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Effects of high-intensity interval training treadmill with changes in inclination on TGF-β2 serum level of overweight male

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Abstract---A third of adults in Indonesia were overweight. Overweight and abdominal obesity also have associations with musculoskeletal disorders, limited respiratory function, and decreased physical function and quality of life, so public health interventions are needed to overcome them. High-Intensity Interval Training (HIIT) has more efficient time and has similar effectiveness as endurance exercise. Transforming Growth Factor-β2 (TGF-β2) is secreted from adipose tissue in response to exercise and improves glucose tolerance. Sixteen overweight subjects who met the study criteria were randomly assigned to the intervention and control groups. The intervention group did HIIT using a treadmill with incline changes for 4 weeks. This research measures weight, body mass index (BMI), waist and hip ratio (WHR), body fat mass, and TGF-β2 serum levels pre- and post-intervention in both groups. There was significant decrease in body fat mass percentage (p=0,002) and WHR (p=0,003) but no significant

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decrease in weight (p=0.51) and BMI (p=0.526) and no significant increase in TGF-β2 serum levels (p=0.208). However, the control got a significant decrease in TGF-β2 serum levels (p=0.029). Although HIIT for 4 weeks did not increase the TGF-β2 serum levels, it is effective to prevent a significant decrease in TGF-β2 serum levels in overweight men.

***Keywords*---**HIIT, Treadmill, inclination changes, TGF-β2, Overweight Men.

Introduction

The rate of overweight continues to increase in both developed and developing countries. Based on the 2018 Basic Health Research (Riskesdas Indonesia) data, the epidemiology of overweight over the age of 18 years is around 13.6%, while the obesity rate in adults is around 21.8%. A third of adults in Indonesia were overweight in 2014. Between 1993 and 2014, the prevalence of overweight among adults doubled from 17.1% to 33.0%. WHO (2004) states that in the Asian population BMI is 23 kg/ m2 or more are classified as overweight individuals and have the risk of comorbidities including type 2 diabetes mellitus and cardiovascular disease. Overweight and abdominal obesity also have associations with musculoskeletal disorders, limited respiratory function, and decreased physical function and quality of life, so public health interventions are needed to overcome them. (Heydary et al., 2012; Speiser et al., 2007; Consultation, 2004; Oddo et al., 2019).

The recommended exercise for overweight individuals is long-term endurance training. Endurance training is an exercise with moderate intensity but with a longer duration. Long duration is a problem, especially regarding exercise compliance for overweight patients because on average, overweight individuals have a sedentary lifestyle and do not have sufficient time to exercise (da Silva et al, 2019; Gueugnon et al., 2012; Buchan et al., 2012).

Exercise with efficient use of time is needed to increase a person's compliance to do regular exercise. Currently being developed High-Intensity Interval Training (HIIT) which is a form of high-intensity physical exercise accompanied by low-intensity intervals that require more efficient time and is expected to be an effective form of physical exercise in reducing the risk of comorbidities (da Silva et al, 2019).).

One study showed that HIIT treadmill training was less boring than cycling. Subjects who did HIIT treadmill exercise with changes in inclination could walk slowly but could increase the intensity of exercise using the incline of the treadmill. A study showed that walking at a relatively slow pace at a moderate incline is a potential exercise strategy that can reduce the risk of musculoskeletal injury/pathological disease while providing appropriate cardiovascular stimulation in overweight adults (Kriel et al., 2018; Ehlen et al., 2011).).

Exercise promotes health and repair in a variety of organ systems and elucidating the mechanisms underlying the beneficial effects of exercise could lead to new therapies. In an in vitro and in vitro study in mice and in vitro in humans, it was shown that Transforming Growth Factor-β2 (TGF-β2) is secreted from adipose tissue in response to exercise and improves glucose tolerance. The study concluded that TGF-β2 is an exercise-induced adipokine in gene expression analysis of post-exercise human subcutaneous adipose tissue biopsies. Lactate, a metabolite released from muscle during exercise, stimulates human adipocyte TGF-β2 expression. Therefore, TGF-β2 stimulated by exercise not only improves glucose tolerance. In a study conducted on mice, TGF-β2 can treat obese mice by lowering blood lipid levels and improving many other aspects of metabolism. Research on the effect of exercise on cytokine levels has been carried out, including on IL-1, TNFα, IL-6, and adiponectin, while research on TGF-β2 is still rare.

