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ORIGINAL PAPER

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EFFECTS OF *LACTOBACILLUS PLANTARUM* ON THE AKT/ENOS CARDIAC PATHWAY IN RATS WITH FRUCTOSE-INDUCED METABOLIC SYNDROME

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ABSTRACT

Background. This study investigated the effects of *Lactobacillus plantarum* supplementation on the Akt/eNOS pathway in the cardiac tissue of rats subjected to a fructose-rich diet, which induced metabolic syndrome.

Materials and methods. Twenty-two male Wistar albino rats were randomly assigned to three groups: (1) control, (2) fructose, and (3) fructose ± *L. plantarum*. Rats in the fructose group were administered a 20% fructose solution in their drinking water for 15 weeks. In the *L. plantarum*-treated group, the probiotic was administered via gastric gavage at a daily dose of 1×10^9 CFU/mL/100 g during the final six weeks of the study.

Results. The administration of *Lactobacillus plantarum* resulted in a significant increase in the levels of Akt ($p < 0.0001$), IRS-1 ($p = 0.0113$), mTOR ($p < 0.0001$), eNOS ($p < 0.0001$), and SIRT1 ($p = 0.0031$) in the cardiac tissue of rats compared to the fructose group. Moreover, a marked reduction in iNOS, NF-κB, and TNF-α levels ($p < 0.0001$) was observed, highlighting the potential of *L. plantarum* to counteract the adverse effects of fructose consumption.

Conclusion. The findings of this study suggest that *L. plantarum* has the potential to reduce inflammation in the heart, enhance insulin sensitivity via the Akt/eNOS pathway, and protect against cardiovascular diseases associated with metabolic syndrome.

Keywords: metabolic syndrome, *Lactobacillus plantarum*, Akt, eNOS, fructose, insulin

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INTRODUCTION

Metabolic syndrome is a collection of risk factors that are linked to the onset of atherosclerosis. It includes glucose intolerance, diabetes mellitus (DM), obesity, essential hypertension, dyslipidemia, and proinflammatory and prothrombotic components. These clinical manifestations arise from insulin resistance and are linked to an elevated risk of cardiovascular disease. Over the past 20 years, there has been a notable global increase in the number of individuals with metabolic syndrome, which is directly linked to rising rates of diabetes and obesity (Fulop et al., 2006). Metabolic syndrome doubles the risk of cardiovascular events, and its overall cardiovascular risk reflects that of its individual components (Oğuz et al., 2020). The primary risk factors for cardiovascular disease linked to metabolic syndrome include low high-density lipoprotein (HDL) levels, elevated blood pressure, and increased fasting plasma glucose.

Research has indicated that a diet rich in fructose, saturated fat, and polyunsaturated fatty acids causes the development of metabolic syndrome in human and animal models (Castro et al., 2015). Specifically, fructose is known to play a role in the progression of metabolic syndrome through the dysregulation of many molecular signaling factors (Rutledge and Adeli, 2007). Fructose consumption and the incidence of metabolic syndrome have both increased dramatically in recent years (Lê and Tappy, 2006).

Consumers are increasingly interested in incorporating bioactive compounds into their diets as functional ingredients because of their various health benefits (Vo and Kim, 2013). Probiotics, prebiotics, and symbiotics represent the most commercially viable functional food components, owing to their demonstrated health benefits (Cruz et al., 2010). Through a variety of methods, including improved barrier function, mucosal immune system regulation, antimicrobial generation, and intestinal microbiota change, probiotic microorganisms have positive effects on the intestinal epithelium (Vanderpool et al., 2008). Probiotics increase the production of intestinal anti-inflammatory cytokines (e.g., interleukin [IL]-10) and decrease the production of proinflammatory cytokines (e.g., IL-1β and tumor necrosis factor-alpha [TNF-α]) (Morita et al., 2002). Furthermore, probiotics have been shown in a number of in vivo and clinical trials to help reduce obesity, insulin resistance, and hepatic steatosis (also known as fatty liver disease). These beneficial impacts on health have been shown to be extremely strain- and species-dependent. Among the strains studied, *Lactobacillus* and *Bifidobacterium* species are the most researched and are generally considered safe probiotics (Ji et al., 2019; Kim et al., 2018).

The human intestinal microbiota is influenced by the host's geographic location, leading to the hypothesis that locally sourced probiotics may be safer and more effective for domestic users (Nagpal et al., 2018). Commonly found in fermented foods and the gastrointestinal system, *Lactobacillus plantarum* is a Gram-positive lactic acid bacteria that is frequently employed in the food sector as a possible first probiotic (Arasu et al., 2016). In addition, *L. plantarum* has traditionally been employed in the fermentation of milk, meat, and vegetables and is thus one of the *Lactobacillus* species with the longest history of use. Indeed, it was consumed by humans long before the term "probiotic" became widespread. Many strains of *L. plantarum* have been shown to have significant effects on intestinal health and metabolic disorders within the gut-heart-brain axis, highlighting their importance (Liu et al., 2018).

