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The difference of VO_{2max} and immune profile (Hmbgl, cortisol, Il-6, Tnf alpha, number of leukocytes, neutrophils and monocytes) in adolescents who were trained and untrained in basketball

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ABSTRACT

This study was conducted to see differences in VO_{2max} values and differences in levels of HMGB1, cortisol, IL6, TNF alpha, and the number of leukocytes, neutrophils and monocytes after moderate intensity aerobic exercise (70-79% MHR) for 12 minutes. The study used a cross-sectional study method and purposive sampling method on 15 basketball-trained students and 15 basketball untrained students at SMAN 1 Banjarbaru with an average age of seventeen years. Measurement of VO_{2max} value was done on the first day with MFT (Multistage Fitness Test / Bleep Test). The levels of HMGB1, cortisol, IL6, TNF alpha, the number of leukocytes, neutrophils, and monocytes were measured on the third day by taking blood after students did aerobic exercise with moderate intensity running for twelve minutes. Data were analysed using the Mann Whitney test for VO_{2max}, cortisol, IL6, TNF Alfa, and leukocyte and monocyte counts and unpaired t test for HMGB1 and neutrophils. The results indicate that the average VO_{2max}, HMGB1, IL6, and cortisol scores of basketballs trained students are higher than those who were not trained. In contrast, the TNF alpha, leukocytes, neutrophils and untrained monocytes are higher in levels and numbers. There are significant differences in trained students basketball and untrained (p < .05) on VO_{2max}, cortisol, and TNF alpha. Not There was a significant difference (p > .05) after intensity aerobic exercise being twelve minutes on students trained in basketball and not trained for HMGB1, IL6, leukocyte count, neutrophils, and monocytes. This study indicates that basketball-trained students have better VO_{2max} scores than untrained basketball students. Cortisol levels were higher in basketball-trained students compared to basketball-untrained students in 12 minutes of moderate-intensity aerobic exercise, while TNFalpha levels were the opposite. 12 minutes of aerobic exercise as a method of choice to increase the body's immune system and show students the homeostatic process is going well.
Keywords: 12 minutes running aerobic exercise; Immune profile; Basketball trained.

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INTRODUCTION

WHO stated that in 2019 the majority of people around the world are not physically active enough to endanger future health so WHO recommends that physical exercise, especially moderate intensity, is done for 150 minutes a week in someone aged 18-64 years (Bennie et. al., 2019). Regular physical exercise is believed to be able to reduce the risk of developing various diseases and increase life expectancy (Sand et. al., 2013; Singh et. al., 2019; Kokkinos et. al., 2012; McKinney et. al., 2016; Mallo, 2019; and Nieman., 2012). One form of physical exercise is a game of basketball. although not as popular as football, basketball is popular with Indonesians (Piepoli et. al., 2016; Singh Chahar, 2014). Basketball uses alternating aerobic-anaerobic metabolism, although anaerobic metabolism is more dominant (Gomes et. al., 2014).

Aerobic exercise is an activity that utilizes the contraction of large muscle groups continuously and regularly over a period of time and utilizes aerobic metabolism as a source of energy. One of the components of aerobic exercise is intensity (Plowman and Smith, 2014). Moderate intensity aerobic exercise, an activity that consumes energy comparable to brisk walking, is the aerobic exercise most frequently studied (Plowman et. al., 2014; WHO, 2010; Patel et. al., 2017). Physical exercise has a relationship to a person's ability to do activities through its effect on VO_{2max} (Habibi et. al., 2014). 12-minute running has been used as one way to measure a person's VO_{2max} value (Bandyopadhyay 2015; Penry et. al., 2011; Das, 2013). In addition, exercise is a stressor that stimulates the neuroendocrine system and immune response (Plowman and Smith, 2014). The correlation between the immune system and exercise was first studied in David Nieman's study which showed a lack of reports of ARI complaints in individuals who routinely do moderate intensity physical exercise (Nieman, 2012; Simpson et. al., 2012). Physical exercise drastically affects the number of leukocytes circulating in the peripheral blood. Leucocytosis that accompanies acute exercise is a transient phenomenon because the number and composition of leukocytes usually returns to normal values within 6-24 hours after an exercise session. Leucocytosis that occurs in physical exercise is mostly caused by the activation of neutrophils and lymphocytes, as well as a small percentage of monocytes (Gleeson et. al., 2013). Physical exercise activates the HPA response, which causes the hypothalamus to release corticotropin releasing hormone (CRH) to cells in the anterior pituitary, which stimulates the release of adrenocorticotropic hormone (ACTH) and stimulates the adrenal cortex to release glucocorticoids (cortisol) into the bloodstream (Habibi et. al., 2014).

