

## Original Article

# The Effect of Moderate-Intensity Aerobic Exercise on IL-8, IL-1 $\beta$ , and NF $\kappa$ B Levels in Women with Type 2 Diabetes

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## ABSTRACT

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**Introduction:** Type 2 diabetes is one of the most common chronic metabolic diseases and is associated with increased inflammatory markers. The present study aimed to investigate the effect of moderate-intensity aerobic exercise on interleukin (IL)-8, IL-1 $\beta$ , and nuclear factor kappa B (NF $\kappa$ B) levels in women with type 2 diabetes.

**Materials and Methods:** This semi-experimental study employed a pretest-posttest design with a control group. Thirty women aged 40 to 50 years with at least three years of diagnosed type 2 diabetes were purposefully selected and randomly assigned to either an exercise group or a control group (n = 15 per group). The exercise group participated in an 8-week progressive aerobic training program (three sessions per week) at 35-75% of maximum heart rate. IL-8 and IL-1 $\beta$  levels were measured using the enzyme-linked immunosorbent assay method, and NF $\kappa$ B activity was assessed through western blot analysis from blood serum samples.

**Results:** The findings indicated a significant decrease in IL-8 and IL-1 $\beta$  levels, and in NF $\kappa$ B activity, in the exercise group compared to the control group after the intervention (p < 0.001). This reduction was statistically significant both within the exercise group compared to the pretest and compared with the control group.

**Conclusion:** This study demonstrates that moderate-intensity aerobic exercise significantly reduces inflammatory markers in women with type 2 diabetes. It appears that aerobic exercise may be recommended as an effective non-pharmacological approach to improve inflammatory status and assist in the management of type 2 diabetes.

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## Introduction

Type 2 diabetes mellitus (T2DM) is one of the most prevalent and critical chronic metabolic disorders in today's world. It not only disrupts glucose metabolism but also leads to a wide range of systemic complications [1]. According to estimates by the World Health Organization (WHO), more than 537 million people worldwide are currently living with diabetes, and this number is projected to exceed 700 million by 2045 [2]. Over 90% of these cases are classified as T2DM, which commonly develops in adulthood due to a sedentary lifestyle, poor dietary habits, obesity, stress, and genetic predisposition [3]. In Iran, the increasing prevalence of T2DM, particularly among middle-aged women, has become a major public health concern [4].

T2DM is a multifactorial chronic disease that affects not only carbohydrate, fat, and protein metabolism but also activates inflammatory pathways leading to vascular disorders, retinal damage, peripheral neuropathy, kidney diseases, and cardiovascular complications [5, 6]. In recent years, there has been growing interest in the role of inflammation in the pathogenesis and progression of T2DM [7]. Research evidence suggests that levels of inflammatory cytokines such as interleukin (IL)-1 $\beta$  and IL-8 are elevated in diabetic patients, along with increased activation of inflammatory signaling pathways such as nuclear factor kappa B (NF $\kappa$ B) in various cell types [8]. These factors play a crucial role in insulin resistance, destruction of pancreatic beta cells, and the acceleration of microvascular and

macrovascular complications associated with diabetes [9].

IL-1 $\beta$  is a key cytokine that initiates and sustains inflammatory responses. By binding to IL-1 receptors and activating NF $\kappa$ B signaling, it promotes the production of additional inflammatory mediators and causes damage to target tissues [10, 11]. IL-8, a potent neutrophil chemoattractant, contributes to immune cell infiltration in peripheral tissues, leading to chronic inflammation and cellular destruction in diabetic patients [12]. NF $\kappa$ B, a major transcription factor regulating inflammatory responses, remains chronically activated in many diabetic individuals and enhances the expression of genes involved in inflammation, apoptosis, cell proliferation, and the immune response [13-15].

