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The Effect of Swimming Exercise on Memory Impairment and Inflammatory Cytokines Caused by Lipopolysaccharide Injection in Male Rats

Farnaz Torabi[1*], Khadijeh Khazaee[1], Mohammad Mohammadi[2]

[1] Department of Physical Education and Sport Sciences, Payame Noor University, Tehran, Iran
[2] Department of Sports Sciences, Faculty of Literature and Humanities, Malayer University, Malayer, Iran

*** Corresponding author email address**: f.torabi@Pnu.ac.ir

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A B S T R A C T

Memory is a fundamental human ability that enables the nervous system to encode, store, and retrieve various types of information. The aim of this study was to examine the effect of swimming exercise on memory impairment and the inflammatory cytokines TNFα and IL1 caused by lipopolysaccharide injection in male rats. In this study, 40 NMRI mice, approximately 70 days old and weighing 23 ± 1 grams, were used. The mice were randomly divided into four groups: a control group (no exercise with saline, no exercise with lipopolysaccharide) and an experimental group (exercise with saline, exercise with lipopolysaccharide). The training program included two phases of swimming: an adaptation phase and a swimming phase. The adaptation phase consisted of three 10-minute sessions with a 10-minute rest interval between each session. The duration of swimming was progressively increased from 20 minutes in the second week to 30 minutes in the third week. The data collected were analyzed using SPSS software with a two-way analysis of variance. The findings of this study demonstrated that swimming significantly reduced the levels of TNFα and IL1 in male rats ($p<0.05$). Lipopolysaccharide reduces spontaneous activity and impairs memory, whereas swimming exercise enhances memory function and spontaneous activity. Therefore, swimming exercise has anti-inflammatory effects and improves memory.

Keywords: *swimming exercise, memory, oxidative stress, inflammation*

1. Introduction

In the recent century, modern lifestyles have become increasingly sedentary, characterized by minimal physical activity and the extensive use of various technologies and conveniences. This shift has contributed to numerous physical and mental health issues, leading to the rise of chronic conditions such as diabetes, obesity, hypertension, and broadly speaking, metabolic syndrome, all of which result in widespread inflammation in the body (1). This

increased inflammation, particularly within the central nervous system (CNS), is linked to various neurological and behavioral disorders (2). Consequently, regular physical activity is essential for a healthy lifestyle, as it helps modulate CNS adaptation, especially in the hippocampus, which plays a vital role in learning and memory (3). However, the precise mechanisms by which exercise reduces inflammatory factors remain somewhat unclear, as pro-inflammatory cytokines can be secreted from various sources, including damaged neurons, astrocytes, and microglia (4). Some studies suggest that physical activity and exercise can influence both peripheral and central inflammation, including the activation of microglia within the CNS (5). Researchers have reported that moderate swimming exercise can alleviate learning and memory impairments caused by LPS by reducing hippocampal cytokine levels and oxidative brain damage (6). The neuroprotective effects of exercise on cognitive function include promoting neurogenesis, enhancing synaptic function, and increasing neural plasticity, which ultimately improves and enhances neurotransmission (7).

