

Influence of Sleep Disruption Before Myocardial Infarction on Cardiac Injury Following Isoproterenol Administration: Insights into Matrix Metalloproteinases

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Received: 06 Dec. 2025; Accepted: 21 Jul. 2026

Abstract- Epidemiological studies have demonstrated an association between sleep duration and various health risk factors, including obesity and cardiovascular diseases, particularly coronary artery disease (CAD). The present study aimed to examine the impact of sleep disruption (SD) prior to myocardial infarction (MI) on cardiac injury, with a specific focus on matrix metalloproteinases (MMPs) as potential mediators of this relationship. Using a controlled animal model, adult male Wistar rats were subjected to chronic SD for 14 days, followed by induction of MI via isoproterenol administration. The activities of MMP-2 and the level of tissue inhibitor of metalloproteinase-1 (TIMP-1) were assessed, alongside histological evaluations of cardiac tissue and oxidative stress markers. Results indicated a significant reduction in the TIMP-1/ β -actin ratio in the MI and SD+MI groups, with a more pronounced decrease in the SD+MI group, and an increase in MMP-2 activity in the MI group compared to the control group, suggesting an imbalance between MMPs and TIMPs that may contribute to myocardial damage. Histological analysis revealed notable disorganization of myocardial fibers, which appeared more severe in the SD+MI group, highlighting the detrimental impact of SD on cardiac tissue integrity. Although no significant alterations in oxidative stress markers were detected, the findings underscore the detrimental role of SD in cardiovascular health and suggest that this imbalance between MMPs and TIMPs could serve as a potential mechanism involved in cardiovascular diseases associated with SD.

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Acta Med Iran 2026;64(9):550-556.

<https://doi.org/10.18502/acta.v64i9.22946>

Keywords: Sleep disruption; Myocardial infarction; Matrix metalloproteinases

Introduction

Epidemiological studies have linked multiple health risk factors to sleep duration, including obesity and cardiovascular diseases (1). Among these, coronary artery disease (CAD) is considered the most common cardiovascular illness (2). Furthermore, recent guidelines for the prevention of cardiovascular diseases emphasize the importance of sleep hygiene (3). Individuals sleeping

fewer than 6 hours or more than 10 hours per night exhibit a significantly higher prevalence of CAD compared with those sleeping 7 to 9 hours (4). It has been reported that sleep disruption (SD) following myocardial infarction (MI) caused cardiac enlargement within 21 days, which was associated with increased oxidative stress, elevated nitric oxide production, and imbalance in the ubiquitin-proteasome system. These changes ultimately contributed to heart failure and cardiac dysfunction (5). An animal

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study demonstrated that inducing a 72-hour SD period prior to MI led to increased ventricular tachycardia and fibrillation (6). The most important consequence of acute cardiac ischemia is left ventricular dysfunction, which is caused by the generation of free radicals, lipid peroxidation, and reduced energy production (7).

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases responsible for extracellular matrix (ECM) degradation (8). These enzymes participate in a variety of physiological activities, such as morphogenesis, growth, and proliferation; however, under pathological conditions such as MI, they become dysregulated and contribute to tissue damage and cardiac injury (8,9). Following MI, ECM remodeling occurs, as evidenced by the upregulation of MMP-2 expression and activity during heart failure (10). Tissue inhibitors of metalloproteinases (TIMPs) are the primary regulators of MMP activity (11). Maintaining ECM integrity and homeostasis is the key physiological function of TIMPs (11). An elevation in MMP activity resulting from an imbalance between MMP and TIMP expression causes improper remodeling, collagen deposition, and the advancement of heart failure (10). MMPs have been identified as predictive indicators of heart failure and as clinical biomarkers linked to left ventricular remodeling (12). Furthermore, SD has been associated with altered MMP expression in various tissues, including bone, joint, and prostate, and studies further suggest that MMPs play a key role in sleep disorder-related injuries, contributing to inflammation, tissue degradation, and impaired function (13-16).

