



Effect of Eight Weeks of Resistance Training with Spirulina Supplementation on Cardiac Troponin T, Brain Natriuretic Peptide, and Creatine Kinase in the Myocardium

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ARTICLE INFO	ABSTRACT
<p><i>Article type:</i> Research Paper</p>	<p>Introduction: Exercise and dietary supplementation influence various biomarkers of myocardial cell damage differently. This study aims to examine the effects of eight weeks of resistance training combined with spirulina supplementation on changes in cardiac troponin T (cTnT), brain natriuretic peptide (BNP), and creatine kinase-myocardial band (CK-MB) to assess myocardial damage.</p>
<p><i>Article History:</i> Received: 18 Oct 2024 Accepted: 19 Nov 2024 Published: 20 Apr 2025</p>	<p>Methods: Thirty-two male Sprague-Dawley rats (150±20 g, 9 weeks old) were randomly assigned into four groups: control (CO), resistance training (RT), spirulina supplementation (SP), and resistance training with spirulina supplementation (RTS). Spirulina was orally administered to SP and RTS groups at a dose of 200 mg/kg/day—the RT protocol involved climbing a 1-meter-high ladder over eight weeks. The cTnT, BNP, and CK-MB expression levels were quantified using real-time PCR.</p>
<p><i>Keywords:</i> Resistance training Spirulina Myocardium Rat cTnT BNP CK-MB</p>	<p>Results: Eight weeks of resistance training led to a significant increase in cTnT (p=0.044), BNP (p=0.001), and CK-MB (p=0.015) levels. In contrast, spirulina supplementation, both alone and combined with resistance training, significantly reduced cTnT (SP: p=0.005; RTS: p=0.001) and BNP (SP: p=0.002; RTS: p=0.0001) levels, with a more significant reduction observed in the RTS group. CK-MB levels also decreased in the SP group (p=0.001) and showed a non-significant trend in the RTS group (p=0.115).</p> <p>Conclusion: Spirulina supplementation mitigates myocardial damage during resistance training by reducing biomarkers indicative of myocardial injury. These findings suggest that spirulina could be a protective agent against cardiac stress induced by resistance exercise.</p>

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Introduction

Lifestyle changes, including the resumption of physical activity, have significantly reduced cardiovascular complications and mortality (1). However, participation in intense physical activities can impair cardiac function and increase the risk of acute myocardial infarction in susceptible individuals (2). Spirulina, a type of algae, constitutes 60-70% protein by weight and is a rich source of vitamins (e.g., beta-carotene and vitamin B12), minerals (e.g., iron), and gamma-linolenic acid (3), (4). It exhibits potent antioxidant and anti-inflammatory properties (5). Research indicates that spirulina supplementation may enhance muscle protein synthesis (6). Zar et al. (2021) examined cardiac muscle gene expression following resistance training and spirulina supplementation. The findings suggest that spirulina supplementation

alone does not significantly influence cardiac hypertrophy gene expression. However, when combined with resistance training, spirulina modulates the expression of genes associated with cardiac hypertrophy signaling pathways (7). Additionally, spirulina has been found to enhance blood glucose uptake in the liver by increasing hexokinase enzyme activity. This activation promotes carbohydrate metabolism and reduces fat synthesis in the body (8). These characteristics suggest that spirulina may be an effective dietary supplement to prevent cell damage and reduce inflammatory markers (9). It can be used in many fields related to health (10). Studies indicate that long-term physical activity can elevate cardiac biomarkers such as cardiac troponin T (cTnT), brain natriuretic peptide (BNP), and creatine kinase-myocardial band (CK-MB) (11). Given that cTnT is a specific marker of myocardial damage (12), monitoring its levels

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post-exercise provides valuable insights into the effects of different exercise intensities on myocardial injury.