So far, research on the effects of exercise-induced TGF-β2 serum was performed almost always in mice. While there is a lack of data in human research. Based on this, the purpose of this study is to test, determine and analyze the effect of increasing TGF-β2 serum after 4 weeks (short-term) of HIIT administration using a treadmill with changes in inclination in male subjects overweight (Takahashi et al., 2019).

Method

Study design

This study used a true experimental method with a pre and post-test randomized control group design, intending to compare TGF-β2 in overweight men after high-intensity interval training with changes in inclination.

Sampling method

Research takes place in the Gymnasium, Medical Rehabilitation Outpatient Clinic, Dr. Soetomo Hospital, and Laboratory of Research and Development of Clinical Pathology at Dr. Soetomo Hospital Surabaya. The sample size in this study was determined based on the sampling formula for hypothesis testing for a population mean (two side test) by Lwanga and Lemeshow. The number of research subjects for each group is a minimum of 8 people. So that the total number of the subjects in this study was at least 16 people.

Overweight patients aged 18–55 years who were in the medical rehabilitation installation at Dr. Soetomo Hospital Surabaya who met the inclusion criteria and was not included in the exclusion criteria were given information about the aims and objectives of the study. Patients were asked to sign an informed consent form if they are willing to become a research subject. The research dropout criteria were subjects are not willing to continue the research for any reason and subjects were unable to complete the exercise according to the established research protocol.