However, the specific effects of chronic sleep disruption prior to MI on the MMP/TIMP balance in cardiac tissue have not been fully elucidated. In this study, we investigated the effects of SD before MI on cardiac injury, with a particular focus on MMPs as potential underlying mechanisms. Exploring these interactions may provide deeper insights into the pathophysiology of myocardial infarction and underscore the critical role of sleep duration in cardiovascular health.

Materials and Methods

Animal housing and care

Thirty-six adult male Wistar rats, free of pathogens and weighing between 200 and 300 g, were employed. The animals were purchased from Tehran University of Medical Sciences and housed in an air-conditioned room maintained at 22-24 °C with a 12-hour light-dark cycle and ad libitum access to food and water. Tehran University of Medical Sciences (Tehran, Iran) granted

authorization for the use of animals in research projects, and all operations involving animals were carried out in accordance with NIH guidelines (Guide for the Care and Use of Laboratory Animals).

Design of study

Rats were randomly assigned to four groups (n=9 per group) Control, Sleep disruption (SD), Myocardial infarction (MI), and Sleep disruption+Myocardial infarction (SD+MI). Rats were placed on water-submerged platforms. Two types of platforms were used: one type allowed the animal to walk around and sleep (large platform), while the other prevented the animals from resting comfortably (small platform). The SD and SD+MI rats were housed on small platforms for 14 days, while the Control and MI rats were placed on a larger platform with sufficient space for sleeping. On days 15 and 16, normal saline injections were administered subcutaneously to the Control and SD groups and injections of isoproterenol (130 mg/kg) were administered subcutaneously to the MI and SD+MI groups. Three days following the second injection, 3 rats were randomly chosen from each group for the evaluation of oxidative markers. Blood samples were taken from all experimental groups between 9:00 am-12:00 pm. Serum was separated by centrifugation at 6000 rpm and 4 °C for 20 minutes and then promptly stored at -80° C. Seven days after the second injection, 3 animals were randomly selected from each group for zymography and Western blot analysis. Cardiac tissue was removed under deep anesthesia, immersed in liquid nitrogen, and preserved at -80 °C. Twenty-eight days after the second injection, 3 animals were employed for histological evaluation. Hearts were placed in 10% formalin, and 6-micron slices were cut from paraffin-embedded blocks.

Induction of chronic SD using a controlled aquarium environment

To induce chronic SD, eight circular platforms (6.5 cm in diameter) were placed inside a metal tank measuring 125 cm×44 cm×44 cm. The aquarium featured eight platforms arranged in two rows of four, with a distance of 10 cm between platforms and a height of 30 cm for each platform. The aquarium was filled with water at 20° C to a depth of approximately 1 cm below the platform's surface. Baskets in the aquarium provided the animals with unrestricted access to food and water (17). Muscle atonia associated with REM sleep prevented the animals from falling asleep, causing them to awaken upon contacting the water. Starting at 2:00 pm on the first day of the protocol, the animals were deprived of sleep for 20

hours per day over a total period of 14 days. Following each 20-hour SD session, the animals were returned to their cages and given 4 hours of rest (10 am-2 pm). The Control and MI groups were placed in the aquarium with a larger surface area, providing sufficient space for the animals to rest.

Induction of MI using isoproterenol

In this study, MI was induced by isoproterenol injection, which served as a non-surgical method to avoid complications associated with surgical induction. On days 15 and 16, isoproterenol was dissolved in normal saline and administered subcutaneously at a dose of 130 mg/kg.

Assessment of plasma malondialdehyde levels

Serum was isolated and kept at -80° C after blood samples were centrifuged for 20 minutes at 6000 rpm and 4° C. Plasma malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances (TBARS) using 1,1,2,2-tetramethoxypropane as a standard.