BNP is a critical biomarker for diagnosing heart failure. However, the long-term effects of resistance training on BNP levels remain unclear, as strength training has received comparatively less attention. One study reported no significant changes in BNP levels in healthy men following 12 weeks of resistance training (13). Conversely, Kandras et al. demonstrated that 4 months of combined resistance and endurance training significantly reduced BNP levels (14). In a separate investigation, researchers examined the impact of marathon running on BNP and cTnT levels in non-professional athletes. The results revealed a significant increase in both biomarkers post-competition. Notably, cTnT levels exceeded the upper reference limit after the race (15). Similarly, Weipert et al. observed a significant rise in cTnT and CK levels following two types of continuous and high-intensity activities in healthy participants (16).

The consumption of sports supplements among athletes has been on the rise. Considering that cardiac troponin T (cTnT) is the gold standard for diagnosing myocardial infarction and its established relationship with brain natriuretic peptide (BNP) and creatine kinase-myocardial band (CK-MB), investigating changes in their levels due to physical activity, especially with spirulina supplementation, can provide valuable insights. Such research may contribute to preventing and managing cardiovascular disorders through physical activity and nutritional supplementation. Calella et al. (2022) demonstrated that spirulina supplementation during submaximal exercise enhances energy production, increases oxygen uptake, and improves exercise tolerance (17). Despite these findings, no studies have specifically examined the combined effects of resistance training and spirulina supplementation on cTnT, BNP, and CK-MB levels. Further research is necessary to evaluate the influence of spirulina on athletic performance, enzyme activity, and the overall health of athletes and active individuals. Resistance exercise, recognized as a non-pharmacological intervention, and spirulina, considered a safe dietary supplement, possess significant biological properties that may impact markers of muscle damage (18). Therefore, the present study aimed to assess the effect of eight

weeks of resistance training combined with spirulina supplementation on cTnT, BNP, and CK levels in the myocardium of male rats.

Material and Methods

Animal and Design

Thirty-two male Sprague-Dawley rats, aged 9 weeks and weighing 290 ± 20 grams, were obtained from the Animal Center of Shiraz University of Medical Sciences and transferred to the laboratory at Marvdasht University. Each rat was housed individually in a transparent polycarbonate cage under controlled environmental conditions, including a temperature of $22 \pm 2^\circ\text{C}$, relative humidity of $55 \pm 4\%$, and a 12-hour light/dark cycle. All animals were fed pelleted food prepared explicitly for animal breeding, reproduction, and stem cell studies during the study. Free access to standard rat food (Parsfeed) and safe drinking water was provided. Humane care was provided according to relevant regulations. The animals were conditioned for two weeks before the experimental procedures. During this period, they were maintained in an environment with moderate room temperature, humidity, and the specified light/dark cycle to acclimate them to the experimental conditions. All animal handling procedures were conducted under the Guidelines for the Care and Use of Laboratory Animals provided by the US Institute of Animal Research (19) and approved by the Ethics Committee of Jahrom University of Medical Sciences (IR.IJMS.REC.1398.011).

To ensure accurate comparisons, the animals were randomly assigned to one of four groups ($n=8$ per group):

Group A: Control (CO)

Group B: Resistance Training (RT)

Group C: Spirulina Supplementation (SP)

Group D: Resistance Training with Spirulina Supplementation (RT+SP).

Resistance Training Protocol

According to Table 1, after purchase, the rats were acclimated to the storage environment for one week. After this adaptation period, a familiarization program involving ladder climbing was conducted over one week, with sessions held every other day. Each session consisted of three to four repetitions performed without attached weights. The ladder, measuring one meter in height and perpendicular to the ground, was used to familiarize the rats with the

climbing procedure. The rats were initially placed on the lowest step of the ladder and guided to use their hind limbs to climb. If the rats refused to climb, gentle tail touches were applied to encourage movement, and this process continued until the rats successfully climbed the ladder. Before the main training sessions started, the rats underwent a warm-up, which involved climbing the ladder three times without weights and with no rest between repetitions. During the

training, the rats performed five repetitions per set with a one-minute rest interval between repetitions. Each training session consisted of three sets with a two-minute rest interval between sets. Training was conducted five days per week for eight weeks. The training intensity was gradually increased, starting at 30% of the rats' body weight and progressing to 100% by the end of the eight weeks (7).