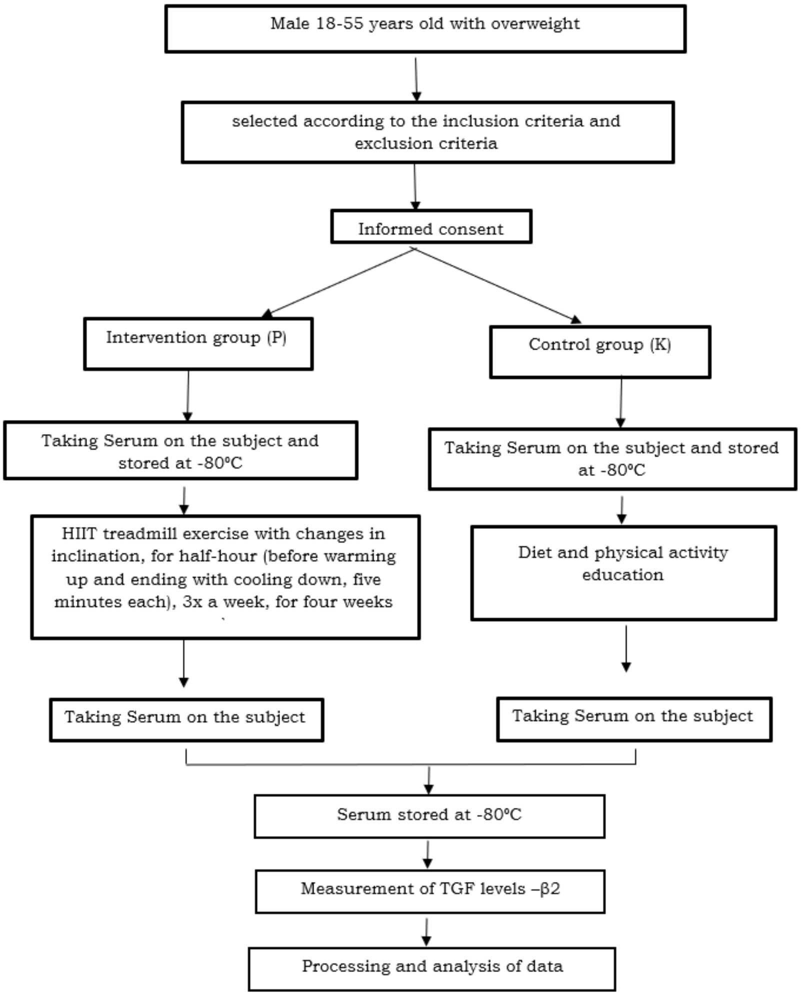

Figure 1. Research Flow

Intervention

In this study, treadmill exercise was performed with HIIT with a change in inclination. Before practice, there are a few things that need to be determined first. The first was a Target Heart Rate (THR) which is High-intensity THR = HR Rest + 80-90% HRR and Low-intensity THR = HR Rest + 30-40% HRR. The THR obtained is used to determine the inclination of the exercise to be used. The second was inclination. The inclination was determined by asking the patient to do a treadmill exercise at a speed of 2.0 mph and then increasing the inclination by 1% every 1 minute until the low-intensity and high-intensity THR were achieved. The low-intensity inclination is the inclination when a low-intensity THR is reached. The high-intensity inclination is the inclination when the high-intensity THR is reached.

After the THR and Inclination were determined, the treadmill program was adjusted using the Naughton method, each heating, and cooling for 5 minutes, intervals of 1 minute, and volume of 15 minutes (total exercise for 40 minutes).

Before starting the exercise, the subject was first given a Covid 19 scoring and then introduced the training protocol during the Covid 19 pandemic as Indonesia Health Ministry Protocol and also the protocol for giving training in the first meeting. Subjects did exercise according to a treadmill program that had been adjusted with a frequency of 3 times a week for 4 weeks.

Before doing the exercises and training tests, vital signs are checked using a sphygmomanometer (Reister®) for blood pressure, a pulse oximeter (Elitech® fox-2) for oxygen saturation and pulse, and explained about the Borg scale. Heart rate monitoring during training uses a polar H10 heart rate sensor that is attached to the subject's chest, connected via Bluetooth to an android device, and monitored using the polar beat application. Monitoring of heart conditions using an electrocardiogram before, during, and after a training test or exercise using 6 leads connected to a computer and monitored through a computer monitor screen.

Outcome measures

Anthropometric measurements were weight, BMI, Waist and Hip Ratio (WHR), and body fat mass percentage. Measurement of TGFβ-2 levels was carried out simultaneously. The examination method was carried out by a one-step process of double-antibody sandwich Enzyme-Linked Immunosorbent Assay (ELISA) to measure TGFβ-2 levels in samples (Bt-Laboratory, 2020).

Data analysis

The data were analyzed computerized with SPSS version 26.0. Shapiro-Wilk test to determine normality data. Levene's Test to determine the data's homogeneity. Paired t-test to compare serum TGF-β2 levels pre and post-intervention. Independent T-test to compare serum TGF-β2 levels in group P compared to group K. If the data are not normally distributed, the data are compared using the non-parametric Wilcoxon and Mann-Whitney u test. Ethical eligibility is submitted to the Ethics Commission for research and basic/clinical science at Dr. Soetomo Hospital Surabaya.

Results

The total subjects of this study were 16 people, divided into two groups (intervention and control) with 8 subjects in each group. All research subjects in the intervention and control groups can complete the study until the end of the study. Statistical tests were carried out at the beginning and end of the study.