METHOD

This research is cross sectional with the population in it is all adolescents of Senior High School 1 (SMAN 1) Banjarbaru. The sample in this study was divided into two, namely the sample taken from 15 basketball players and 15 non-basketball players at the State Senior High School I (SMAN I) in Banjarbaru. The sample is cooperative and has filled in the consent form to become the research subject. Purposive sampling method was used to take samples according to the inclusion criteria, namely (a) Male, (b) Age 15-18 Years, (c) Physically Healthy, meaning that at the time of the study the probands was not sick or infected and had no history of heart or lung disease. and allergies, (d) Cooperative, research subjects can be invited to collaborate to carry out research procedures, (e) Do not smoke, (f) Do not take drugs that affect the number of leukocytes, monocytes, and neutrophils at least 2 days before blood collection, (g) Have a normal body mass index (BMI) (20-25). The sample of teenage basketball players is students of SMAN 1 Banjarbaru who are members of a basketball sports club and routinely do basketball practice at least three times a week for one hour per training session for a year. The study will be stopped on subjects who experience signs of fatigue during exercise so that they are unable to complete the exercise.

The research was conducted for 3 days. On the first day, VO_{2max} measurements were carried out using the MFT (Multistage fitness test). Subjects rested on the second day. Blood sampling for analysis of the number of leukocytes, monocytes, neutrophils, and cortisol levels were checked on the third day. Blood samples were taken as much as 5 cc in the brachial vein after twelve minutes of moderate intensity aerobic exercise. Before exercise, the research subject will be calculated its maximum pulse rate (MHR) using the Tanaka formula. After knowing the MHR, the research subjects will use pulse oximetry and warm up in the form of running in groups consisting of 3 people per group until they reach the target of 70 -79% MHR. After reaching the target of 70 -79% MHR, the subject will continue to run for twelve minutes at the same rhythm. Blood sampling and analysis of the subjects were carried out by trained personnel from the Prodia laboratory. Trained health workers always accompany training sessions to prevent life-threatening things from occurring. The Mann Whitney test was used for VO_{2max}, cortisol, IL6, TNF Alfa, leukocyte count, and monocyte counts. Unpaired t test for HMGB1 and Neutrophil.

RESULTS

Table 1. Characteristics of research subjects.

Characteristic (Mean ± SD)	Basket group (Trained Basketball Student n=15)	Non Basket Group (Untrained Basketball Student n=15)
Age (Years)	16.93±0.258	17.07±0.495
SO2 (%)	97.27±2.576	96.93± 3.731
Pulse per minute	84.20±11.384	92.93±12.981
BMI (kg / m2)	21.65±2.104	21.68±5.911
Height (cm)	167.37±8.067	172.00±5.305
Weight (kg)	61.13±10.034	64.33±18.289
Systolic blood pressure (mmHg)	127.53±14.282	132.20±13.078
Diastolic blood pressure (mmHg)	78.73±7,601	82.87±9.797

Table 2. Research data.

Marker	Trained Basketball (n = 15)		Untrained Basketball (n = 15)	
	Mean	SD	Mean	SD
VO_2 max	37.49	26,1	28,85	18,8
HMGB1	2180,6	166.4	2168,9	295.8
Cortisol	1440,1	1863.1	26,3	16.1
IL6	58,50	26,1	52,75	18,8
TNF Alpha	532,0	667,2	963,7	1144,8
Leucocytes	7,99	2,04	8,90	2,79
Neutrophyls	47,40	9.03	49,40	9.70
Monocytes	6,88	1,56	7,12	3.51

Sample characteristics were based on age, oxygen saturation, body mass index, pulse, height, weight, systolic and diastolic blood pressure. The mean sample was 17 years old and the mean value of pulse rate, body weight, body mass index, systolic and diastolic blood pressure of basketball-trained students were lower than those of non-basketball trained students. Pulse, body mass index, oxygen saturation, systolic and diastolic blood pressure were within normal limits. The table above shows that students who are trained in basketball are fitter than those who are not trained in basketball.

