

ORIGINAL PAPER

doi: 10.5455/medarh.2023.77.4-7

MED ARCH. 2023 FEB; 77(1): 4-7

RECEIVED: DEC 06, 2022

ACCEPTED: FEB 10, 2023

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Effect of Moderate Intensity Continuous Training and Slow Type Interval Training to Gene Expression of TGF- β in Type 2 Diabetes Mellitus Model Wistar Rats

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ABSTRACT

Background: Physical exercise is a process of increasing contraction of skeletal muscles involving several organ systems to increase energy formation and it is one of the pillars for the management of type 2 diabetes mellitus (T2DM). T2DM affects the expression of the TGF- β gene. **Objective:** This study aims to determine the effect of moderate intensity continuous training and slow interval training in expression of TGF- β gene in wistar rats T2DM model. **Methods:** A moderate intensity continuous training physical activity given to the diabetic rat group in the form of running on a treadmill with a running speed of 20 m/min for 30 minutes while the slow type interval training physical activity given to the diabetic rat group was in the form of running on a treadmill with a running speed of 20 m/minute for 10 repetition sessions, with a duration of 2 minutes per session with interspersed active breaks for 1 minute. TGF- β gene expression was measured using real-time methods. **Results:** The results in the continuous group, TGF- β significant decreased $p < 0.05$. In the interval group, TGF- β insignificant decreased $p > 0.05$. Moderate intensity continuous training was insignificantly better at decreasing the expression of the TGF- β genes. **Conclusion:** This research concluded that there is an effect of moderate intensity continuous training and slow interval training on the expression of TGF- β genes in wistar rats T2DM. **Keywords:** continuous training, interval training, TGF- β , rats, type 2 diabetes mellitus.

1. BACKGROUND

Diabetes Mellitus (DM) is a chronic disease characterized by blood glucose levels exceeding normal limits caused by a relative or absolute deficiency of the hormone insulin. The World Health Organization (WHO) said that the prevalence of DM is increasing worldwide and 90-95% of DM cases are T2DM (1, 2). Diabetic Nephropathy is a microvascular complication of T1DM and T2DM (3). Diabetic Nephropathy is characterized by the presence of persistent albuminuria accompanied by an increase in the expression of the TGF- β gene (4-6). TGF- β plays an important role in maintaining the stability of the vascular structure, therefore it is closely related to T2DM. TGF- β plays an important role with several angiogenic factors such as VEGF, PDGF, and TNF- α this protein plays a role in accelerating the neovascularization process in chronic hyperglycemia conditions (7-9). Physical exercise that recommended for T2DM by ADA care was moderate intensity continuous training, but previous studies showed that interval training has positive effect to insulin resistance (10-12). However, there has never been a reported effectiveness of the two exercises on blood sugar control and expression of the TGF- β in T2DM patients. In connection with the previous description, a study was conducted on the effect of continuous training of moderate intensity and slow type interval training on the expression of TGF- β activity in T2DM model wistar rats.

2. OBJECTIVE

This study aims to analyze the effect of moderate continuous training and slow interval training in expression of TGF-β gene in T2DM model wistar rats.

3. MATERIAL AND METHODS

Participants

This experimental study used thirty six healthy male Wistar rats, aged 8 weeks, 180-200 gram in weight, housed in cages with temperature 22-25o, 12h/12h light-dark cycle. Rat were given normal feed for 3 days after that T2DM model was made by providing high fat diet for 5 weeks. After 5 weeks T2DM model fasting for 1 night and injecting low dose streptozotocin (30mg/kg in 0.1 citrate buffer, pH 4.5) intraperitoneal. Injecting streptozotocin repeat after 1 week. After 1 week second inject, T2DM model was determined when fasting blood glucose was >200 mg/dl. Group were divided into control group, interval groups i.e slow interval training (SIT) and continuous groups i.e moderate continuous training (MCT). Exercise protocol was performed for 8 weeks, 3 times per week. T2DM model were execution under sedation (ketamine 30 mg i.p). TGF-β gene expression analysis by real-time PCR.

Procedure and ethical considerations

The study used experimental animals of T2DM models that were grouped into control groups and experimental groups. The grouping of study objects was carried out randomly, namely: the diabetic group that was not treated as a control group (K1), the diabetic group that was given moderate intensity continuous training (K2) treatment, and the diabetic group that was given a slow type interval training (K3) treatment. The subjects used as experimental animals were male rats of the wistar strain. All groups of rats were given a feed that had been determined to support obesity needs for 5 weeks. Then all existing groups were injected with STZ to be modeled as T2DM mice. The parameters taken during the study of experimental animals were body weight before and after being given high-fat feeding treatment, Blood sugar levels before and after STZ induction, Blood sugar levels after the formation of the T2DM model and after physical activity. This study is a purely experimental study using experimental animal models with a posttest with control group design to determine the effect of various physical exercises on the expression of the TGF-β in wistar rats modeled on T2DM after treatment. The research was approved by The Health Research Ethical Committee of Faculty of Medicine Universitas Sumatera Utara. The research was conducted by applying ethical standards for the use of experimental animals with the principle of 3R (Replacement, Reduction, Refinement) the treatment must be based on the principle of animal welfare, namely 5 Freedoms (5F); Freedom from hunger and thirst, Freedom from discomfort, Freedom from pain, injury or disease, Freedom from fear and distress, Freedom to express normal behaviour.