The protein kinase B (Akt)/endothelial nitric oxide synthase (eNOS) pathway plays a pivotal role in regulating cellular processes, particularly in the production of nitric oxide (NO). This pathway begins with phosphatidylinositol-3-kinase (PI3K)/Akt signaling and continues with the activation of eNOS. External stimuli, such as growth factors (e.g., VEGF) or pharmacological agents, trigger the activation of PI3K, which subsequently leads to the phosphorylation of Akt. Phosphorylated Akt enhances eNOS activity by phosphorylating the enzyme at the Ser1177 site. Activated eNOS then catalyzes the conversion of L-arginine into nitric oxide and L-citrulline. NO facilitates the relaxation of vascular smooth muscles, thereby regulating vascular tone, and also exerts anti-inflammatory effects. The Akt/eNOS pathway is crucial for maintaining vascular health, supporting cellular repair mechanisms, and mitigating oxidative stress. Dysregulation of this pathway is significantly implicated in the pathogenesis of metabolic syndrome, diabetes, and other cardiovascular diseases. Therefore, the

modulation of the Akt/eNOS pathway is considered a critical target for both pharmacological and natural therapeutic approaches (Guan and Wang, 2021).

This study aims to deepen our understanding of the cardiovascular effects of probiotic lactic acid bacteria, with a special emphasis on how they influence insulin signaling pathways in the context of a fructose-rich diet. Focusing on *L. plantarum*, it seeks to assess how supplementation affects the Akt/eNOS pathway in the cardiac tissue of rats on a fructose diet. Despite extensive research on the metabolic implications of fructose, its specific effects on cardiovascular signaling pathways, such as Akt/eNOS, have not been adequately explored. This study examines how *L. plantarum* supplementation modulates these pathways, potentially offering novel therapeutic insights. The investigation of the influence of *L. plantarum* on this particular pathway is intended to reveal potential therapeutic advantages and action mechanisms that could help prevent the cardiovascular issues linked to high-fructose diets. Although metabolic syndrome has been widely studied, limited attention has been given to the molecular pathways underlying its cardiovascular effects, particularly the role of the Akt/eNOS pathway. This study addresses this gap by investigating the potential therapeutic effects of *L. plantarum* supplementation on the Akt/eNOS signaling pathway in a fructose-induced metabolic syndrome model.

MATERIALS AND METHODS

Animals

The study involved twenty-two healthy Wistar albino male rats weighing between 50 and 70 grams, which were obtained from the Gazi University Laboratory of Experimental Animals (Ankara, Turkey). These rats were kept in rooms where the temperature and humidity were strictly controlled, complemented by a consistent 12-hour light/12-hour dark cycle. During the study, rats were given standard pellet chow and water ad libitum. Rats in all groups were fed standard rat chow (dry matter: 88%, protein: 23%, cellulose: 7%, crude ash: 8, HCl insoluble ash: 2%, calcium: 1.5%, phosphorus: 0.9%, sodium: 0.7%, salt (NaCl): 1%, methionine: 0.3, lysine: 1).

Weekly records of the rats' body weight and food and liquid consumption were kept to monitor their health and dietary intake closely. The care and maintenance of the rats was strictly monitored to ensure ethical and humane treatment. This study was carried out in accordance with ethical rules, respecting animal welfare and animal rights, after receiving approval from the Gazi University Animal Experiments Local Ethics Committee (G.Ü.ET-18.080) regarding its compliance with the principles in the ethics committee directive.

Metabolic syndrome

In the study, twenty-two male Wistar albino rats were randomly divided into three groups using the randomization protocol: 1) a control group (n = 8), receiving a standard diet; 2) a fructose group (n = 7), receiving fructose solution in their drinking water; and 3) a fructose ± *L. plantarum* group (n = 7), receiving fructose solution supplemented with *L. plantarum* via gastric gavage at a daily dose of 1×10^9 CFU/mL/100 g body weight during the last six weeks of the experiment. To simulate metabolic syndrome, the rats in the fructose and fructose plus *L. plantarum* groups received their drinking water mixed with a 20% (w/v) fructose solution, accessible ad libitum for 15 weeks. This solution was freshly prepared every day to ensure consistency (Yildirim et al., 2019). The control group, meanwhile, was not subjected to the metabolic syndrome model and was given drinking water without fructose. At the end of the treatment period, the rats were euthanized following the administration of general anesthesia using xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (50 mg/kg). Anesthesia was administered intraperitoneally, ensuring a deep plane of anesthesia (loss of reflex response) before euthanasia. Heart tissues were promptly excised and stored at –80°C for subsequent analysis.