Table 1. Exercise protocol

week	Number of sessions per week	Courses	Repetition	Rest between periods (minutes)	Rest between repetitions (minutes)	Weight percentage relative to body weight
First	5	3	5	2	1	30
Second	5	3	5	2	1	40
Third	5	3	5	2	1	50
Fourth	5	3	5	2	1	60
The fifth	5	3	5	2	1	70
The sixth	5	3	5	2	1	80
The seventh	5	3	5	2	1	90
Eighth	5	3	5	2	1	100

Spirulina Supplement

Spirulina has garnered significant attention, with a protein content higher than legumes and meat—approximately 70 grams per 100 grams of dry weight (dw). Its consumption is attributed to its diverse chemical composition, including proteins, minerals, phenolics, and essential fatty acids, which offer numerous health benefits (20). For the SP and SP+RT groups, spirulina was added to their drinking water 24 hours before the start of the study and continued daily at a dose of 200 mg/kg until the end of the eighth week (20).

Sampling

Sampling of animals from the groups was performed 24 hours after the final training session. The mice were anesthetized for approximately 5 minutes using an injection of 10% ketamine (50 mg/kg body weight) and 2% xylazine (10 mg/kg body weight) to facilitate parameter measurement. The heart was then surgically removed from the chest cavity, and the

left ventricle was isolated. The left ventricular tissue was immediately placed in nitrogen tanks and subsequently transferred to an -80°C freezer for ribonucleic acid (RNA) extraction (7).

RNA Isolation and Real-Time PCR Analysis

Total RNA was extracted from tissue samples using an RNA extraction kit (Cinnagen Inc., Iran). The RNA's purity, integrity, and concentration were assessed by measuring the optical density at 260/280 nm and performing 1% agarose gel electrophoresis. Complementary DNA (cDNA) was synthesized from 1 µg of RNA using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas Inc.). Real-time PCR was conducted following the protocol of the Real Q Plus 2x Master Mix Green (Ampliqon Inc.) using the Applied Biosystems StepOne™ Instrument (ABI, StepOne, USA) (19).

Real-time PCR, using primer pairs listed in Table 2, was used to analyze the expression of BNP, cTnT, and CK.

Table 2. Real-time PCR (qPCR) primer pairs used in the study.

Genes	Primer Sequences	Sizes (bp)
Tnnt2 (cTnT)	Forward: 5'- ACGACAACCAGAAAGTCTCCA-3' Reverse: 5'- CCAGACAGGAGTCTGCATCG-3'	163
Nppb (BNP)	Forward: 5'- TTAGTCTCAAGACAGCGCC-3' Reverse: 5'- TAAAACAACCTCAGCCCGTCA-3'	142
Creatine kinase (CK)	Forward: 5'- CCATGGAGAAAGGAGGCAATA-3' Reverse: 5'- CCTTCTGAAGATCTCCTCAATCT-3'	84

Statistical Analysis

Statistical analysis was conducted using the Kolmogorov-Smirnov test to evaluate the data distribution among research groups. To assess the effects of interventions, a two-way ANOVA was performed using SPSS software (version 18), with a significance level set at ($P \leq 0.05$) (7, 19, 21).

Ethical Statement

The study was approved by the Ethics Committee of Jahrom University of Medical Sciences (IR.JUMS.REC.1398.011) and was conducted in strict compliance with the United States Institute of Animal Research Guidelines for the Care and Use of Laboratory Animals.