Statistical analysis

The data normality test was carried out with the Shapiro-Wilk test. To see the average difference of each

variable measured between groups will use the one way ANOVA test when the data is normally distributed and if it is not normally distributed, it will use the Kruskal-Wallis test. Test results with $p < 0.05$ are considered meaningful (significant).

4. RESULTS

Clinical characteristics

Mean blood sugar levels of male white rats of wistar strain before and after STZ induction can be seen in Table 1. Based on the Normality Test using the Shapiro-Wilk Test, the results of normal distribution data were obtained so that to determine the difference in the average blood sugar levels before and after STZ induction, a dependent t-test was carried out.

Based on Table 1 above, there was a change in the blood sugar levels of male white rats of the wistar strain before the induction of STZ (above 200 mg / dL showed that the study subjects had become T2DM model mice. With dependent t there was a significant difference in the average blood sugar level after induction in each group ($P = 0.001$).

The measurement results of the TGF-β gene during the administration of the 8-week exercise intervention can be seen in Table 2.

From Table 2 it is known that the average cycling threshold K3 is higher than cycling threshold K1 ($K3$ vs $K1$: 1.70 ± 0.06 vs 1.64 ± 0.05 ; $P = 0.016$). Thus it can be

| Group | Blood sugar before induction (g) Mean ± SD | Blood sugar after induction (g) Mean ± SD | P value |
|-------|--|---|---------|
| K1 | 111.00 ± 13.12 | 311.88 ± 27.38 | 0.001 |
| K2 | 100.44 ± 11.96 | 265.33 ± 15.67 | 0.001 |
| K3 | 101.11 ± 12.01 | 290.11 ± 12.33 | 0.001 |

Table 1. Average Blood Sugar Levels of experimental animals before and after induction of STZ. K1 = control group, K2 = continuous group, K3 = interval group.

| Group | Cycling Treshold Mean ± SD | P value |
|-------|----------------------------|---------|
| K1 | 1.64 ± 0.05 | 0.016 |
| K3 | 1.70 ± 0.06 | |

Table 2. Effect of moderate intensity continuous training on TGF-β gene expression. K1 = control group, K3 = interval group.

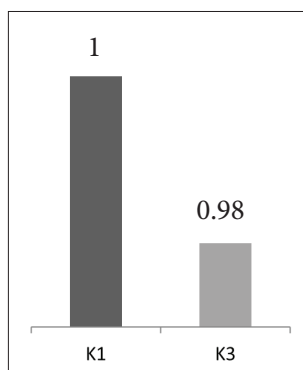


Figure 1. Decreased expression of TGF-β after continuous training. K1 = control group, K3 = interval group.

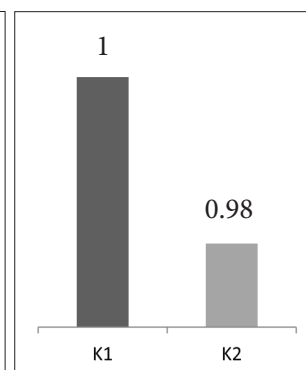


Figure 2. Decreased expression of TGF-β after interval training. K1 = control group, K2 = continuous group.

| Group | Cycling Treshold Mean ± SD | P value |
|-------|----------------------------|---------|
| K1 | -0.07 ± 0.01 | 0.061 |
| K2 | -0.04 ± 0.06 | |

Table 3. Effect of slow type interval training on TGF-β gene expression. K1 = control group, K2 = continuous group.

| Group | Cycling Treshold Mean ± SD | P value |
|-------|----------------------------|---------|
| K2 | -0.04 ± 0.06 | 0.965 |
| K3 | -0.05 ± 0.01 | |

Table 4. Effect of slow type intervals training with moderate intensity continuous training on TGF-β gene expression. K2 = continuous group, K3 = interval group.

said that the intervention of moderate intensity continuous training for 8 weeks can decrease the expression of the TGF-β gene. Using the livask formula, it was found that the decrease in TGF-β gene expression in the moderate intensity continuous training group was 0.98 times compared to the control group. Statistically, differences in TGF-β gene expression in the K3 and K1 groups are meaningful.

The measurement results of the TGF-β gene during the administration of the 8-week exercise intervention in interval training can be seen in Table 3.

From Tabel 3 it is known that the average cycling threshold K2 is higher than cycling threshold K1 (K2 vs K1: -0.04 ± 0.06 vs -0.07 ± 0.01; P = 0.061). Thus it can be said that the intervention of slow type interval training for 8 weeks can decrease the expression of the TGF-β gene. Using the livask formula, it was found that the decrease in TGF-β gene expression in the slow type interval training group was 0.98 times compared to the control group. However, statistically the difference in TGF-β gene expression in the K2 and K1 groups was not meaningful.