Production of *L. plantarum* strains

L. plantarum (ATCC: 14917) strains were cultured under sterile conditions at 30°C in a 150 rpm shaker using Man, Rogosa, and Sharpe (MRS) media (Oxoid; Unipath Ltd., UK). To ensure sterility and consistency, contamination checks were conducted weekly by streaking cultures on sterile agar plates. CFU counts were validated using serial dilution and plating techniques, ensuring an accurate dose of 1×10^9 CFU/mL per 100 g BW. Stock cultures were kept in

MRS medium with 20% (v/v) glycerol at –80°C. The cultures were grown at 600 nm (cell density) to an optical density of 1.0 (1×10^8 CFU/mL) after being inoculated with 1.5 mL of glycerol stock culture in Erlenmeyer flasks containing 20 mL MRS and shaken at 35 rpm at 35°C ±1°C. After the culture had been divided into 10 mL tubes (1×10^9 CFU), the bacteria were collected at 4°C for 5 minutes at 5,000 g. After being cleaned with an isotonic saline solution, the cell pellets were lyophilized in a freeze-dryer.

Western blot analysis

Western blotting was used to analyze the protein levels of Akt, eNOS, iNOS, IRS-1, SIRT1, mTOR, NF-κB, and TNF-α in the heart tissue of the rats using rat-specific antibodies, as specified in a previous study (Gencoglu et al., 2015). To minimize bias, the researchers conducting protein analysis via Western blotting were blinded to group assignments. This ensured objective data collection and analysis. Briefly, the heart samples of the rats were homogenized in the cold chain with the aid of a glass–glass homogenizer and sonicated for 10 minutes at 4°C in a lysis buffer containing 1X protease and phosphatase inhibitors to ensure protein integrity. Measurements were taken using a NanoDrop spectrophotometer (MaestroGen, Las Vegas, NV, USA) to calculate the total protein content of the heart tissue samples from each group. Working samples that contained equal amounts of protein (20 μg) were transferred to a 12% acrylamide that contained gel by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to a nitrocellulose membrane with the Trans-Blot Turbo Transfer System (Bio-Rad, Life Sciences Research, Hercules, CA, USA). The membranes were then washed five times with phosphate-buffered saline (PBS) that contained 0.1% Tween (PBS; blocking solution). Prior to the application of the primary antibody, nitrocellulose membranes were incubated with PBS containing 1% bovine serum albumin for two hours at room temperature to block nonspecific binding. Subsequently, the membranes were incubated overnight with primary antibodies targeting Akt, eNOS, iNOS, IRS-1, SIRT1, mTOR, NF-κB, and TNF-α (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The following day, the membranes were rinsed five times with PBS buffer and then incubated with a goat anti-mouse secondary antibody (1:1000 dilution, Abcam, Cambridge, UK) at room temperature for two hours. The blotting reaction was stopped with diaminobenzidine solution that contained 3% H_2O_2 after washing five times with PBS buffer solution. The protein levels were analyzed as percent relative values in comparison to the control group after being densitometrically assessed using an image analysis system (Image J; National Institute of Health, Bethesda, MD, USA) and verified with the values found on β-actin blots.

Statistical analyses

SPPS ver. 22 (IBM Corp., Armonk, NY, USA) was used to analyze the study data (Hair et al., 2010). The sample size calculation determined that a total of 21 rats ($n = 7$ per group) was required to achieve a statistical power of 85%, with a significance level of 0.05 (type I error) and an effect size of 0.80, following the methodology described by Faul et al. (2007). The Levine test was used to test the homogeneity of the variances, which is a prerequisite for using parametric tests on the data, and the Shapiro–Wilk test was used to check the assumption of normality using a one-way analysis of variance to determine the differences between the groups. The variables were expressed as the mean ± standard error. The Tukey post-hoc test was then used for multiple comparisons. The cutoff value for a statistically significant difference was 0.05.

RESULTS AND DISCUSSION

Following fructose administration in rats with metabolic syndrome, the Akt levels in their heart tissue were observed to be 100.00 ±3.98, 61.48 ±3.01, and 76.64 ±3.40 in the control, fructose-exposed, and fructose-exposed with *L. plantarum* supplementation groups, respectively (Fig. 1A, Table 1). Akt levels in the fructose-exposed group exhibited a decrease of 38.5% compared to the control group (Fig. 1A; $p < 0.0001$). *L. plantarum* supplementation for 6 weeks alongside fructose led to a 24.7% increase in Akt levels within cardiac tissue (Fig. 1A; $p < 0.0001$). However, despite this rise in Akt levels in rats whose diets were supplemented with *L. plantarum*, they remained 23.4% lower than those in the control group (Fig. 1A; $p < 0.0001$). Additionally, IRS-1 levels in heart tissues exhibited reductions of 53.8% ($p < 0.0001$) and 33.6% ($p = 0.0001$) with respect to the control group in the