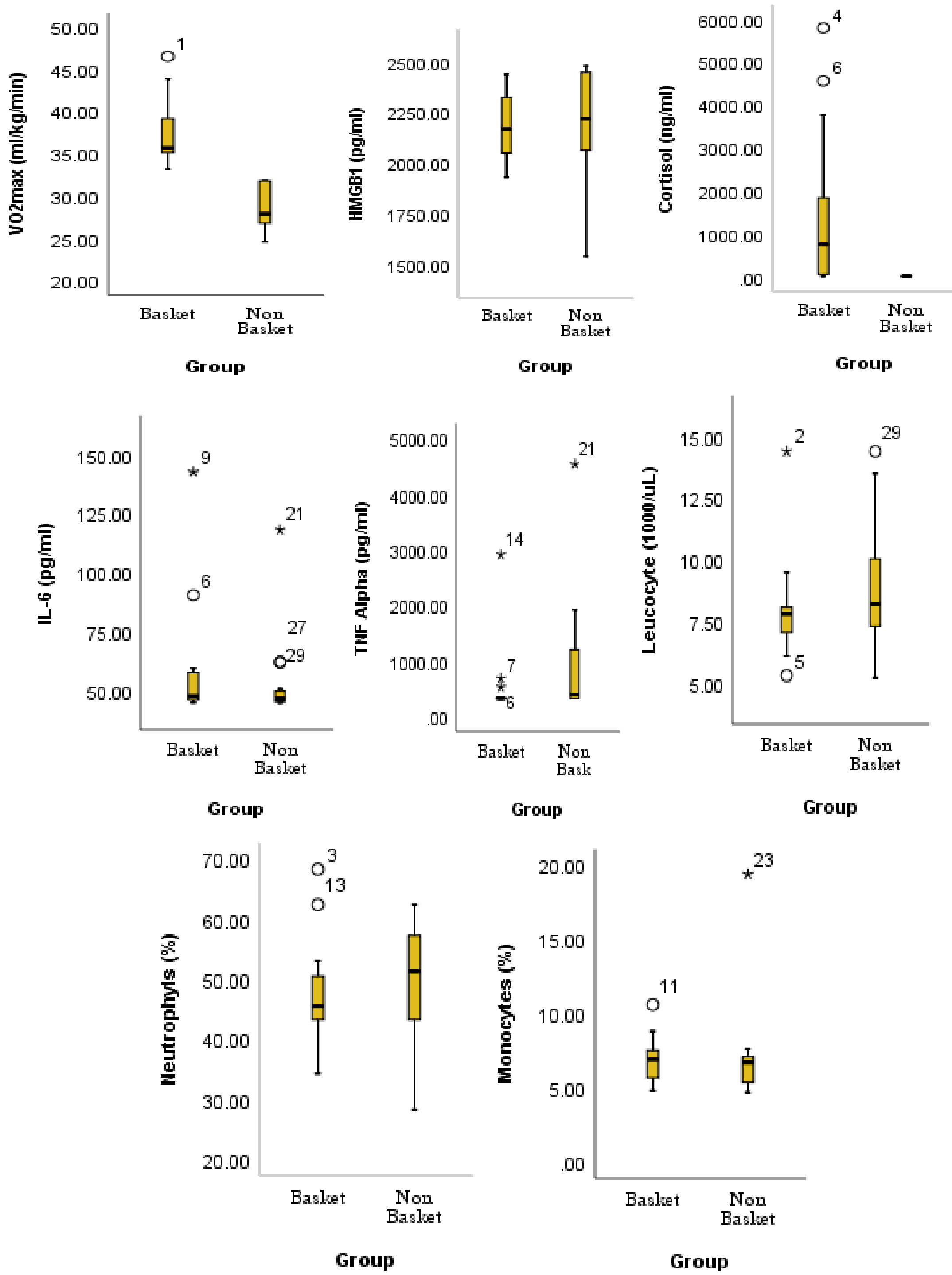

Figure 1. Boxplot graph of VO_{2max}, HMGB1, Cortisol, IL6, TNF Alpha, Leukocyte, Neutrophil, and Monocyte values.