Results

BNP Changes

The results of the two-way ANOVA for changes in BNP demonstrated a significant difference between the groups. The most notable changes in BNP were observed in the spirulina supplementation group ($P=0.001$). Additionally, compared to the control group, exercise alone caused a significant increase in BNP levels ($P=0.001$). Conversely, significant decreases in BNP levels were observed in the spirulina group ($P=0.002$) and the exercise + spirulina group ($P<0.0001$), with the most significant reduction occurring in the exercise + spirulina group (Figure 1).

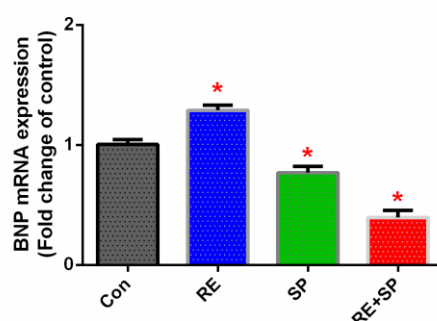


Figure 1. Changes in bnp in cardiac muscle after eight weeks of resistance training and Consumption of spirulina. Con; Control, SP; Spirulina, RE; Resistance Exercise, SP +RE; Spirulina + Resistance Exercise. Data are presented as the mean \pm standard error of the mean. *p value less than 0.05 considered as significant.

cTnT Changes

The results of the two-way ANOVA for cTnT changes revealed a significant difference between the groups. The most notable changes in cTnT levels were observed in the exercise group ($P=0.044$). Additionally, compared to the control group, exercise alone caused a significant

increase in cTnT levels ($P=0.003$). In contrast, significant decreases in cTnT levels were observed in the spirulina group ($P=0.005$) and the exercise + spirulina group ($P=0.001$), with the most significant reduction occurring in the exercise + spirulina group (Figure 2).

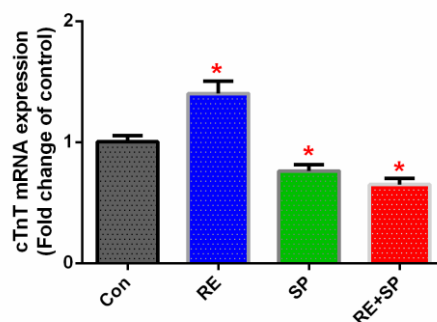


Figure 2. Changes in cTnT in cardiac muscle after eight weeks of resistance training and Consumption of spirulina. Con; Control, SP; Spirulina, RE; Resistance Exercise, SP +RE; Spirulina + Resistance Exercise. Data are presented as the mean \pm standard error of the mean. *p value less than 0.05 considered as significant.

ck Changes

The results of the two-way ANOVA for CK changes indicated a significant difference between the groups. The most substantial changes in CK levels were observed in the spirulina group ($P=0.001$). Additionally, compared to the control group, exercise alone

caused a significant increase in CK levels ($P=0.015$). In contrast, a significant decrease in CK levels was observed in the spirulina group ($P=0.001$), and a decrease was also noted in the exercise + spirulina group ($P=0.115$). However, this reduction was not statistically significant (Figure 3).

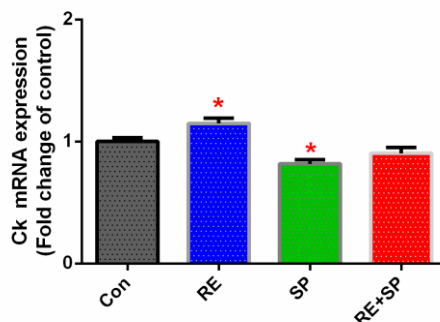


Figure 3. Changes in ck in cardiac muscle after eight weeks of resistance training and Consumption of spirulina. Con; Control, SP; Spirulina, RE; Resistance Exercise, SP +RE; Spirulina + Resistance Exercise. Data are presented as the mean \pm standard error of the mean. *p value less than 0.05 considered as significant.