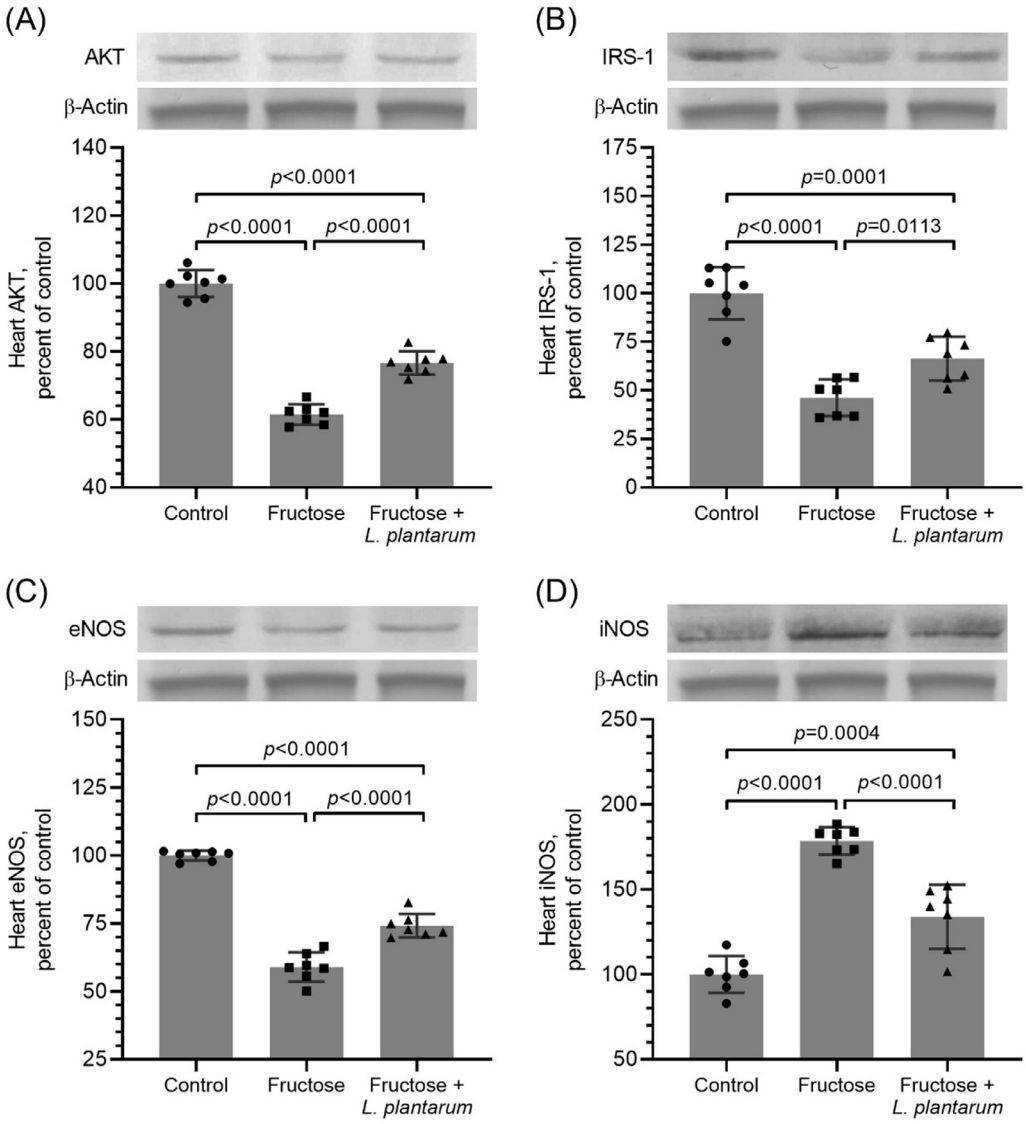

Fig. 1. Effect of *Lactobacillus plantarum* administration on cardiac tissue protein levels in rats with metabolic syndrome. The proteins analyzed include Protein Kinase B (Akt, Panel A), Insulin Receptor Substrate 1 (IRS-1, Panel B), Endothelial Nitric Oxide Synthase (eNOS, Panel C), and Inducible Nitric Oxide Synthase (iNOS, Panel D). Western blotting was performed in triplicate. Data are presented as the mean ±standard deviation for each group, expressed as a percentage of the control. β-actin was used as a loading control. A one-way analysis of variance (ANOVA) was applied for group comparisons, and pairwise comparisons were made using Tukey's post-hoc test. Statistical significance was set at $p < 0.05$

fructose and fructose + *L. plantarum* groups, respectively (Fig. 1B). Importantly, the administration of *L. plantarum* resulted in a notable increase of 43.7% in IRS-1 protein levels within the heart tissue of rats with metabolic syndrome (Fig. 1B; $p = 0.0113$).

Relative to the control group, eNOS levels in heart tissue decreased by 41.0% in the fructose group (Fig. 1C; $p < 0.0001$), while iNOS levels increased by 78.6% (Fig. 1D; $p < 0.0001$). The administration of *L. plantarum* resulted in a 25.7% elevation in eNOS

levels in heart tissue compared to the fructose group (Fig. 1C; $p < 0.0001$) and a reduction of iNOS levels by 25.0% (Fig. 1D; $p < 0.0001$). Interestingly, eNOS levels were 25.8% lower (Fig. 1C; $p < 0.0001$), and iNOS levels were 33.9% higher (Fig. 1D; $p = 0.0004$) in the fructose + *L. plantarum* group than in the control group.