The measurement results of the TGF-β gene during the administration of the 8-week exercise intervention can be seen in Table 4.

From Table 4 it is known that the average cycling threshold K2 is higher than cycling threshold K3 (K3 vs K2: -0.05 ± 0.01 vs -0.04 ± 0.06; P=0.965). Thus it can be said that the intervention of moderate intensity continuous training for 8 weeks can decrease the expression of the TGF-β gene. Gene expression calculations showed a decrease in TGF-β gene expression in the group given continuous training compared to the group given interval training. However, statistically the difference in TGF-β gene expression in the K2 and K3 groups was not meaningful.

5. DISCUSSION

Diabetes Mellitus is a metabolic syndrome with an increase in T2DM cases characterized by an increasing prevalence of T2DM worldwide whose data has increased and is relatively large. For the Indonesian region, this is also the same, where T2DM is ranked 7th as a disease that has complications to the cardiovascular system and has the potential to cause death. This is what causes the need for many more in-depth studies from various studies of research aspects of the findings of treatment

from T2DM to prevent some complications that can arise and have the potential to cause death.

The characteristics of the subjects of the study observed were: gender, weight, age and blood sugar levels. Previous studies have stated that insulin resistance disorders can cause glucose intolerance so that pathophysiologically it can cause T2DM.

The characteristics of the research subject are important things to pay attention to in this study (Table 1). Statistical tests showed that by comparing blood sugar levels before and after STZ induction, it showed a significant difference (P<0.05).

This is also in line with this study which was designed using a male white rat trial of the wistar strain to make it easier to provide the intervention provided. Male white rats made in the T2DM model by giving inducator substances in the form of Streptozosin (STZ) were given several physical activity intervention treatments in accordance with several pillars of treatment management for T2DM sufferers, one of which was to provide physical activity. The type of physical activity intervention provided was in the form of interval training and continuous training compared to the control group. The effect of the intervention for the final result was the observation of the gene expression TGF-β.

The results showed that in the continuous group, the significant results were found on the decrease in TGF-β in the continuous group and statistically obtained results p < 0.05. Likewise, in the interval group, results of a decrease in TGF-β in the interval group and statistically obtained a p > 0.05.

In this study, there was a significant decrease in TGF-β after 8 weeks of moderate intensity continuous training in T2DM model wistar rats, this is in line with research conducted by Faramarz et al in 2020 with results that showed exercise increased protein levels from pro-angiogenic factors while lowering protein levels of anti-angiogenic factors, one of which was TGF-β in T2DM animal models. This change is followed by an increase in capillary density and a decrease in interstitial fibrosis in the left ventricle (13-15).

In this study, results were also found that moderate intensity continuous training was better at reducing TGF-β compared to slow type interval training. This is in line with research conducted by Faramarz et al in 2020 with results that showed moderate intensity continuous training was better at increasing angiogenic factors, one of which was TGF-β and lowering blood glucose and fibrosis in diabetic model rats. Moderate intensity continuous training provides a good increase in TGF-β in diabetic cardiomyopathy conditions (13, 16, 17).

T2DM can increase TGF-β gene expression, and regular physical exercise can decreased the expression, by controlling glucose metabolism (18-21).

Physical exercise improves metabolic health and is the first step for the management and prevention of T2DM. One of the important systemic advantages of physical exercise is that it becomes an idea for anti-inflammatory effects. Some of the anti-inflammatory effects on prolonged physical exercise are lowering fat tissue, but

there is also evidence to suggest physical exercise can directly affect systemic inflammatory cell and mediators. Interventional studies that have been carried out report that physical exercises can reduce the circulatory level of pro-inflammatory markers (21-23).

6. CONCLUSION

Based on the results of the experimental research that has been done, it can be concluded that there is an effect of moderate intensity continuous training and slow type interval training to gene expression of TGF- β in T2DM model wistar rats. Moderate intensity continuous training decreased expression of the TGF- β gene after 8 weeks of exercise in T2DM model wistar rats ($p = 0.016$). Slow type interval training decreased expression of the TGF- β gene after 8 weeks of exercise in wistar strain rats modeled T2DM ($p = 0.061$). Moderate intensity continuous training were better at decreasing TGF- β gene expression than slow type interval training in T2DM model wistar rats ($p = 0.965$).

- **Acknowledgments.** The authors would like to express their gratitude to Molecular Biology Integrated Laboratory Faculty of Medicine Universitas Sumatera Utara for providing the opportunity for us to conduct this experimental study.
- **Author's contribution:** The author gave substantial contribution to the conception, in design of in the acquisition, analysis and interpretation of data for the article. The author had role in drafting the article and revising it critically for important intellectual content. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
- **Conflicts of interest:** There are no conflicts of interest.
- **Financial support and sponsorship:** None.

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