Discussion

The results of the present study demonstrated that resistance training significantly increased BNP levels. These findings align with those of Krupika et al. (22) and Sheikhan et al. (23) which reported a significant increase in BNP concentration following exercise. In the study by Sheikhan et al. (2011), which investigated the effects of resistance training and acute aerobic exercise in professional athletes, BNP levels showed a significant increase (23). The elevation in BNP levels observed after exercise may indicate heightened cardiac activity (24). Bardbar et al. (2012) suggested that myocardial damage could result from prolonged resistance training (25). It is well established that the demand for blood flow in cardiac muscle tissue increases during exercise. Intense exercise, however, can lead to transient myocardial ischemia, disrupting ventricular function (26). This increase in ventricular wall tension in response to overload may stimulate BNP secretion from the left ventricle (27). Additionally, the mechanical tension applied to cardiomyocytes is transmitted to intracellular mechanical sensors, activating p38, a member of the MAPK family. Activation of p38 triggers the transcription factor NF- κ B, which translocates to the nucleus. In the nucleus, NF- κ B binds to specific DNA sequences, leading to the transcription of preproBNP mRNA. The

transcribed mRNA is exported from the nucleus, where ribosomes synthesize proBNP in the cytoplasm. This signaling pathway ultimately increases BNP concentrations in the bloodstream (28).

Conversely, the findings of some studies, such as those by Ahmadi et al. (2017), Rengers et al. (2018), and Waltz et al. (2014), are not consistent with the results of the present research. Ahmadi et al. (2017) reported no significant differences in BNP values between continuous and intermittent aerobic activities, with both types of exercise imposing similar loads on the myocardium (29). Similarly, Rengers et al. (2018) found that eight weeks of resistance training, performed three sessions per week, significantly reduced BNP levels in older adults. They also concluded that resistance training improved markers of myocardial damage in this population (30). Waltz et al. (2014) examined the effects of 12 weeks of low- and moderate-intensity resistance training on BNP levels in healthy elderly individuals and observed no significant changes in BNP levels following the intervention (31). However, some studies have reported decreased BNP levels in response to exercise (26). This reduction may be attributed to improved hemodynamic balance in the myocardium, enhanced systolic function, BNP clearance via renal receptors, reduced sympathetic activity, and better oxygen delivery

to the myocardium. The disparity between the findings of the present study and those of opposing studies could be explained by differences in exercise intensity, type of activity, or characteristics of the research subjects. The increased secretion of cardiac natriuretic peptides is recognized as a crucial compensatory mechanism. It works by inhibiting the renin-angiotensin-aldosterone system and sympathetic nervous activity, promoting sodium excretion, suppressing abnormal vascular cell growth, and enhancing vascular dilation (32).

According to the results, there was a significant decrease in BNP in the spirulina and the exercise and spirulina groups, and the reduction was more significant in the exercise and spirulina groups. In line with the previous results, Marumoto et al. (2012) stated that the level of BNP in cardiac infarction patients increased significantly immediately after a session of dynamic resistance training (33). In addition, an increase in BNP after heavy resistance training and indoor soccer sessions was reported by Carranza-Garcia et al. (2011) (22). Regarding the possible mechanism of action that causes the synergistic effect of exercise and spirulina, spirulina supplement is helpful for exercise performance, and it can inhibit the activity of free radicals through the activation of the NRF2 signaling pathway considering its high antioxidant enzyme (34) and inhibition of lipid peroxidation (35) can affect sports performance (34). Spirulina supplements can probably cause better efficiency and stimulation of enzyme synthesis, and their antioxidant effects can improve growth performance and health (36). Contrary to Ahmadizad et al. (2013), they did not observe a change in the BNP level of healthy men after 12 weeks of resistance training (14). Differences in the training duration and type may be the reasons for the differences in results. According to Partdas et al. (2012), the increase in sympathetic tone during resistance activities causes micromolecular disorders, and the blood flow in the coronary artery increases with the increase in the diameter of the vessels of the heart muscles, increasing the heart rate and the contraction of the heart wall. On the other hand, stimulating alpha and beta-adrenergic receptors causes narrowing and dilation of heart vessels during resistance activities. Together, these factors cause ischemia of the heart muscle, which increases the tension in the left ventricular wall