It is important to note that memory is not confined to a single area of the brain but is instead interwoven throughout the brain via neuronal connections, distributed in a diffuse manner (3). The role of neuroinflammation as a risk factor for neurological diseases is becoming increasingly clear, with evidence suggesting that inflammation plays a significant role in the development of pathological conditions in both the central and peripheral nervous systems (8). Cytokines are key regulators of the immune system, capable of exerting localized effects on target organs (9). Inflammation is one of the most severe manifestations of immune-related diseases, marked by the production of potent cytokines, and it plays a role in many diseases by causing excessive inflammatory infiltration and neural tissue damage in the brain (10). Inflammation is an innate immune response designed to protect the body, and in the context of neural inflammation, microglia, cytokines, chemokines, and associated molecular processes are critical players, leading to the recruitment of immune cells, edema, cellular damage, and ultimately, cell death (11). Inflammatory cytokines, particularly TNFα, IL1, and IL6—referred to as the "inflammation triad"—are found in elevated levels in the affected tissues of certain chronic inflammatory diseases, where their endocrine effects are mediated by high concentrations of cytokines like TNFα, IL6, IL1, and TGBG (12). Lipopolysaccharide (LPS) is a stable and potent endotoxin that can exacerbate damage to dopaminergic neurons in the presence of microglia and is known to induce inflammation (13). Consequently, exercise has been shown to enhance hippocampal function, potentially reversing neurological disorders (14). Physical exercise increases cerebral blood flow, leading to the proliferation of brain cells in the hippocampal region and the secretion of beneficial molecules (15). Alazobi and colleagues (2019) reported that swimming exercise could improve hippocampal function and significantly reverse the progression of neurobehavioral disorders (16). Therefore, this study aims to explore whether swimming exercise affects memory impairment and the inflammatory cytokines TNFα and IL1 following lipopolysaccharide injection in male rats. The research investigates the impact of swimming exercise on memory impairment and inflammatory cytokines TNFα and IL1 induced by lipopolysaccharide injection in male rats.

2. Methods and Materials

2.1. Study Design and Participants

The research design was experimental. Forty NMRI strain rats, approximately 70 days old and weighing 18 to 20 grams, were purchased from the Iran Pasteur Institute and transferred to the animal facility at the Salari Institute for Cognitive and Behavioral Disorders. The animals were housed under a 12-hour light/12-hour dark cycle with adequate food and water provided. The animals were then divided into four groups of 10:

1. Control Group 1: Animals received no exercise and were administered saline (the solvent for lipopolysaccharide).
2. Control Group 2: Animals received no exercise but were administered lipopolysaccharide.
3. Experimental Group 3: Animals received exercise and were administered saline.
4. Experimental Group 4: Animals received exercise and were administered lipopolysaccharide.

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2.2. *Swimming Exercise Protocol*

The animals were placed in a circular tank (80 cm in diameter and 30 cm in height) filled with water maintained at a temperature of 32 ± 1°C. To prevent the animals from floating, four wave-making motors were positioned at different angles in the tank. The protocol consisted of two phases: the adaptation phase and the swimming exercise phase. During the adaptation phase, to reduce stress from the water, the animals were placed in shallow water (5 cm deep) for 10 minutes daily during the first week to acclimate to the procedure. In the second week, the swimming protocol involved three 10-minute sessions with 10-minute rest intervals in 10 cm of water. In the third week, the animals swam in three 10-minute sessions with 10-minute rest intervals in 15 cm of water, totaling 30 minutes of swimming. From the third week onward, the number of daily swimming sessions was increased. This exercise was conducted daily between 1 PM and 4 PM. After swimming, the animals were dried with a towel and warmed for 10 to 15 minutes using an electric heater. The non-exercise animals (control group) were placed in a similar circular tank without water for the same duration as the exercised animals. After the exercise sessions, the animals received an intraperitoneal injection of lipopolysaccharide (LPS) at a dose of 250 micrograms per kilogram in a volume of 100 microliters for nine days. The LPS was dissolved in saline. Subsequently, memory-related behavioral tests were conducted. On the first, second, and third days after the LPS injection, memory behaviors were assessed using three behavioral tests.

2.3. *Memory Behavioral Tests in the Research*

Spatial memory tests were measured using the T-maze apparatus, working memory was assessed with the Y-maze apparatus, and cognitive memory was evaluated using the novel object recognition test, all following standard procedures.

2.4. *Brain Dissection*

After completing the memory behavioral tests, the rats were placed under deep anesthesia. Blood was then drained from the brain, the brain was extracted, and the hippocampus was isolated. The samples were subsequently used to measure the inflammatory cytokines TNFα and IL1 using the ELISA technique, according to the protocol provided in the kit by the RD company.