Table 1
Characteristics and homogeneity of research subjects in both groups

Variable	Intervention n = 8 (Mean + SD)	Control n = 8 (Mean + SD)	Price p
Age (years)	31.13 + 2.42	35.25 + 2.71	0.680
Weight (Kg)	78.13 + 12.67	76.50 + 12.72	0.839
Height (cm)	166.38 + 5.45	168.00 + 7.19	0.731

BMI (kg/cm^2)	28.13 + 3.54	26.93 + 2.62	0.849
WHR			
% body fat mass	0.89 + 0.05	0.87 + 0.03	0.085
(%)	18.67 + 4.69	16.94 + 3.28	0.179
TGF-β2 basal	131.54 + 33.99	140.84 + 23.54	0.455
(ng/L)			

Note: the numbers for age, height, weight, BMI, WHR, and TGF-β2 are the average ± deviation; the p-value means if $p < 0.05$

Based on the results of the homogeneity test of age, weight, height, BMI, WHR, percentage of body fat mass, and TGF-β2. The baseline between the intervention group and the control group can be seen that the two groups of research subjects have the same value on each variable that will be used to measure the level of serum TGF-β2 in overweight men.

Table 2
Difference Test Results Pre and post in the intervention group

Condition	Mean + SD	Mean Difference	*P*
Weight (kg) pre[a]	78.13±12.67	1.63	0.51
Weight (kg) post[a]	76.50 + 12.72		
BMI (kg/cm2)pre[a]	28.13±3.54	1.2	0.526
BMI (kg/cm2)post[a]	26.93 + 2.62		
WHR pre[a]	0.89±.05	0.02	0.003*
WHR post[a]	0.87 + 0.03		
% body fat mass_pre[a]	18.67 + 4.69	1.73	0.002*
% body fat mass post[a]	16.94 + 3.28		
TGF-β2 (ng/L) pre[b]	131.54 + 33.99	18.3	0.208
TGF-β2 (ng/L) post[b]	113.21 + 34.77		

Note: [a]: Paired Data T-Test, [b]: Wilcoxon test, *Significantly different if $p<0.05$

Table 3
Test Results Differences in Serum TGF-β2 Levels in the Intervention Group After 30 Minutes Post-Exercise

	TGF-β2 (ng/L)basal	TGF-β230 minutes Post Workout	Mean Difference	*P*
Pre[a]	131.54 + 33.99	123.90 + 38.31	7.64	0.732
Post[b]	113.21 + 34.77	103.31 + 37.49	9.9	0.575

Note: [a]: Paired T-test, b: Wilcoxon. test *Significantly different if $p<0.05$

Table 4
TGF-β2 Difference Test Results Pre and Post Intervention

Group	Condition	Mean + SD	Mean Difference	*P*
Intervention[b]	Pre (ng/L)	131.54 ± 33.99	18.33	0.208
	Post (ng/L)	113.21±34.77		
Control[a]	Pre (ng/L)	140.84 + 23.54	30.26	0.029*

Group	Condition	Mean + SD	Mean Difference	*P*
	Post (ng/L)	110.58±28.59		

Note: [a]: Paired Data T-test, b: Wilcoxon test, *Significantly different if p<0.05

Level serum TGF-β2 in the control group pre and post had a p-value <0.05 (0.029), so there was a significant difference in serum TGF-β2 levels pre and post in the control group. Where the measurement results before (140.84) had a higher average serum TGF-β2 level than after (110.58) with a difference of 30.26. This indicates a significant decrease in serum TGF-β2 levels in someone who does not do HIIT exercise.

Table 5
Test Results Differences in Serum TGF-β2 Levels Between Intervention and Control Groups

Condition	Intervention	Mean + SD	Mean Difference	*P*
Pre[a] (ng/L)	Intervention	131.54 + 33.99	9.3	0.535
	Control	140.84 + 23.54		
Post[b] (ng/L)	Intervention	113.21±34.77	2.63	0.834
	Control	110.58±28.59		

Note: [a]: Unpaired Data T-test b: Mann Whitney test *Significantly different if p<0.05

Based on Table 5, it can be seen that serum TGF-β2 levels in the intervention and control groups before exercise had a p-value (0.535) > 0.05 so there was no significant difference in serum TGF-β2 levels before exercise in the intervention and control groups. This indicates that serum TGF-β2 levels before exercise in the intervention or control groups were the same. Serum TGF-β2 levels in the intervention and control groups after exercise has a p-value (0.834) > 0.05 so there is no significant difference in serum TGF-β2 levels after exercise in the intervention and control groups.