and causes BNP to be secreted (37). In addition, the release of angiotensin II and catecholamine hormones due to resistance exercise probably causes the release of BNP (38). In addition to these factors, the difference between the results in the present research and previous research may be due to the difference in the tests, the intensity of the training, or the number of samples. Pacino et al. consider the reduction of BNP to improve the systolic function of the heart, decrease the sympathetic nervous tone, and improve oxygen supply to the heart muscle tissue (39). Calella et al. (2022) found that spirulina supplementation during submaximal exercise has the potential to generate energy, increase oxygen uptake, and improve exercise tolerance (17). Studies on the benefits of spirulina supplements on heart muscle damage indicators in active, healthy people are rare, and more research is needed to evaluate the effects of spirulina consumption on sports performance, enzyme activity, and the health of athletes and active people (17). This significant decrease in BNP in the spirulina group and the exercise and spirulina group shows that the consumption of spirulina alone and in combination with resistance training may not lead to necrosis of cardiac cells or changes in the membrane permeability of those cells. It is important to note that drawing definitive conclusions about the effects of such exercises on specific indicators of cardiac cell damage is premature, and further research is necessary to determine the precise protective effects of spirulina supplementation on cardiac cells. However, it is noteworthy that in the current study, resistance training alone, without spirulina supplementation, resulted in a significant increase in cardiac damage markers, confirming that resistance training can induce myocardial damage. Consequently, exercise physiologists should exercise caution and avoid prescribing resistance exercises for individuals with cardiovascular conditions.

Exercise caused a significant increase in cTnT levels. In the spirulina group and the exercise and spirulina group, the amount of cTnT decreased significantly, which was more important in the exercise and spirulina group. Participating in high-intensity physical activities harms heart function and is not safe. This relative risk of heart cell damage increases during intense physical activity, so sports physiologists are looking for suitable exercise programs that prevent heart

damage in addition to the benefits of cardiovascular adaptations (40). In line with the present study's findings, Carranza et al. (2011) investigated the effect of a heavy resistance training session and soccer match on cTnT levels in experienced athletes. Heavy resistance training significantly increased cTnT levels. The results of their study showed that intermittent exercise leads to disturbances in cardiac biomarkers along with myocyte damage (39). In this regard, Chang et al. (2015) investigated the cTnT of cTnT in male rats and observed a significant increase compared to the initial time (41). The results of this research are consistent with the present study. Sports activity increases cardiovascular adaptations and causes heart muscle damage and fatigue (40). In Chang et al.'s research, exercise increased cTnT inhibitor, which indicates heart damage. Therefore, it is essential to carefully evaluate all aspects of a training program before implementation. However, the present study's findings suggest that consuming spirulina supplements alone and in combination with resistance training significantly reduces indicators of cardiac damage. Therefore, it is possible to partially prevent heart damage caused by resistance training by taking spirulina supplements and resistance training. According to the suggestion of the American College of Sports Medicine, the best treatment is prevention through sports activity (42).

The significant decrease in cTnT observed in the spirulina group, and the exercise plus spirulina group suggests that spirulina supplementation, whether used alone or in combination with resistance training, may not lead to necrosis of heart cells or alterations in their membrane permeability. However, it is premature to draw definitive conclusions regarding the impact of such interventions on specific indicators of cardiac cell damage. Further research is necessary to fully elucidate the protective effects of spirulina supplementation on cardiac cells. The duration of physical activity is a critical factor in training programs, as evidence indicates a positive correlation between the length of exercise sessions and increased cTnT levels (43). The present study's findings demonstrated a significant increase in CK-MB levels following 8 weeks of resistance training. This observation aligns with the studies conducted by Rengarz et al. (2018) and Rodrigues et al. (2010). Rengarz et