The levels of mTOR in the heart tissue of the fructose group decreased by 44.4% compared to the control group (Fig. 2A; $p < 0.0001$). However, treatment

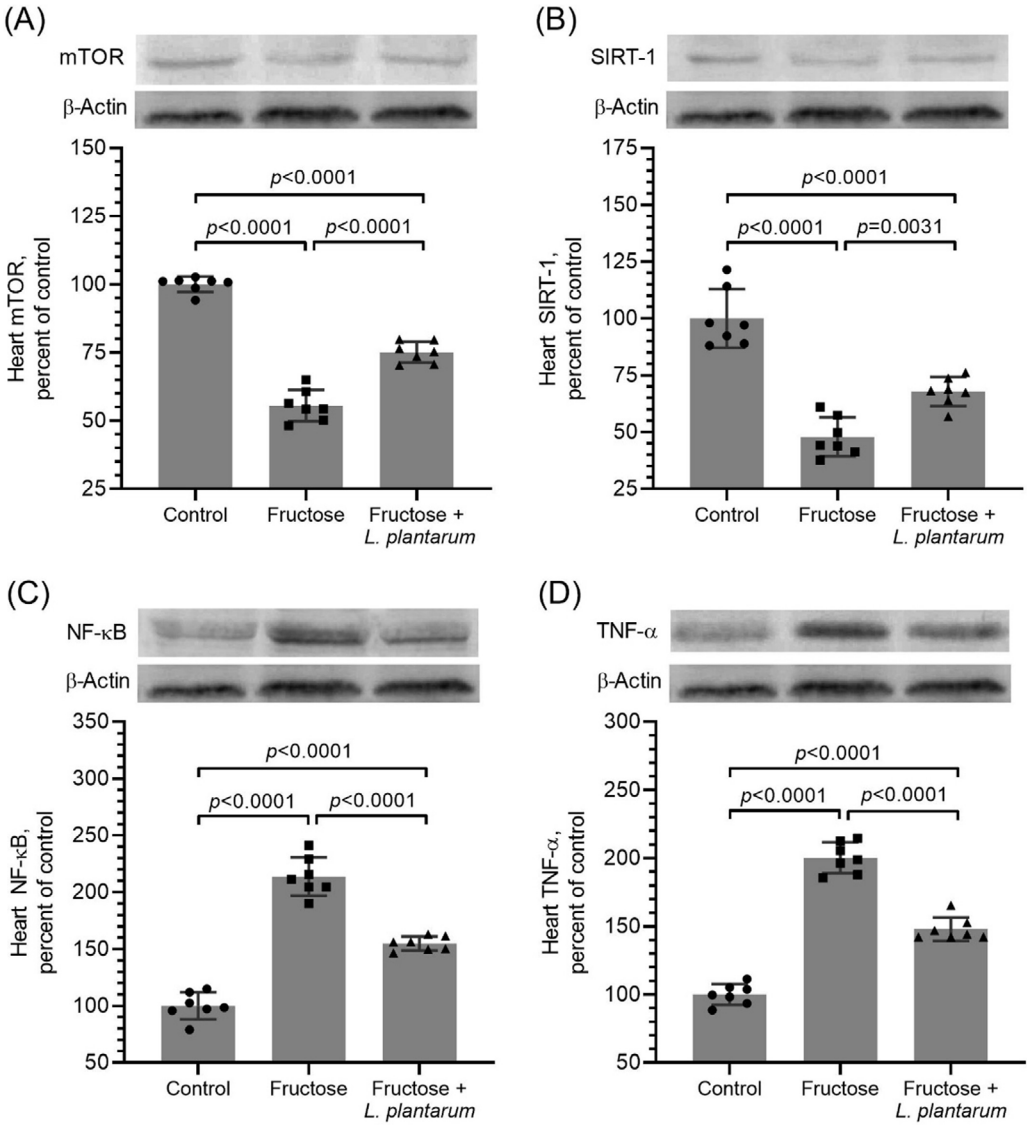

Fig. 2. Effect of *Lactobacillus plantarum* administration on cardiac tissue protein levels in rats with metabolic syndrome. The proteins analyzed include Mammalian Target of Rapamycin (mTOR, Panel A), Sirtuin 1 (SIRT-1, Panel B), Nuclear Factor Kappa B (NF-κB, Panel C), and Tumor Necrosis Factor-alpha (TNF-α, Panel D). Western blotting was performed in triplicate. Data are presented as the mean ±standard deviation for each group, expressed as a percentage of the control. β-actin was used as a loading control. A one-way analysis of variance (ANOVA) was applied for group comparisons, and pairwise comparisons were made using Tukey's post-hoc test. Statistical significance was set at $p < 0.05$