Given the significant role of these factors in the exacerbation of diabetic complications, reducing their levels may be an effective strategy in disease management. Among the non-pharmacological interventions, regular physical activity and structured exercise have shown promising results in modulating inflammatory and metabolic processes [16-18]. Aerobic exercise, particularly moderate-intensity exercise, not only improves body composition, reduces fat mass, and enhances insulin sensitivity but also exerts anti-inflammatory effects by downregulating pro-inflammatory cytokines, modulating immune responses, and improving overall health status in diabetic patients [19-21]. While some previous studies have reported reductions in IL-6, tumor necrosis factor-alpha (TNF- $\alpha$ ), and C-reactive protein (CRP) following

aerobic training [22-24], fewer investigations have explored the specific effects on IL-1 $\beta$ , IL-8, and NF $\kappa$ B, especially among middle-aged women with T2DM, a physiologically and immunologically vulnerable population [24, 25]. Moreover, many earlier studies have suffered from limitations such as lack of a controlled design, short intervention periods, or failure to simultaneously examine multiple key inflammatory markers. Therefore, identifying the specific effects of moderate-intensity aerobic exercise on these three critical inflammatory components could provide a foundational step toward designing more effective therapeutic interventions for diabetic patients.

Accordingly, the present study was designed to investigate the effects of moderate-intensity aerobic exercise on serum IL-8 and IL-1 $\beta$  levels and NF $\kappa$ B activity in women with T2DM. The aim is to provide robust scientific evidence supporting the use of exercise as an adjunct to pharmacological treatment to control systemic inflammation and reduce secondary complications in this patient population.

## Materials and Methods

This study used a quasi-experimental, pretest-posttest design with a control group. A total of 30 women with T2DM, aged 40-50 years, and with at least 3 years of membership at the Yazd Province Diabetes Center, were selected through purposive and convenience sampling. Considering the limited target population and similarity to previous studies, this sample size was deemed adequate to assess the effects of moderate-intensity aerobic exercise. Eligible participants were then randomly assigned to either

a control group (n = 15) or an exercise group (n = 15) to investigate the effects of training on serum levels of IL-8, IL-1 $\beta$ , and NF $\kappa$ B activity.

Inclusion criteria were: Age between 40–50 years; diagnosis of T2DM for at least three years; fasting blood glucose level above 126 mg/dL; use of similar antidiabetic medications; adherence to a dietary plan recommended by the diabetes center; non-smoking status; absence of cardiovascular, hepatic, or infectious diseases that could influence inflammatory markers; and no participation in regular or intense exercise within the past three months. Exclusion criteria included: unwillingness to continue participation; non-compliance with the training program or interventions; development of serious or dangerous complications; unintended changes in medication or treatment regimen; emergence of new medical conditions; and physical or psychological conditions unsuitable for continuing the study. Participants who missed training sessions or sample collections were also excluded.

All participants voluntarily joined the study after being fully informed of the procedures and signing a written informed consent form.

Serum IL-8 and IL-1 $\beta$  levels were measured using the enzyme-linked immunosorbent assay technique. NF $\kappa$ B activity was assessed by western blot or similar methods to determine its phosphorylation status in serum samples. One week prior to the start of the intervention, participants attended a familiarization session in which they were instructed on safety guidelines and the proper use of the treadmill device. The aerobic exercise protocol

consisted of progressive treadmill running with gradually increasing intensity. In the first and second weeks, participants exercised at 35–45% of their maximum heart rate (HRmax); during weeks three and four at 45–55% HRmax; weeks five and six at 55–65% HRmax; and in the final two weeks (seven and eight) at 65–75% HRmax. Each session lasted 50 minutes, comprising 10 minutes of warm-up, 30 minutes of continuous main activity, and 10 minutes of cool-down. Participants completed three sessions per week. A summary of the exercise protocol is presented in Table 1 [26].

### Statistical analysis

Data were analyzed using SPSS software version 26. The normality of the data was assessed using the Kolmogorov–Smirnov test. Paired sample t-tests and independent sample t-tests were used to compare the means within and between groups, respectively. A significance level of  $p < 0.05$  was considered for all statistical tests.

## Results

In both the control and exercise groups, the mean age of the participants was 45 years with a standard deviation (SD) of 3 years. The mean body weight was 60 kg (SD = 5 kg), and the average height was 160 cm (SD = 4 cm). Additionally, the body mass index (BMI) was reported as 23.4 with a SD of 1.2 in both groups.

