level of mRNA SREBP-1c and serum and hepatic TG content. Additionally, AMPK phosphorylation not only reduced LXR-dependent SREBP-1c transcription but also directly reduced SREBP-1c nuclear translocation and the transcription of related genes such as FASN and HMGCOA-R. These results indicated that rice bran supplementation, rich in bioactive compounds such as anthocyanins, dietary fiber, and minerals, improves lipid metabolism {Liu, 2020 #31871}. The recommendations of the World Health Organization indicate that a healthy diet should contain more than 25 grams of fiber. Most European countries recommend consuming 25 to 30 grams of fiber per day. Additionally, fiber (usually water-soluble) in whole grains has a synergistic effect through prebiotics and beneficial gut bacteria such as lactobacilli and bifidobacteria, which can convert anthocyanins into phenolic acids {Yang, 2021 #31873}. Chronic disorders are closely related to a high-fat diet. Diseases such as obesity, diabetes, hyperlipidemia, and metabolic syndrome may also modulate systemic lipids and increase the risk of coronary heart disease and non-alcoholic fatty liver disease (NAFLD). NAFLD is considered a hepatic manifestation of metabolic syndrome and is characterized by the excessive accumulation of lipids in the liver. Although exogenous dietary fat is likely to contribute to liver fat accumulation, there is evidence that endogenous lipid synthesis may also play an important role in NAFLD {Yang, 2021 #31873}. Lipidomic evaluation of liver biopsies showed high levels of esterified and non-esterified cholesterol in the livers of overweight patients with NAFLD. Surprisingly, an inconsistent increase in HMG-CoA reductase expression was observed in the livers of obese patients with NAFLD. Increased cholesterol biosynthesis has also been observed in patients with NAFLD regardless of obesity. Therefore, it seems that in the context of NAFLD, traditional regulation of sterols in hepatic cholesterol synthesis may be ineffective {Wu, 2013 #31875}. The liver plays a major role. New cholesterol synthesis is regulated by the rate-limiting enzyme HMGCR, which converts HMGCR to mevalonate. Under physiological conditions, HMGCR activity is regulated by multiple mechanisms at the transcriptional level by sterol regulatory element (SREBP). SREBPs are transcription factors that play a crucial role in controlling cholesterol and fatty acid biosynthesis {Ma, 2017 #31869}. Three isoforms, including SREBP-1a, SREBP-1c, and SREBP-2, are encoded by a single gene and a different gene, respectively. The liver expresses all three SREBP isoforms, but it is SREBP-2 that selectively activates new cholesterol synthesis by inducing HMGCR expression and other enzymes involved in the cholesterol biosynthesis pathway. Under physiological conditions, SREBP-2 activation is regulated by cellular sterols, including cholesterol. When cellular sterol levels increase, SREBP-2 remains in the endoplasmic reticulum {Wu, 2013 #31875}. Conversely, when cellular sterol levels decrease, SREBP-2 is transported from the endoplasmic reticulum to the Golgi apparatus, a step critical for SREBP-2 maturation and subsequent nuclear localization. When SREBP-2 enters the nucleus, it binds to the sterol regulatory element of DNA and activates the HMGCR gene {Ma, 2017 #31869}. Increased hepatic fatty acid and cholesterol biosynthesis was observed in mice fed

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with a high-fat diet. In one study, feeding a high-fat diet (60% kcal from fat) was observed to induce oxidative stress and increase hepatic triglyceride and total cholesterol levels in mice. Regulating HMGCR activity is the primary approach for controlling new cholesterol synthesis, while abnormal activation can lead to hepatic cholesterol accumulation and hypercholesterolemia, and using rice bran supplements may be a way to reduce cholesterol levels {Wu, 2013 #31875}.

The results of the present study showed that aerobic exercise combined with the consumption of ethanolic extract of rice bran led to increased expression of the acetyl-CoA carboxylase gene in rats fed with a high-fat diet. Similar to acetyl-CoA carboxylase synthesis pathways, its catabolic pathways are also diverse. The most common pathway for circulating acetyl-CoA carboxylase catabolism is ATP production through the mitochondrial TCA cycle {Wu, 2013 #31875}. Additionally, certain cells, including liver cells, can synthesize ketones through acetyl-CoA carboxylase to meet energy metabolism needs. Mitochondrial hydroxymethylglutaryl-CoA synthase (HMGCS) condenses acetyl-CoA carboxylase with acetoacetyl-CoA to form HMGCR, through which hydroxymethylglutaryl-CoA lyase (HMGCLoace) is released {Yang, 2021 #31873}. Acetyl-CoA carboxylase in the cytoplasm can be used for protein modification by histone acetyltransferases (HATs), N-acetyltransferases (NATs), and lysine acetyltransferases (KATs) {Makhmudova, 2021 #31868}. In the cytoplasm of liver cells, acetyl-CoA can be hydrolyzed by acyl-CoA thioesterase 12 (ACOT12) to coenzyme A and acetate (Wu et al., 2013). Similar to the early stages of ketone synthesis, two molecules of cytoplasmic acetyl-CoA carboxylase condense and form acetyl-CoA, which reacts with a third acetyl-CoA to form HMGCR. HMGCR is reduced to mevalonate by hydroxymethylglutaryl-CoA reductase (HMGCR). A series of enzymatic reactions convert mevalonate to farnesyl pyrophosphate. The condensation reaction of two farnesyl pyrophosphate molecules produces squalene {Makhmudova, 2021 #31868}. Acetyl-CoA carboxylase can also be converted to malonyl coenzyme A by acetyl-CoA carboxylase and then paired with acetyl-CoA carboxylase to a domain of the acyl carrier protein in the multifunctional enzyme fatty acid synthase (FASN) {de Lima, 2022 #31882}.

Overall, it can be stated that aerobic exercise, as a positive stimulus, improves the expression levels of HMGCR and acetyl-CoA carboxylase genes through various cellular and molecular mechanisms. Additionally, the results of the present study showed that regardless of aerobic exercise, the addition of rice bran extract can also affect this pathway and reduce the complications of a high-fat diet.

Authors' Contributions

S.M. conceptualized the study, designed the research methodology, and supervised the overall implementation of the experiments. M.A.A., the corresponding author, conducted the data analysis, interpreted the results, and led the drafting and revising of the manuscript. S.R.M. assisted with the recruitment and care of the animal subjects, facilitated the aerobic exercise protocol, and contributed to data collection. M.P. supported the administration of the ethanolic extract of rice bran and helped with data analysis. S.R.A. assisted in the molecular biology techniques, including the measurement of gene expression, and contributed to the literature review. All authors participated in discussing the findings, critically reviewed the manuscript for important intellectual content, and approved the final version for publication.

Declaration

In order to correct and improve the academic writing of our paper, we have used the language model ChatGPT.

Transparency Statement

Data are available for research purposes upon reasonable request to the corresponding author.

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Declaration of Interest

The authors report no conflict of interest.

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Ethics Considerations

The study placed a high emphasis on ethical considerations. Informed consent obtained from all participants, ensuring they are fully aware of the nature of the study and their role in it. Confidentiality strictly maintained, with data anonymized to protect individual privacy. The study adhered to the ethical guidelines for research with human subjects as outlined in the Declaration of Helsinki. This article is derived from the first author's master's thesis at Lahijan Branch, Islamic Azad University, Lahijan, Iran, and has an ethics code IR.IAU.LIAU.REC.1401.017 from the Ethics Committee of Islamic Azad University, Lahijan Branch. We would like to express our deepest appreciation to all participants in this study.

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