with *L. plantarum* increased the levels of mTOR in heart tissue by 35.1% compared to those of the fructose group (Fig. 2A; $p < 0.0001$). Supplementation with *L. plantarum* in rats led to higher mTOR levels in heart tissue than in the fructose group, yet these levels remained 24.9% below those of the control group (Fig. 2A; $p < 0.001$). In rats with metabolic syndrome, SIRT 1 protein levels in the heart tissue decreased by 52.1% ($p < 0.0001$) and 32.1% ($p < 0.0001$) for the fructose and fructose + *L. plantarum* groups, respectively, when compared to those of the control group (Fig. 2B). Nevertheless, *L. plantarum* supplementation increased the level of SIRT-1 protein in heart tissue by 41.7% in the rats with fructose-induced metabolic syndrome (Fig. 2B; $p = 0.0031$).

The inflammatory index of NF-κB levels in the heart tissue of rats in the fructose group exhibited a significant increase of 113.8% compared to the control group (Fig. 2C; $p < 0.0001$). In contrast, NF-κB levels in the fructose + *L. plantarum* group were reduced by 27.6% relative to the fructose group (Fig. 2C; $p < 0.0001$), although they remained 54.8% higher than those in the control group (Fig. 2C; $p < 0.0001$). Similarly, TNF-α protein levels in the heart tissue of the fructose group were twice as high as those in the control group (Fig. 2D; $p < 0.0001$). In the fructose + *L. plantarum* group, TNF-α levels increased by 48% compared to those of the control group (Fig. 2D; $p < 0.0001$), yet they were 26.1% lower than those of the fructose group (Fig. 2D; $p < 0.0001$).

Table 1. Comparative Analysis of Relative Protein Expression Levels Across Experimental Groups (Control, Fructose, and Fructose + *L. plantarum*)

	Control	Fructose	Fructose + *L. plantarum*
Akt (relative)	100%	61.5%	76.6%
IRS-1 (relative)	100%	46.2%	66.7%
eNOS (relative)	100%	59.0%	74.7%
TNF-α (relative)	100%	200%	147.9%

Clinical and experimental studies conducted on humans and other animals have shown that excessive consumption of fructose causes metabolic syndrome and increases susceptibility to DM (Miller and Adeli, 2008). Metabolic syndrome can also play a role in the occurrence of factors that cause a prothrombotic state, atherosclerosis, and heart attack (Grundy, 2016); however, because of intestinal dysbiosis, chronic inflammatory disorders (e.g., obesity, diabetes, and metabolic syndrome) may be more severe. Various probiotic supplements can prevent the adverse outcomes of metabolic syndrome by helping to regulate the intestinal microbiota (Xavier-Santos et al., 2020). The current study's findings demonstrated that *L. plantarum* has a regulatory effect at the molecular level that may protect against heart problems in rats with fructose-induced metabolic syndrome.

Previous experimental studies have shown that fructose administration is a suitable method by which to construct an experimental metabolic syndrome model (Réggami et al., 2021). Inflammatory reactions have significant effects on the progression of cardiovascular diseases, and it has been shown that inflammatory parameters (e.g., C-reactive protein, IL-6, and TNF-α) increase in those with metabolic syndrome (Rochlani et al., 2017). The consumption of high levels of fructose also triggers the formation of NF-κB–mediated inflammatory reactions and can increase the amount of proinflammatory cytokines (e.g., TNF-α, IL-1β, IL-18, and IL-6) in the heart as well as in the sera (Cheng et al., 2021). In line with these results, Xie (2017) has reported that NF-κB, IL-1β, and TNF-α levels in the heart were significantly increased in mice fed a high-fructose diet compared to that in a control group (Xie, 2017).

Dysbiosis in the intestinal microbiota is among the causes of obesity, diabetes, and metabolic syndrome because of the increased levels of lipopolysaccharides (LPSs) in intestinal dysbiosis, and this increase results in metabolic endotoxemia. Metabolic endotoxemia can exacerbate inflammatory reactions by increasing the release of inflammatory progenitor cytokines (e.g., IL-1β, IL-6, and TNF-α) along with NF-κB (Xavier-Santos et al., 2020); however, probiotic supplements can alleviate cardiac inflammation that results from fructose-induced metabolic syndrome by controlling the heart-intestinal axis (Cheng et al., 2021). Previous studies have shown that different *Lactobacillus* species reduce oxidative stress, insulin

resistance, and inflammatory states in experimental animals treated with high levels of fructose (Huang et al., 2019). *L. plantarum* can prevent LPS-induced inflammation in the intestines by decreasing the levels of NF-κB, IL-1β, IL-6, and TNF-α and increasing the release of anti-inflammatory cytokines (e.g., IL-10) (Liu et al., 2018). The results of the present study also reveal that *L. plantarum* helps to reduce NF-κB and TNF-α levels in heart tissue, which is in agreement with studies that have focused on different tissues (e.g., serum, liver (Huang et al., 2019), and intestines (Jeong et al., 2018)).