These findings indicate that the two groups were relatively homogeneous in terms of baseline demographic characteristics. In this section, the changes in IL-8 levels in patients

with T2DM following moderate-intensity aerobic exercise were examined.

A paired-samples t-test was used to compare mean IL-8 levels before and after the intervention within both the experimental (exercise) and control groups.

### Exercise group

In the exercise group, the mean difference in serum IL-8 levels before and after the aerobic training was  $-19.46$  pg/mL, indicating a significant reduction in IL-8 following the intervention. The SD of this difference was  $6.58$  pg/mL, and the standard error of the mean (SEM) was  $1.70$  pg/mL. The 95% confidence interval (CI) for the mean difference ranged from  $-23.11$  to  $-15.82$  pg/mL, confirming the statistical significance of the decrease. The paired t-test value was  $-11.45$  with a  $p$ -value  $< 0.001$ , indicating a highly significant effect of moderate-intensity aerobic exercise on IL-8 levels.

### Control group

In the control group, the mean difference in serum IL-8 levels between the pre-test and post-test was  $0.79$  pg/mL, indicating no meaningful change following the study period. The SD of this difference was  $3.25$  pg/mL, and the SEM was  $0.84$  pg/mL. The 95% CI for the mean difference ranged from  $-1.01$  to  $2.59$  pg/mL, which includes zero, indicating that the observed change was not statistically significant. The paired t-test yielded a  $p = 0.364$ , confirming the absence of a significant change in IL-8 levels in the control group. Overall, these findings indicate that the significant reduction in IL-8 was observed only

in the exercise group, whereas no significant alteration occurred in the control group.

#### **Independent sample t-test for between-group differences in IL-8 changes**

To examine whether IL-8 levels differed significantly between the exercise and control groups, an independent-samples t-test was performed. The detailed results of this test are presented in Table 1. Levene's test for equality of variances showed that the significance value was 0.002. Therefore, the null hypothesis of equal variances was rejected, and the analysis was conducted using the "equal variances not assumed" results. The independent samples *t*-test indicated a *t*-value of -10.683 and a significance level of 0.0001. Hence, there is a statistically significant difference between the exercise and control groups regarding IL-8 levels. These results demonstrate that IL-8 levels differ significantly between the two groups. The mean difference between the groups was -20.2500, indicating a significant reduction in IL-8 in the exercise group compared to the control group. The 95% CI for the mean difference (assuming unequal variances) ranged from -24.1984 to -16.3016. These values indicate a significant negative difference in IL-8 levels between the groups. Table 2 examines the descriptive statistics of IL-8, IL-1 $\beta$ , and NF $\kappa$ B levels in the exercise and control groups at pre-test and post-test. Table 3 examines the paired sample *t*-test results for changes in IL-8 levels in the exercise and control groups between pre-test and post-test. Table 4 examines the independent samples *t*-test results comparing IL-8 changes between the exercise and control groups. Table 5 examines the paired sample *t*-test results for

changes in IL-1 $\beta$  levels in the exercise and control groups between pre-test and post-test. Table 6 examines the independent samples *t*-test results comparing IL-1 $\beta$  changes between the exercise and control groups. Table 7 examines the paired sample *t*-test results for changes in NF $\kappa$ B levels in the exercise and control groups between pre-test and post-test. In this section, changes in IL-1 $\beta$  levels in patients with T2DM following moderate-intensity aerobic exercise were examined.

A paired-samples *t*-test was used to compare mean IL-1 $\beta$  levels before and after the intervention within both the experimental (exercise) and control groups.

#### **Exercise group**

In the exercise group, the mean difference in IL-1 $\beta$  levels before and after aerobic training was -4.37, indicating a significant reduction in IL-1 $\beta$  levels following the intervention. The SD of this difference was 1.18, and the standard error of the mean was 0.30. The 95% CI for the difference ranged from -5.02 to -3.72, confirming the statistical significance of the reduction. The paired *t*-test value was -14.33, with a *p*-value of 0.0001, indicating highly significant results.