2.5. *Data Analysis*

Descriptive and inferential statistical tests were used for data analysis. Descriptive statistics were employed to calculate measures of central tendency, mean, and standard deviation of the variables, while inferential statistics were used to compare the means between groups. The data were analyzed using SPSS-26 software, applying two-way ANOVA with a significance level set at 0.05 or below.

3. Findings and Results

Table 1 provides general descriptive information, including the mean and standard deviation, for both the non-exercise group and the swimming exercise group in memory-related behavioral tests and inflammatory cytokines (TNFα – IL1). As shown in Table 1, there is a significant difference in the levels of TNFα and IL1 among at least one group, as determined by the two-way ANOVA test ($p \leq 0.05$). However, no significant differences were observed between the groups in the memory-related tests ($p > 0.05$).

Table 1

Mean and Standard Deviation of Research Variables (Memory Variables and Inflammatory Cytokines TNFα – IL1)

Variable	Group	Research Groups		F value	P value
		Without Exercise	With Exercise		
New Object Detection System	Saline Injection Group	0.74±0.09	0.76±0.10	2.938	0.951
	Lipopolysaccharide Injection Group	0.60±0.09	0.72±0.11		
Memory Behavioral Test, Mazes-shaped Device	Saline Injection Group	0.007±0.94	0.008±0.46	0.612	0.439
	Lipopolysaccharide Injection Group	0.406±0.56	0.407±0.04		
Memory Behavioral Test, Y-Maze Device	Saline Injection Group	0.507±0.21	0.508±0.29	0.001	0.970
	Lipopolysaccharide Injection Group	0.106±0.37	0.307±0.66		

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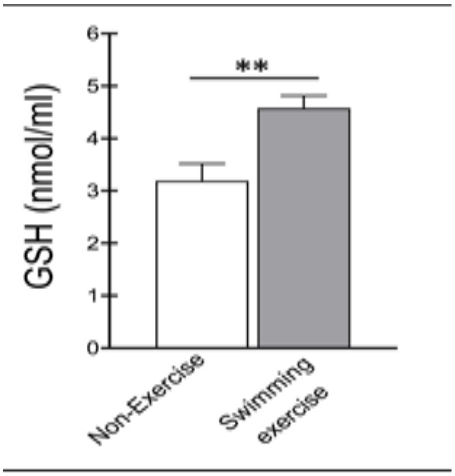
TNFα	Saline Injection Group	0.501±0.21	0.001±0.47	5.542	0.024
	Lipopolysaccharide Injection Group	0.602±0.29	0.901±0.75		
IL1	Saline Injection Group	0.909±0.85	0.201±0.34	5.231	0.028
	Lipopolysaccharide Injection Group	0.601±0.75	0.109±0.79		

Figure 1

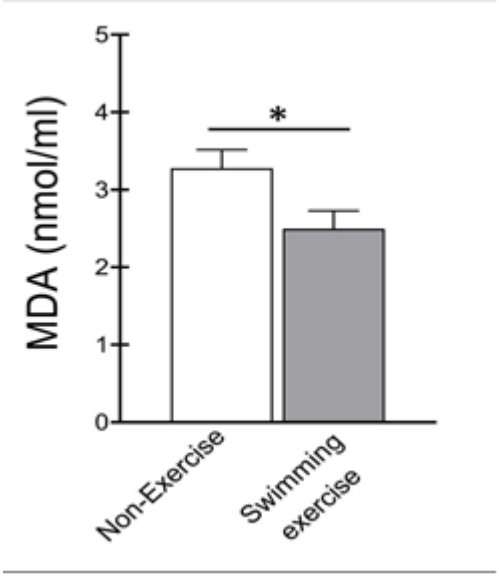
Glutathione



As illustrated in Figure 1, glutathione is an antioxidant that has beneficial effects, particularly when its levels are elevated, potentially reducing anxiety. The activity of glutathione is inversely related to malondialdehyde; in other words, when malondialdehyde levels are high, glutathione levels tend to be low. In this study, swimming exercise has been shown to increase glutathione levels, indicating that swimming may help reduce oxidative stress.