Downregulation of eNOS, which plays an important role in the regulation of vascular tonus, occurs in many vascular disorders, including metabolic syndrome and DM (Muniyappa and Sowers, 2013). On the contrary, iNOS, which is an inflammatory marker, may increase in fructose-induced metabolic syndrome (Babacanoglu et al., 2013). In the present study, although the eNOS levels in the heart tissue decreased after fructose administration, the iNOS levels exhibited a relative increase compared to those in the control group, possibly because of the increased inflammatory factors (TNF-α and NF-κB) (Silva et al., 2016). *L. plantarum*, which was administered after metabolic syndrome had been induced by fructose, may have reduced the levels of TNF-α and NF-κB, increasing eNOS levels and decreasing iNOS levels.

In the current study, *L. plantarum* supplementation significantly improved insulin signaling and reduced inflammation, as evidenced by elevated Akt, eNOS, and IRS-1 levels and decreased NF-κB and TNF-α expression. These results are consistent with previous studies demonstrating probiotics' ability to modulate insulin and inflammatory pathways (Jeong and Kim, 2019; Liu et al., 2018). The enhanced eNOS levels, for instance, suggest improved endothelial function, which is critical for maintaining vascular health in metabolic syndrome. Moreover, the reduction in NF-κB and TNF-α aligns with the anti-inflammatory properties of probiotics, likely mediated by the modulation of gut microbiota and systemic cytokine profiles (Huang et al., 2019).

The supplementation of *L. plantarum* significantly modulated key molecular markers involved in insulin signaling and inflammation. For example, the increase in Akt and eNOS levels suggests enhanced insulin sensitivity and improved endothelial function, which are critical in mitigating the cardiovascular effects of metabolic syndrome. Similarly, elevated IRS-1 levels indicate the potential restoration of upstream insulin receptor signaling. These findings align with the hypothesis that probiotics like *L. plantarum* can counteract the deleterious effects of high-fructose diets through both anti-inflammatory and insulin-sensitizing mechanisms.

The decreased Akt kinase activity and eNOS phosphorylation in experimental animals with metabolic syndrome may lead to the formation of insulin resistance, which then leads to cardiovascular disorders (Huang, 2009). It has been reported that insulin resistance that occurs in mice after metabolic syndrome is induced by a high-fat and high-fructose diet is mainly caused by the disruption of the IRS-1 phosphoinositide 3-kinase (PI3K)/Akt signaling pathway (Jeong and Kim, 2019). Akt plays a vital role in eNOS phosphorylation; therefore, the interruption of the IRS-1/PI3K/Akt pathway can significantly reduce eNOS activity in the vascular endothelium (Kim et al., 2006). Oral *L. plantarum* administration can be effective in protecting the beta cells of the pancreas, increasing insulin sensitivity, and balancing insulin and leptin levels in the sera (Li et al., 2016). The data obtained from the present study show that IRS-1 and Akt protein levels decreased in rats with metabolic syndrome, which agrees with data from previous studies (Jeong and Kim, 2019). The addition of *L. plantarum* to the diet of these rats increased the levels of these proteins, most likely because of its regulatory effects on the IRS-1/Akt/eNOS pathway in the heart (Sumlu et al., 2022). In addition, the reduction in serum insulin levels following *L. plantarum* administration supports the hypothesis that this probiotic bacterium helps mitigate insulin resistance.

It has been reported that the rapamycin pathway plays an important role in metabolic diseases of mTOR, and inhibition of this pathway can prolong survival in animal models (Passtoors et al., 2013). High fructose administration may cause increased activation of the mTOR pathway in the liver and thus trigger hepatic insulin resistance and inflammatory

reactions (Wang et al., 2022). On the other hand, Akt also provides phosphorylation of mTOR. The activation of the Akt/mTOR pathway in the insulin signaling pathway of the heart decreases after diet-induced obesity in rats (Medeiros et al., 2011); however, it is not clear whether mTOR activation or inhibition exhibits the same change under different pathological conditions (Sciarretta et al., 2022). The administration of *L. plantarum* to treat fructose-induced metabolic syndrome most likely modulates the cardiac IRS-1/Akt/mTOR pathway, potentially enhancing insulin sensitivity by increasing mTOR activation (Yoon, 2017).

Sirtuins (SIRT-1-7) are involved in the regulation of energy metabolism. SIRT-1 can be effective in preventing NF-κB-mediated inflammatory states in many tissues, including heart tissue. Its activation may decrease after insulin resistance, and TNF-α, IL-6, and iNOS levels increase as a result of obesity, metabolic syndrome, and diabetes (Vachharajani et al., 2016). Experimental studies have reported that short- or long-term administration of fructose in rats reduces the expression of SIRT-1 protein in the liver and heart (Boskovic et al., 2019; Rebollo et al., 2014). The anti-inflammatory effects of *L. plantarum*, which was used in the present study, may have increased the levels of SIRT-1 protein in the heart (Huang et al., 2019; Jeong et al., 2018). *L. plantarum*'s beneficial effects may be attributed to its capacity to modulate gut microbiota composition, reducing metabolic endotoxemia and inflammatory cytokine release. This modulation likely contributes to the observed restoration of the Akt/eNOS pathway and reduction in oxidative stress in cardiac tissue. Future studies should explore these mechanisms in greater detail, particularly through metagenomic and metabolomic analyses.