Discussion

The initial total number of research subjects was 16 subjects who were randomly divided into 2 groups, 8 subjects in the control group and 8 subjects intervention group who did an aerobic exercise program with a high-intensity interval treadmill (HR rest + 80-90% HR reserve) with changes in inclination during 30 minutes (before warming up and ending with cooling down, 5 minutes each), 3x a week, for 4 weeks.

In the anthropometric results, there were significant differences in WHR and body fat mass percentage pre and post-intervention (p-value < 0.05) while in weight and BMI there was no significant decrease (p-value > 0.05). The results of anthropometry and body fat mass in this study are in accordance with the meta-analysis by Wewege et al. (2017) who concluded that short-term high-intensity exercise can induce improvements in body composition in overweight and obese individuals without significant changes in body weight. The reason for this is the very high number of adrenaline receptors in the abdominal fat tissue. Simultaneously, the production of hormones such as epinephrine, norepinephrine, and dopamine promotes muscle development while fat is constantly being broken down. Short-term and extreme stress on muscle fibers leads to increased muscle development. This can explain why short-term high-intensity exercise can induce improvements in body composition without being accompanied by changes in body weight because a decrease in fat mass is followed by an increase in muscle mass (Shehata and Mahmoud, 2018).

Excess body weight results in progressive adipocyte enlargement, and reduced blood supply to adipose tissue, resulting in tissue hypoxia (Cinti et al., 2005). Hypoxia will lead to necrosis and infiltration of macrophages into adipose tissue that initiates overproduction of inflammatory factors such as inflammatory chemokines. Local inflammatory conditions in adipose tissue will initiate systemic inflammation associated with obesity-related morbidity (Trayhurn et al., 2004).

In overweight subjects, adipocytes and macrophages are the main sources of increased synthesis and secretion of pro-inflammatory cytokines (IL-1, TNFα, IL-6) (Wang and He, 2018). In adipose tissue, obesity mainly releases pro-inflammatory cytokines including TNF-, IL-6, leptin, visfatin, resistin, angiotensin II, and plasminogen activator inhibitor 1 (Makki et al., 2013).

TNF- is a potent proinflammatory cytokine, mainly secreted from myeloid cells via activation of the MAPK and NFkB signaling pathways, resulting in the release of other inflammatory cytokines, such as IL-1β and IL-6. It was the first WAT (white adipose tissue)-derived inflammatory cytokine reported to be involved in the initiation and development of insulin resistance (Makki, 2013).

Most adipokines are pro-inflammatory, whereas small amounts of anti-inflammatory adipokines, including adiponectin, are beneficial in reducing obesity complications. Adipokine dysregulation seen in obesity is associated with the pathogenesis of various disease processes. Plasma adiponectin levels are negatively associated with the accumulation of body fat, especially visceral fat, and low plasma adiponectin levels in obese individuals. Clinical studies show a close relationship between low adiponectin levels and many obesity-related disorders (Ohashi et al., 2014).

TGF-β2 is an exercise-induced adipokine that can improve glucose tolerance and insulin sensitivity, increase fatty acid absorption and oxidation, and stimulate glucose uptake in skeletal muscle, heart, and Brown adipose tissue (BAT). TGF-β2 is an adipokine that also has anti-inflammatory effects and plays a role in suppressing the inflammatory response of macrophages. In a study on mice, the role of TGF-β2 in macrophage infiltration was mentioned. These findings suggest that TGF-β2 can alter macrophage polarization and attenuate HFD-induced inflammation in adipose tissue (Takahashi et al., 2019).

TGF-β2 levels in addition to being influenced by high levels of lactate are also influenced by the presence of wounds. This is because TGF has an important role in the wound healing process as one of the factors driving the formation of fibroblasts during wound healing. TGF-β also acts as a potent inhibitor of cell proliferation, playing an important role in tumor suppression (Pakyari et al., 2013; Kubiczkova et al., 2012)

High-intensity interval training (HIIT) can be highly lactate-requiring and may result in the production of high blood lactate levels. Previous studies have shown that lactate is a strong metabolic stimulus, which is important for adaptation (Wiewelhove et al., 2018).