The mean VO_{2max}, HMGB1, IL6, and cortisol scores of basketball-trained students were higher than untrained. In contrast, the value of TNF alpha, leukocytes, neutrophils and monocytes who were not trained was higher than that of the untrained.

VO_{2max}

The average VO_{2max} of students trained in basketball was 37.49 ± 26.1 ml / kg / minute higher than that of untrained basketball which was 28.85 ± 18.8 ml / kg / minute. With the Mann Whitney test, the VO_{2max} value obtained statistically significant differences in the VO_{2max} value after 12 minutes of moderate intensity running training for basketball trained and untrained students $p = .000$ ($p < .05$).

HMGB1 concentration

Mean HMGB1 concentration of basketball trained students 2180.6 ± 166.4 pg / ml was higher than the untrained 2168.9 ± 295.7 pg / ml. There was a significant difference in HMGB1 levels after 12 minutes of moderate intensity exercise in students who were trained in basketball and untrained with basketball $p = .895$ ($p > .05$).

Cortisol concentration

Mean cortisol concentration of basketball-trained students was greater than that of untrained basketball students. The mean blood cortisol concentration of basketball trained students was 1440.1 ng / ml higher than untrained, which was 26.3 ng / ml. In this study, it was found statistically significant results with the Mann Whitney test and there was a significant difference in cortisol levels after twelve minutes of moderate intensity exercise for basketball trained and untrained students $p = .000$ ($p < .05$).

IL-6 concentration

The average IL-6 basketball trained students (58.50 pg / ml) was greater than that of unbasketball students (52.75 pg / ml). With the Mann Whitney test, there was no significant difference between the two $p = .395$ ($p > .05$).

TNF Alpha concentration

The average value of the TNF-Alfa concentration of basketball players was lower than that of non-basketball students. The mean value of TNF Alpha concentration for basketball trained students was 532 pg / ml and untrained 963.7 pg / ml. The Mann Whitney test showed a significant difference between the two or found statistically significant differences in TNF alpha levels after 12 minutes of moderate intensity running exercise for basketball trained and untrained students $p = .000$ ($p < .05$).

Number of leukocytes, neutrophils, and monocytes

The mean number of basketball trained leukocytes was 7.99 ± 2.04 (10^3 / µL) and untrained basketball 8.9 ± 2.79 (10^3 / µL); basketball trained neutrophils 47.4 ± 9.03 percent and untrained basketball 49.7 ± 9.70 percent; basketball-trained monocytes 6.88 ± 1.56 percent and untrained basketball 7.12 ± 3.51 percent.

In this study, it was found that the results were not statistically significant on the difference in the number of leucocytes ($p = .35$) and monocytes ($p = 0564$) with the Mann Whitney and neutrophil test ($p = .454$) with unpaired t test after twelve of moderate intensity exercise students are basketball trained and untrained ($p > .05$).

DISCUSSION

Statistical tests in this study showed significant differences in VO_{2max}, cortisol and TNF alpha levels between basketball-trained and untrained students. While other aspects, namely HMGB1, IL6, leukocytes, monocytes, and neutrophils, there were no statistically significant differences between basketball-trained and untrained basketball students.