al. reported that 8 weeks of resistance training (3 sets of 10 repetitions at 70% 1RM, with a 1-minute rest interval) performed three times per week significantly elevated CK-MB levels (30). Similarly, Rodrigues et al. indicated that CK levels increased significantly after two resistance training sessions at 80% 1RM (44). In contrast, Ranjbar et al. (2017) found that intermittent and continuous aerobic exercise had no significant effect on CK-MB levels in healthy men, with participants completing 40 minutes of either continuous or intermittent aerobic treadmill activity per session (45). Some studies suggest long-term or high-intensity physical activity activates the stress response and induces pathological changes such as apoptosis in striated muscles (skeletal and cardiac), liver, and kidneys (46). Although the precise mechanisms driving cell death in various organs during and after exercise are not fully understood, experts hypothesize that factors such as the training regimen and reactive oxygen species (ROS) play a significant role in this process (47).

CK-MB levels decreased in the spirulina and the exercise plus spirulina groups, with a significant reduction observed in the spirulina group. As previously mentioned, various studies have demonstrated that exercise and nutritional supplements have varying effects on BNP, cTnT, and CK-MB levels. Researchers have reported diverse findings based on these variables. Given the prevalence of heart attacks and sudden deaths in sports, examining the effects of such exercises and supplements is crucial for understanding the causes of sports-related cardiac injuries. However, whether spirulina supplementation influences CK-MB gene expression changes after eight weeks of resistance training in male rats remains unclear. No studies have specifically investigated the combined effects of resistance training and spirulina supplementation or spirulina alone on CK-MB levels. Jalali et al. (2023) reported that spirulina supplementation and resistance training did not significantly affect gene expression or increase liver enzymes but contributed to adaptive responses related to metabolic damage (48). Similarly, Kashif et al. (2013) examined the impact of short-term hydroxymethylbutyrate supplementation on creatine kinase levels following resistance activities in young athletes. Their results indicated that the supplement reduced creatine

kinase, a marker of muscle damage, but was not effective as an independent factor in reducing muscle damage markers after resistance training (49). Limited research exists on the effects of spirulina supplementation on gene expression during exercise. The potential indirect role of spirulina in controlling gene expression requires further investigation (50). The present study has limitations, such as missing measurements for other cardiac risk factors, including inflammatory biomarkers. Further studies are needed to comprehensively establish the effects of spirulina supplementation combined with resistance training on cardiac cell damage indicators. Future research should explore various resistance exercises with different intensities and investigate diverse sports.

Conclusion

The findings indicate that resistance training increases the levels of BNP, cTnT, and CK-MB, which are markers of myocardial damage, suggesting that resistance training elevates indicators of cardiac injury. However, the concurrent use of spirulina supplementation with resistance training significantly reduced the levels of these markers, implying that spirulina can mitigate myocardial damage. This highlights spirulina supplementation as a potential strategy to prevent myocardial damage during resistance training by reducing markers of cardiac cell injury induced by such exercise. Based on these findings, it is recommended to incorporate spirulina supplementation alongside resistance training to prevent the rise in myocardial damage indicators associated with resistance exercises. However, the study had limitations, including using a single supplement dose and lacking diverse methods for assessing gene expression. Future research should evaluate the effects of various supplement dosages and utilize alternative gene expression measurement techniques.

Declarations

Ethical Statement

The study was approved by Jahrom University of Medical Science's ethics committee and carried out in strict accordance with the United States Institute of Animal Research guidelines for the care and use of laboratory animals by Animal Care (IR.JUMS.REC.1398.011).

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CRediT Authorship Contribution Statement

Fatemeh Ahmadi: Supervision, Resources Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Conflicts of Interest

The authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data are available on request.

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