Figure 2

Malondialdehyde



As depicted in Figure 2, malondialdehyde is a marker that rises when oxidative stress in the brain or body is significantly elevated. Any substance or activity that can lower malondialdehyde levels is beneficial for the body. In

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this case, we observe that swimming has resulted in a reduction of malondialdehyde, which is a positive sign. Therefore, swimming may help reduce oxidative stress.

4. Discussion and Conclusion

The intermittent parameters, by themselves, showed significant improvement in cognitive function and levels of IL-18 and TNF-α compared to the group that exercised. Additionally, it was shown in the four groups under study that the two groups not engaged in exercise had lower cognitive performance compared to the saline group, and even more so in the group exposed to LPS. Among the groups following the swimming exercise protocol, the group that both exercised and received LPS demonstrated that swimming had a significant positive impact on cognitive function. This study highlighted that swimming can have substantial effects on memory and cognitive performance arising from spontaneous alternation. Moreover, it was found that swimming could influence the levels of IL-1 and TNF-α, helping to keep inflammation induced by lipopolysaccharide at a lower level. The results also showed that swimming exercise did not have a significant impact on cognitive, spatial, and functional memory impairment caused by lipopolysaccharide injection in male mice. This finding is consistent with the results of studies by Jian-Hu et al. (2019), Al-Zoubi et al. (2019), Zhang et al. (2024), and Jin et al. (2014). Studies have demonstrated that physical activity and exercise can be beneficial for overall health and cognitive function, and can also enhance learning ability and memory performance (17).

Given that cytokines trigger a highly inflammatory response characterized not only by an increase in pro-inflammatory markers but also by mitochondrial dysfunction, and oxidative and nitric oxide species disturbances, it's evident that LPS can directly access the brain (18). Oxidative stress plays a crucial role in many physiological and pathological conditions, where intracellular oxidative homeostasis is tightly regulated by the production of reactive oxygen species. Increased oxidative stress can alter lipids, DNA, and proteins, leading to cellular inflammation and programmed cell death (Wang et al., 2020). Swimming exercise has beneficial effects and can reduce systemic inflammation; regular exercise in individuals with MS has been shown to lower inflammatory cytokine levels (19, 20). Research has also demonstrated that swimming reduces memory impairment during pregnancy (17, 21). Key inflammatory factors include the cytokines IL-1 and TNF-α. Recent human and animal studies have shown that an increase in these cytokines in the body and brain can lead to memory decline. Increased inflammation, particularly in the central nervous system, can result in neurological and behavioral disorders. Physical activity has the potential to modulate microglial activation in the central nervous system. Low-intensity exercise is sufficient to induce anti-microglial effects by regulating the expression of various factors and is recognized as a risk-modifying agent for reducing memory and learning deficits in neurological diseases.

Based on the results of this study, we conclude that swimming during pregnancy prevents long-term pregnancy-related memory impairment and enhances memory function through increased cellular proliferation in the hippocampus. Given that this study focused on memory and inflammation induced by lipopolysaccharide, it is suggested that future research should also explore other diseases related to inflammation and oxidative stress. Additionally, future studies should investigate the mechanisms through which physical activity influences psychological behaviors and memory. The results of this research suggest that exercise and physical activity positively impact cognitive performance and memory factors, and engaging in regular swimming and physical exercise can help prevent cognitive and memory impairments.

Authors' Contributions

F.T. and K.K. designed the study and developed the experimental protocols. F.T. was responsible for the animal handling and execution of the swimming exercise regimen. K.K. conducted the biochemical analyses and measured the levels of inflammatory cytokines. M.M. contributed to the data analysis and interpretation of the results. All authors were involved in drafting the manuscript, and M.M. provided critical revisions. The final manuscript was reviewed and approved by all authors.

Declaration

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

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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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Ethical Considerations

The study adhered to the ethical guidelines for research with human subjects as outlined in the Declaration of Helsinki (Ethics Code: IR.PNU.REC.1401.207).

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