#### **Control group**

In the control group, the mean difference in IL-1 $\beta$  levels was -0.55, indicating a significant reduction. The SD was 0.93, and the standard error of the mean was 0.24. The 95% CI for the difference ranged from -1.07 to -0.03, which does not include zero, confirming the significance of the change. The paired *t*-test value was -2.29, with a *p*-value of 0.038, indicating statistical significance.

**Table 1.** Training program specifications

Week	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Intensity (% of HRmax)	35–45	35–45	45–55	45–55	55–65	55–65	65–75	65–75
Duration per session (min)	40–50	40–50	40–50	40–50	40–50	40–50	40–50	40–50
Sessions per week	3	3	3	3	3	3	3	3

**Table 2.** Descriptive statistics of research variables in exercise and control groups (pre-test and post-test)

Variable	Group	Phase	Minimum	Maximum	Mean	Standard deviation
IL-8 (pg/mL)	Exercise	Pre-test	68.27	138.84	106.2360	24.6050
	Exercise	Post-test	44.42	121.71	86.7747	23.5465
	Control	Pre-test	50.01	145.85	88.3293	30.8719
	Control	Post-test	51.29	145.05	89.1180	30.2178
IL-1 $\beta$ (pg/mL)	Exercise	Pre-test	11.76	29.12	19.2233	6.2515
	Exercise	Post-test	7.50	23.60	14.8527	5.3966
	Control	Pre-test	10.36	29.38	19.2440	6.4326
	Control	Post-test	9.49	29.30	18.6933	6.3612
NF $\kappa$ B (AU)	Exercise	Pre-test	21.97	49.47	36.6573	8.6546
	Exercise	Post-test	11.95	42.89	26.6920	8.7247
	Control	Pre-test	20.59	49.88	35.8020	9.9451
	Control	Post-test	19.25	50.91	35.5333	10.6934

**Table 3.** Paired sample t-test results for IL-8 (pg/mL) changes in exercise and control groups

Group	Paired Differences	Mean Difference	Standard Deviation	Standard Error Mean	95% Confidence Interval of the Difference	t	df	p-value (2-tailed)
Exercise	Post.IL8- Pre.IL8	-19.4613	6.5811	1.6992	-23.1058 to -15.8169	-11.453	14	0.0001
Control	Post.IL8- Pre.IL8	0.7887	3.2536	0.8401	-1.0131 to 2.5905	0.939	14	0.364

**Table 4.** Independent samples t-test results for comparing IL-8 (pg/mL) changes between exercise and control groups

Levene's test for equality of variances	F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Standard error difference	95% Confidence interval of the difference
Equal variances assumed	12.303	0.002	-10.683	28	0.0001	-20.2500	1.8955	-24.1329 to -16.3671
Equal variances not assumed			-10.683	20.458	0.0001	-20.2500	1.8955	-24.1984 to -16.3016

**Table 5.** Paired sample t-test results for IL-1 $\beta$  (pg/mL) changes in exercise and control groups

Group	Paired differences	Mean difference	Standard deviation	Standard error mean	95% Confidence interval of the difference	t	df	p-value (2-tailed)
Exercise	Post IL-1 $\beta$ - Pre IL-1 $\beta$	-4.37	1.18	0.30	-5.02 to -3.72	-14.33	14	0.0001
Control	Post IL-1 $\beta$ - Pre IL-1 $\beta$	-0.55	0.93	0.24	-1.07 to -0.03	-2.29	14	0.038

**Table 6.** Independent samples t-test results for comparing IL-1 $\beta$  (pg/mL) changes between exercise and control groups

Levene's Test for Equality of Variances	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Standard Error Difference	95% Confidence Interval of the Difference
Equal variances assumed	1.871	0.182	-9.834	28	0.0001	-3.8200	0.3884	-4.6157 to -3.0243
Equal variances not assumed			-9.834	26.561	0.0001	-3.8200	0.3884	-4.6176 to -3.0224