CONCLUSIONS

The findings of this study indicate that *L. plantarum* (ATCC: 14917), administered in a fructose-induced metabolic syndrome model in rats, has the potential to mitigate cardiac inflammation, enhance insulin sensitivity through the IRS-1/Akt/eNOS pathway, and reduce the risk of metabolic syndrome-associated cardiovascular disorders by upregulating SIRT-1 levels in the heart. In order to support these findings, clinical studies should be carried out and the effects of different strains of *L. plantarum* should be clarified.

FUNDING

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DECLARATIONS

Data statement
All data supporting this study has been included in this manuscript.

Ethical Approval
This study was carried out in accordance with ethical rules, respecting animal welfare and animal rights, after receiving approval from the Gazi University Animal Experiments Local Ethics Committee (G.Ü.ET-18.080) regarding its compliance with the principles in the ethics committee directive.

Competing Interests
The authors declare no conflicts of interest.

SUPPLEMENT

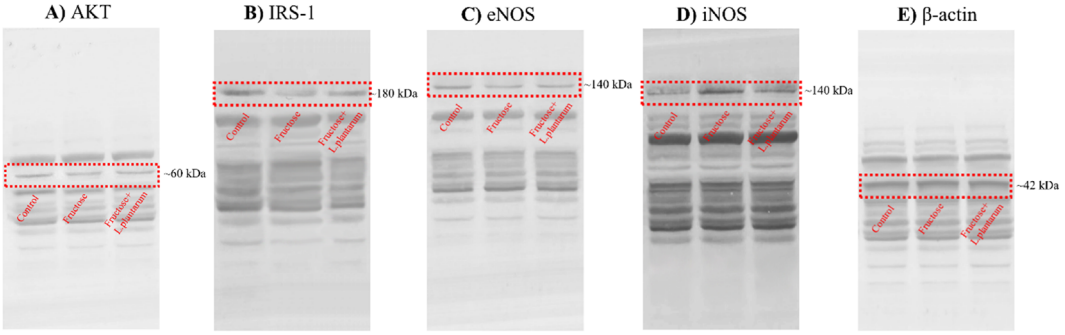

Fig. S1. Full immunoblots of the effect of the administration of *Lactobacillus plantarum* to metabolic syndrome rats on heart tissue AKT (A), IRS-1 (B), eNOS (C), iNOS (D), and (E) β-actin. Each immunoblot is representative of three independent experiments. The results shown in Fig. 1 are delineated by red dotted rectangles. MW (in kDa) are indicated. AKT, the protein kinase B; IRS-1, insulin receptor substrate 1; eNOS, endothelial nitric oxide synthase; iNOS, inducible-NO synthase

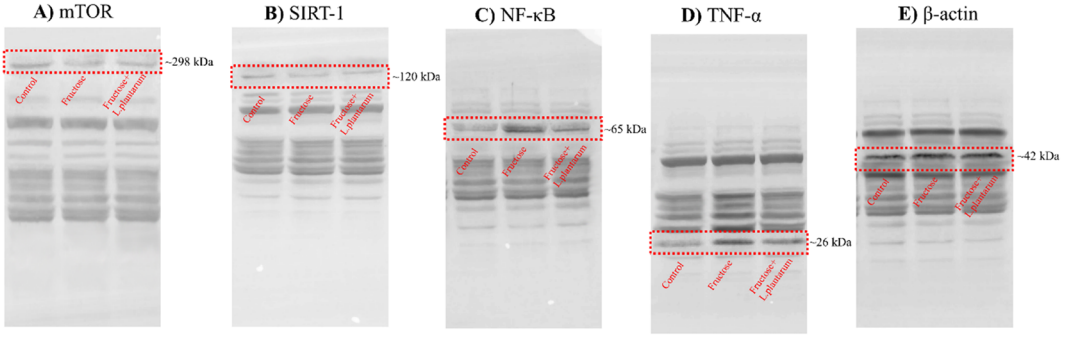

Fig. S2. Full immunoblots of the effect of the administration of *Lactobacillus plantarum* to metabolic syndrome rats on heart tissue mTOR (A), SIRT-1 (B), NF-κB (C), TNF-α (D), and (E) β-actin Each immunoblot is representative of three independent experiments. The results shown in Fig. 2 are delineated by red dotted rectangles. MW (in kDa) are indicated. mTOR, the mammalian target of rapamycin; SIRT1, Sirtuin-1; NF-κB, nuclear factor kappa B; TNF-α tumor necrosis factor α

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