Assessment of MMP-2 activity using gelatin zymography

MMP-2 gelatinase activity was assessed using gelatin zymography. Briefly, rat hearts were collected, and protein concentration was measured using the BCA assay kit. Samples with equal protein concentrations were mixed with 5X loading buffer. SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was performed on a 4% (w/v) polyacrylamide stacking gel and a 7.5% separating gel copolymerized with 4 mg/mL porcine skin gelatin type A. The gel was then twice washed for 30 minutes at room temperature in a washing buffer that contained Triton-X-100, Tris-HCl pH 7.5, CaCl₂, and ZnCl₂. After that, it was rinsed for ten minutes at 37 °C in an incubator. The gel was incubated in an incubation solution containing cofactors necessary for MMP gelatinase activity for 24 hours at 37° C. A solution including methanol, acetic acid, and Coomassie brilliant blue R-250 was used to stain the gel for an hour. The same buffer but without the dye was used for the destaining procedure. The MMP proteolytic activity appeared as clear bands against a blue background. Using the ImageJ program, the intensity of these bands was analyzed.

Assessment of TIMP-1 using Western blot analysis

Initially, the left ventricular tissue was divided into small fragments on ice, followed by homogenization in

lysis buffer supplemented with protease and phosphatase inhibitors. The homogenized samples were centrifuged at 4° C for 5 minutes at 12,000 rpm, and the protein concentration of the supernatant was determined using the BCA assay kit. To denature proteins, samples were boiled at 100° C for 5 minutes. Protein separation was performed by SDS-PAGE, and subsequently transferred onto polyvinylidene difluoride (PVDF) membranes. To block nonspecific binding sites, membranes were incubated with 5% skimmed milk for 2 hours at room temperature. They were then exposed to primary antibodies overnight at 4° C, followed by incubation with HRP-conjugated secondary antibodies for 90 minutes at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) reagent (CW BIO, Beijing, China), and quantification was carried out with ImageJ software.

Histological evaluation of cardiac tissue using hematoxylin and eosin staining

After deparaffinization, tissue sections were stained with hematoxylin and eosin (H&E) and examined under an Olympus BX-50 light microscope (Olympus Corporation, Tokyo, Japan). H&E staining allowed for the examination of general tissue architecture. Histological examination was performed at 40× magnification, and images were captured using a digital camera attached to the microscope.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software (version 8.4.0.671). One-way analysis of variance (ANOVA) was used, followed by Tukey's post hoc test for multiple comparisons. All data are presented as mean±standard error of the mean (SEM). Statistical significance was set at $P < 0.05$.

Results

Impact of chronic SD before MI on survival and mortality

There was no death in the Control or SD groups. During the trial, 3 animal deaths occurred: 1 in the MI group died on day 20 (1/9), and 2 in the SD+MI group died; one died 48 hours after the second isoproterenol injection (day 18 of the protocol), and the other died on day 4 of the protocol (prior to MI induction) (2/9).

Impact of chronic SD before MI on TIMP-1/β-actin

As shown in Figure 1, there was no significant difference in the ratio of TIMP-1 to β-actin (TIMP-1/β-

actin) between the SD and the Control groups. In comparison to the Control and SD groups, the MI group's TIMP-1/ β -actin ratio was significantly lower ($P<0.001$). Furthermore, there was a significant drop in the TIMP-1/ β -actin ratio in the SD+MI group as compared to the Control group ($P<0.001$), the SD group ($P<0.001$), and the MI group ($P<0.05$).

Impact of chronic SD before MI on MMP-2 activity

MMP-2 activity was measured by zymography analysis, and the results showed that there was no significant difference in MMP-2 activity between the SD and Control groups. The MI group exhibited a statistically significant increase in enzyme activity when compared to the Control group ($P<0.01$) and no significant change was observed compared to the SD group. Additionally, there was no difference in this enzyme's activity in the SD+MI group when compared to all experimental groups (Figure

2).

Impact of chronic SD before MI on cardiac tissue changes

Hematoxylin and Eosin (H&E) staining revealed that animals in both the Control and SD groups exhibited an orderly and normal arrangement of myocardial fibers without any obvious damage. Noticeable disorganization of the myocardial fibers, the existence of hemorrhagic foci, and increased intercellular space were seen at 28 days after the final injection in the MI group. These changes were exacerbated in the SD+MI group (Figure 3).

Impact of chronic SD before MI on oxidative stress

As shown in Figure 4, MDA, as an oxidative stress biomarker, showed no significant differences between the experimental groups.