**Table 7.** Paired sample t-test results for NFkB (AU) changes in exercise and control groups

Group	Paired differences	Mean difference	Standard deviation	Standard error mean	95% Confidence interval of the difference	t	df	p-value (2-tailed)
Exercise	Post NFkB Pre NFkB	-9.97	2.65	0.68	-11.43 to -8.50	-14.58	14	0.0001
Control	Post NFkB Pre NFkB	-0.27	1.11	0.28	-0.88 to 0.35	-0.93	14	0.366

**Table 8.** Independent samples t-test results for comparing NFkB (AU) changes between exercise and control groups

Levene's test for equality of variances	F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Standard error difference	95% Confidence interval of the difference
Equal variances assumed	9.155	0.005	-13.080	28	0.0001	-9.6967	0.7414	-11.2153 to -8.1781
Equal variances not assumed			-13.080	18.801	0.0001	-9.6967	0.7414	-11.2495 to -8.1439

### Independent samples t-test for between-group differences in IL-1 $\beta$ changes

In this section, an independent-samples t-test was performed to assess significant differences in IL-1 $\beta$  levels between the exercise and control groups. The detailed results of this test are presented in Table 7. Levene's test for equality of variances showed a significance value of 0.182. Therefore, the null hypothesis of equal variances was accepted, and the analysis was conducted using the "equal variances assumed" results. The t-test results indicated a *t* value of -9.834 and a significance level of 0.0001. Thus, there is a statistically significant difference between the exercise and control groups regarding IL-1 $\beta$  levels. These results demonstrate that the changes in IL-1 $\beta$  levels differ significantly between the two groups. The mean difference between the groups was -3.82, indicating a significant reduction in IL-1 $\beta$  in the exercise group compared to the control group. The 95% CI for the mean difference (assuming equal variances) ranged from -4.6157 to -3.0243. These values indicate a significant negative difference in IL-1 $\beta$  levels between the groups. In this section, changes in NF $\kappa$ B levels in patients with T2DM following moderate-intensity aerobic exercise were examined.

A paired sample *t*-test was used to compare the mean NF $\kappa$ B levels before and after the intervention within both the experimental (exercise) and control groups.

### Exercise group

In the exercise group, the mean difference in NF $\kappa$ B levels before and after aerobic training

was -9.97, indicating a significant reduction in NF $\kappa$ B levels following the intervention. The SD of this difference was 2.65, and the standard error of the mean was 0.68. The 95% CI for the difference ranged from -11.43 to -8.50, confirming the statistical significance of the reduction. The paired t-test value was -14.58, with a p-value of 0.0001, indicating highly significant results.

### Control group

In the control group, the mean difference in NF $\kappa$ B levels was -0.27, indicating a slight reduction in NF $\kappa$ B levels. The SD was 1.11, and the standard error of the mean was 0.28. The 95% CI for the difference ranged from -0.88 to 0.35, which includes zero, indicating that this change is not statistically significant. The paired t-test value was -0.93, with a p-value of 0.366, suggesting that the observed change in the control group is not statistically significant and is likely due to chance.

### Independent samples t-test for between-group differences in NF $\kappa$ B changes

In this section, an independent-samples t-test was conducted to assess significant differences in NF $\kappa$ B levels between the exercise and control groups. The detailed results of this test are presented in Table 8.

Levene's test for equality of variances showed a Significant Value of 0.005. Therefore, the null hypothesis of equal variances is rejected. Hence, the results from the "equal variances not assumed" row are used for analysis. The t-test results indicate a *t*-value of -13.080 and a Significant of 0.0001. Therefore, there is a significant difference between the exercise

and control groups in NF $\kappa$ B levels. These results demonstrate that NF $\kappa$ B changes differ significantly between the two groups. The mean difference between groups was -9.6967, indicating a significant reduction of NF $\kappa$ B in the exercise group compared to the control group. The 95% CI for the mean difference ranged from -11.24945 to -8.14388 under the assumption of unequal variances. These values indicate a significant negative difference in NF $\kappa$ B levels between the groups.