Running at a moderate intensity (treadmill speed 21 m/min) increased blood lactate and activated the concentration of transforming growth factor-β (TGF-β) in rat cerebrospinal fluid (CSF) (Yamada et al., 2012). Lactate released from fibroblastic foci can trigger extracellular activation of latent TGF-, which, by binding to the TGF-β2 receptor, causes activation of hypoxia-inducible factor (HIF)-1α and increased expression of LDH-5 (Tuder et al., 2012).

Exercise can increase the expression of messenger RNA TGF-ß in human subcutaneous white adipose tissue (scWAT). TGF-β2 is an exercise-induced adipokine that can improve glucose tolerance and insulin sensitivity, increase fatty acid absorption and oxidation, and stimulate glucose uptake in skeletal muscle, heart, and Brown adipose tissue (BAT). Treatment with recombinant TGF-β2 in vivo improved the effects of a high-fat diet in mice, decreased fat mass, and decreased inflammation in WAT (White Adipose Tissue). The mechanism by which exercise increases TGF-β2 in scWAT involves lactate stimulation in the gene expression phase.

In this study, the levels of TGF-β2 basal level in the intervention group after 30 minutes of exercise (acute effect) decreased but was not significant, as well as a decrease in levels of basal TGF-β2 intervention group after 4 weeks of exercise but not significant. This is not in accordance with the research of Yazdani et al. (2020) which showed a significant increase in TGF-β2 protein expression in HIIT-treated diabetic rats. However, in this study, mice were given a control diet and performed HIIT for 8 weeks. Existence differences in intensity, exercise duration, sampling time, and metabolic state of the subjects were likely the cause of the different results.

A study by Cao et al., 2011 concluded that the effect of diet on TGF-β2. Diets high in pectin fiber in foods can increase TGF-β2 levels. This study has limitations in controlling the subject's diet which can ultimately affect the results of the study. Another study by Yu and Welge (2013) stated that TGF-β2 stimulation can be reduced by giving antioxidants, including vitamin E, vitamin C, and vitamin B1. This research was conducted during the Covid-19 pandemic, so it is possible for most of the samples to take anti-oxidant supplements.

In a study conducted by Timmons et al. (2005) 24 sedentary people concluded that TGF-β2 and TGF-β Receptor2 were substantially increased in the group that had a high response to exercise (VO2 max increased significantly after exercise). These observations suggest that the response of ECM (extracellular matrix)-related genes in the high response group to exercise is due to greater tissue remodeling, which will contribute to a greater increase in aerobic capacity. The exercise given in this study was 4 times per week (each for 45 minutes) with an intensity of 75% VO2 peak using cycle ergo for 6 weeks and TGF-β2 was measured using a vastus lateralis muscle biopsy taken 24 hours after exercise.

TGF-β binds to a carrier protein, 2-macroglobulin, non-covalently as a latent form in the bloodstream. The active (free) form and total serum TGF- levels (free + latent) were 2.1 ± 0.58 ng/ml and 136 ± 5.59 ng/ml, respectively, indicating that the levels of the latent form of TGF-β were approximately 65 times higher than the active form in the bloodstream (Khan et al., 2016).

In this study, the measurement using the ELISA method, TGF-β2 as measured in serum is total TGF-β2, while TGF-β2 which plays a role in anti-inflammatory activity is active, so further research is needed to assess post-exercise active TGF-2 or use samples in target organs such as muscles. Based on this, it can be concluded that examination of the target organ will produce a more representative TGF-β2 value, however, this is difficult to do because it gives discomfort to the subject, side effects of larger sampling intervention, and more costs.