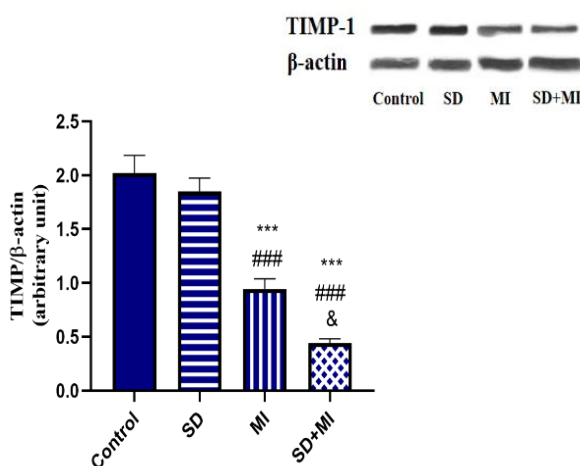


Figure 1. Western blot analysis of TIMP-1/ β -actin ratio in cardiac tissue seven days after the final isoproterenol injection ($n=3$). Data are presented as mean $\pm$ SEM. *** $P<0.001$ vs. Control group; ### $P<0.001$ vs. SD group; & $P<0.05$ vs. MI group. MI: Myocardial Infarction; SD: Sleep Disruption

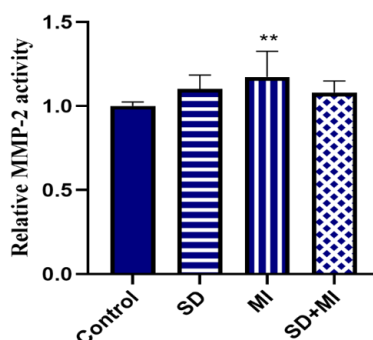


Figure 2. MMP-2 gelatinase activity in cardiac tissue seven days after the final isoproterenol injection ($n=3$). Data are presented as mean $\pm$ SEM. ** $P<0.01$ vs. Control group. MI: Myocardial infarction; SD: Sleep Disruption

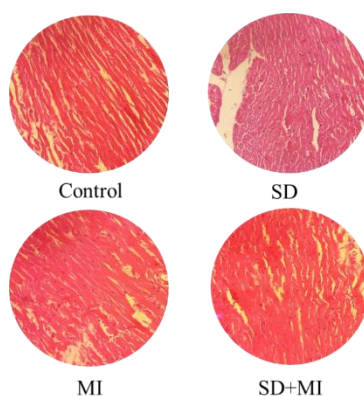


Figure 3. Representative hematoxylin and eosin (H&E) staining of myocardial tissue sections (6- μ m thick) from Control, SD, MI, and SD+MI groups at 28 days after the final isoproterenol injection (magnification: $\times 40$; $n=3$). MI: Myocardial Infarction; SD: Sleep Disruption

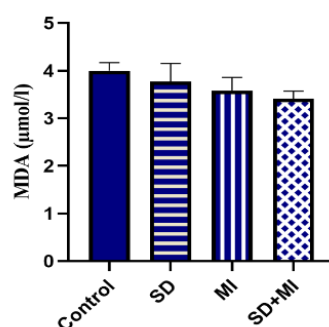


Figure 4. Serum malondialdehyde (MDA) levels in experimental groups 3 days after the final isoproterenol injection ($n=3$). Data are presented as mean $\pm$ SEM. MI: Myocardial infarction; SD: Sleep Disruption

Discussion

An imbalance between MMPs and TIMPs can cause ECM degradation since they have a role in maintaining the homeostasis and the integrity of the ECM. Initial MI induces inflammation, decreases TIMP levels, increases MMP activity, and leads to scar tissue formation and ECM stiffness in the injured area, a process that typically begins within the first week after MI (18,19).