## Discussion

The present study found that 8 weeks of moderate-intensity aerobic exercise significantly reduced levels of IL-8, IL-1 $\beta$ , and NF $\kappa$ B activity in women with T2DM. These results highlight the notable anti-inflammatory effects of aerobic exercise in this patient population and are consistent with previous research. Regarding IL-8 reduction, the results showed a significant decrease in IL-8 levels in the exercise group, both compared with baseline and with the control group ( $p < 0.001$ ). IL-8 is a key pro-inflammatory cytokine involved in neutrophil recruitment, increased vascular permeability, and chronic inflammation. In diabetic patients, IL-8 levels are chronically elevated and have been associated with complications such as retinopathy and neuropathy. The current study's findings align with previous reports of decreased IL-8 after aerobic training.

The mechanism behind this reduction may involve improved insulin sensitivity, reduced oxidative stress, and modulation of NF $\kappa$ B

signaling pathways. Similarly, IL-1 $\beta$  results showed a significant reduction in the exercise group, whereas changes in the control group were minimal. IL-1 $\beta$  is a major initiator of inflammatory responses, associated with pancreatic beta-cell destruction and insulin resistance. The reduction of IL-1 $\beta$  following the exercise intervention reflects the positive effects of physical activity on cytokine balance and systemic inflammation. These findings agree with previous studies reporting reductions in IL-1 $\beta$ , increased adiponectin, and inhibition of the NF $\kappa$ B pathway following exercise.

A key finding of this research was the significant decrease in NF $\kappa$ B activity in the exercise group. NF $\kappa$ B is a central transcription factor that regulates inflammatory processes by promoting the expression of genes such as IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$ , thereby perpetuating inflammation. Elevated NF $\kappa$ B activity in T2DM is linked to tissue damage, endothelial permeability, and insulin resistance. The reduction in NF $\kappa$ B activity post-exercise may result from inhibition of reactive oxygen species-dependent inflammatory pathways, enhanced antioxidant levels, and improved mitochondrial function. These findings are in line with related studies.

Overall, the results support the effective role of moderate-intensity aerobic exercise in modulating immune responses and reducing chronic inflammation in T2DM patients. Several physiological mechanisms may contribute, including:

- Increased secretion of anti-inflammatory myokines from activated skeletal muscles;

- Enhanced mitochondrial function and reduced free radical production;
- Reduction of visceral fat, a source of many inflammatory cytokines;
- Improved insulin sensitivity and glucose metabolism.

It is noteworthy that these changes were observed after only 8 weeks of intervention and are likely to be more sustained with continued exercise and lifestyle modifications. However, limitations of this study include a relatively small sample size, a lack of assessment of hormonal inflammatory markers, restriction to one gender (female), and a narrow age range, all of which limit the generalizability of the results. Future studies with larger samples, multiple inflammatory markers, both genders, and different age groups are recommended to gain more comprehensive insights.

## Conclusion

Our findings suggest that aerobic exercise not only improves metabolic indices but also plays a crucial role in modulating chronic inflammatory responses. Therefore, it can be considered a complementary non-pharmacological approach for better inflammatory control and reduction of diabetes-related complications. Reducing inflammatory factors in diabetic patients improves their quality of life and decreases the risk of cardiovascular, renal, ocular, and neurological complications associated with diabetes. Consequently, this study emphasizes

the importance of incorporating regular aerobic exercise into rehabilitation and care programs for patients with diabetes. Moderate-intensity aerobic exercise, through anti-inflammatory effects, modulation of the immune response, improved insulin sensitivity, and reduced oxidative stress, plays an important role in managing T2DM. Health and exercise professionals are encouraged to include this type of training as part of multifaceted interventions for diabetic patients.

## Ethical Considerations

The ethical principles of this study were approved by the Ethics Committee of Islamic Azad University, Khorasgan Branch (Approval No. IR.IAU.KHUISH.1403.511). Ethical standards, including informed consent, privacy, and confidentiality, were upheld. Participants were informed about the study and were free to withdraw at any time without consequences.

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None.

## Conflict of Interest

The authors declare no conflict of interest.

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## Data Availability Statement

The data presented in this study are available on request from the corresponding author.

## Authors' Contributions

A.B.M: Collection of materials and sources; F.T: Research management and scientific editing; K.J. D: Research consultant; E.B: Data analysis; All authors have read, approved the final manuscript, and accept responsibility for the research.

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