In a study conducted by Takahashi et al. (2019) mentioned that serum concentration of TGF-β2 in healthy subjects before (Pre) and after (Post) 12 weeks of moderate-intensity exercise; n=9 showed no significant increase in serum TGF-β2. This study clearly demonstrated that exercise significantly increased TGF-2 in human scWAT (subcutaneous adipose tissue), and lactate increased TGF-β2 in human adipocytes. However, in the same study, the effect of exercise on serum TGF-β2 concentrations was more variable. The study concluded that there were differences in intensity, exercise duration, sampling time, and the metabolic state of the subjects as the cause of the variation in results (Takahashi et al., 2019).

This study uses an intervention duration of 4 weeks of intervention based on previous studies stating that giving HIIT for 4 weeks can induce improvement in VO2 max and can improve body composition by decreasing fat mass. Where on the research of Timmons et al. (2005) stated that TGF-β2 and TGF-β Receptor2 were substantially increased in the group that had a high response to VO2 max production. However, in a study conducted by Alkahtani et al., 2013 regarding the effect of HIIT for 4 weeks on blood lactate levels in obese men, it was concluded that lactate levels increased in the first week of HIIT but decreased after 4 weeks of intervention. Serum lactate levels are an important component that increases serum TGF-β2. In this study, the absence of a significant increase in TGF-β2 could be due to decreased lactate levels after the 4th week of exercise.

The results of this study showed that there was no increase in the levels of TGF-β2 in the intervention group after being given High-Intensity Interval Training (HIIT) for 4 weeks. This is in accordance with the research of Zebrowska et al (2019) which stated that there was no significant increase in TGF-β baseline in healthy male subjects with normoxia after 3 weeks of HIIT exercise. This study showed a significant increase in TGF-β2 in hypoxic subjects (exercise in a hypoxic chamber). This indicates that hypoxic conditions affect serum TGF-β2 levels (Zebrowska et al., 2019).

The results of this study differ from the study of Takahashi et al (2019) which showed that exercise increased TGF-β2 in scWAT. However, in this study, the subjects used were healthy young men (BMI 20-25) who were given HIIT training for 12 weeks (Takahashi et al., 2019). Differences in the condition of the subjects and the duration of the intervention may be factors that influence the absence of changes in TGF-β2 levels in this study.

In the control group, there was a significant decrease in TGF-β2. Levels at the start of the study compared after 4 weeks. This shows a dysregulation effect of TGF-β2 in untreated control subjects. Based on this result we can get information that even though HIIT doesn't effective in increasing TGF-β2 serum levels but it is effective to prevent a significant decrease in TGF-β2 serum levels in overweight men.

Several studies showed that there was an increase in TGF-β2 after the subjects were given HIIT intervention, but other studies found no difference. Both are important informative results, inconsistencies of this kind provide valuable insights. Several biases can be the cause of the varying results in several studies. In this study, we identified several biases, such as age, gender, hormonal, tumor, and wound healing. We have anticipated this bias by excluding and conducting homogeneity tests on subjects. However, the weakness in this study was that it was not possible to anticipate the subject's diet which could affect the results of the study.

Additionally, this study has several limitations, including did not use double blinds so that the trainer and the subject knew the intervention they received and bias could occur. the intervention group is known to the research subject so that it can affect the subject's motivation when doing the exercise program. Researchers find it difficult to monitor daily physical activity and diet which can affect research results.

Conclusion

HIIT is not effective to increase TGF-β2 serum levels in overweight men. However, the HIIT treadmill with a change in inclination for 4 weeks is effective to prevent a significant decrease in TGF-β2 serum levels in overweight men. Further research is needed on HIIT with a comparison of moderate-intensity continuous exercise. It is necessary to do further research on HIIT with changes in inclination with a larger number of samples, a longer time, and examination of levels of TGF-β2on subcutaneous adipose tissue or muscle biopsy. High-intensity interval treadmill exercise with changes in inclination for 4 weeks cannot be used as an exercise therapy option to improve body composition and decrease body fat mass, but to increase levels of TGF-β2in male patients with overweight need further examination.

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Conflict of Interest
The authors declared no conflict of interest.

Author Contribution
All authors equally contributed to preparing this article.

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