In the present investigation, seven days after the final isoproterenol injection, we observed a significant decrease in the TIMP-1/ β -actin ratio in the MI group relative to the Control and SD groups. Moreover, the SD+MI group exhibited an even greater reduction in this ratio compared with both the Control and SD groups, and the level was also lower than that observed in the MI group. Inducing SD alone did not alter TIMP-1/ β -actin levels. There was a significant increase in MMP-2 activity in the MI group when compared to the Control group. However, there was no significant difference in the SD+MI group compared with the Control, SD, or MI groups. This pattern implies that while SD prior to MI

aggravates TIMP-1 downregulation, its influence on MMP-2 activation is limited, pointing to distinct regulatory pathways for these matrix modulators. Furthermore, histological studies using H&E staining showed that the destruction of myocardial fibers in the MI and SD+MI groups increased, with this damage being more pronounced in the SD+MI group, underscoring the detrimental effect of pre-infarction SD on myocardial tissue.

It has been reported that chronic SD triggers the ERK pathway, which raises the mRNA and protein levels of MMP-1, MMP-3, and MMP-13 in animals' temporomandibular joint and causes pathological alterations that are detrimental to the joint (15). According to a 2024 study, SD in animals revealed a connection between MMP-9 and the circadian rhythm-related genes PER and TIMELESS within the hippocampal regions (20). It has been reported that SD can impair MMP-9 function in cortical and hippocampal regions. Consequently, sufficient sleep plays an active role in regulating molecules associated with synaptic plasticity, particularly through the modulation of MMP-9

(21).

Based on research conducted by Rajani *et al.*, we selected day 7 after isoproterenol injection for our MMP activity examination via zymography, as this study showed that MMP activity is detectable on this day following ischemia (22). A study involving 40 males suffering from insomnia found that the MMP-2/TIMP-1 ratio increased in patients with chronic insomnia when compared to healthy people, while the MMP-9/TIMP-1 ratio did not change (23). It should be noted that measuring MMP activities in the initial stages of ischemia induction and during the second or fourth week following injection may provide different outcomes. Based on the results of Western blotting and the significant drop in the TIMP-1/ β -actin ratio in the MI and SD+MI groups compared with the Control and SD groups, it can be inferred that SD before MI disrupts the balance between MMPs and TIMP-1 in the cardiac remodeling process. This imbalance may underlie the histological evidence of aggravated myocardial changes observed in the SD+MI group.

While some studies have reported an association between SD and increased oxidative stress, the specific relationship between SD before MI and oxidative stress in the heart is not yet fully understood. Our study does not provide consistent evidence of significant changes in oxidative stress specifically in the context of MI and SD. A study on the Wistar rat model reported similar results to our study, showing no difference in antioxidant enzymatic activities after SD in the brain or peripheral tissues such as the liver and skeletal muscles (24).

It is important to note that the effects of SD on MMPs and oxidative stress can be complex and may depend on various factors, including the severity and duration of SD, different sleep patterns among species, assessment tests, and experimental conditions.

At this stage, it is appropriate to acknowledge that while SD prior to MI is clearly associated with an imbalance between MMP-2 and TIMP-1, its impact on oxidative stress remains uncertain and warrants further investigation. Importantly, the observation that mortality was absent in the Control and SD groups but occurred after MI induction, with early deaths in the SD+MI group, suggests that SD before MI not only modulates molecular pathways but also exacerbates vulnerability to post-MI complications.

This study has some limitations. First, MMP-2 and TIMP-1 were assessed only at a single time point (day 7), so changes at earlier or later stages were not captured. Second, the rat model with isoproterenol-induced MI may not fully reflect human disease. Finally, only limited

biomarkers of oxidative stress were measured, which may not provide a complete picture. Future studies with multiple time points and broader biomarker panels are needed to confirm and extend these findings.

In conclusion, SD prior to MI may worsen cardiac injury by disturbing the balance between MMP-2 and TIMP-1. Although oxidative stress markers did not show significant changes, histological findings revealed greater myocardial fiber disorganization in the SD+MI group. These results highlight the importance of sleep quality in cardiovascular health and suggest that targeting MMP-2 and TIMP-1 could be a potential therapeutic approach. Further studies with broader biomarker assessments are needed to confirm these findings.

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