Physical activity results in changes in physiological functions of the body, both temporary changes (responses), and changes that are permanent (Mulyono, 2016). This also occurs in people who do exercise or exercise, a physiological response occurs so that the body's homeostasis is well maintained. The increase in workload due to exercise will cause an increase in the workload of the organ systems, for example in contracting skeletal muscles an increase in glucose uptake from the blood, an increase in body metabolism, one of which is heat dissipation as a compensation characterized by sweating, an increase in the amount of energy needed, and the cardiovascular system occurs. increased heart rate due to pumping blood for more supply to all organs (Giriwijoyo, 2007). The occurrence of a physiological response in the musculoskeletal system as an adaptation to physical exercise that is carried out occurs by involving many molecular pathways that play a role in regulating muscle contraction and the biosynthetic pathway for ATP formation, such as mitogen activated protein kinases (MAPKs), AMP activated protein kinase (AMPK), Sirtuins (SIRTs), and oxygen sensors prolyl hydroxylases (PHDs) (Camera et. al., 2016; Fan and Evan, 2017; Kjøbsted et. al., 2018). All these changes require a good balance so that the work of the organs and the condition of the body is not disturbed, that is what is called homeostasis. Thus, in athletes, if the homeostatic response fails, it will reduce their physical performance and performance (Giriwijoyo and Sidik, 2012; Ashadi, 2014).

VO_{2Max}

The VO_{2max} value of basketball trained adolescents was higher than that of untrained basketball adolescents. This is in line with the research of Buchan et al. In this study, it was found that physical exercise three times a week for seven weeks with moderate intensity can increase the VO_{2Max} value compared to those who do not do physical exercise in adolescents (p = .000). The study explains that this is probably due to the effect of moderate intensity physical exercise on cardiac output, where physical exercise causes an increase in cardiac output due to an increase in stroke volume (Buchan et. al., 2011).

Macpherson et. al. research, 2011 explains two factors that cause an increase in VO_{2max}, namely central and peripheral factors. Centrally, the increase in VO_{2max} was due to an increase in stroke volume and a slight increase in the maximal heart rate. Peripherally, the increase in VO_{2max} occurs due to increased arterial-venous oxygen differences that are influenced by oxygen transport to active muscle fibres, local enzyme adaptation, and mitochondrial density. Peripheral increase in VO_{2max} occurs in sprint interval training (Lee et. al., 2014).

HMGB1

The results of this study showed that there was no significant difference in HMGB1 levels after doing physical exercise in the form of running 12 minutes between trained basketball and not.

Research by Goh et. al. in 7 healthy young men with a 3-week high-intensity training program and simultaneously aerobic and resistance training performed for 3 consecutive days each week. HMGB1 plasma increased from Pre to Post (p < .05). Monocytes increased from Pre to Post in 3 weeks; HMGB1 is positively correlated with monocytes, indicating the higher release of alarmin after strenuous exercise may involve an increase in circulating monocytes (Goh et. al., 2020).

The human body has endogenous danger signals to prevent secondary inflammatory responses due to the release of intracellular inflammatory factors into the extracellular area, namely damage-associated molecular patterns (DAMP). High mobility group box 1 (HMGB1) is a protein from DAMP which is a sign of muscle cell damage and causes immune cell mobilization to the trauma site. (Fan and Evans, 2017). HMGB1 levels will return to their original concentration after 30 minutes of rest after exercise (Huldani, 2016).

Overall, DAMP triggers the release of massive cytokines including TNF-α, IL-1, IL-6, IL-8, IL-12 and IFN types I and II. These mediators amplify the activation, maturation, proliferation, and recruitment of immune cells at the trauma site, causing indirect activation of innate and adaptive immune cells such as DC or T cells (Mickael et. al., 2018).

HMGB1 exposure to human monocyte cultures will stimulate several pro-inflammatory cytokines such as tumour necrosis factor (TNF), interleukin (IL) -1, IL-6, IL-8 and macrophage inflammatory protein (MIP) -1. The kinetic responses to the LPS-mediated release of HMGB1 and TNF were very different. HMGB1-stimulated TNF release was biphasic with a delayed second wave, whereas LPS-mediated TNF release only occurred in the initial monophasic mode (Huan et. al., 2015).

Cortisol

The results of this study indicated that there was a significant difference in cortisol levels after doing physical exercise in the form of running 12 minutes between trained basketball and not (p = .000). The basketball-trained cortisol value (1440.1 ng / uL) was higher than the untrained (26.3 ng / uL) this was probably the homeostatic process not due to the stressor. And the average value of HMGB1 is also bigger. So that cortisol here functions as an anti-inflammatory and suppresses proinflammatory cell cytokines such as IL6, TNF Alpha, Leukocytes, Neutrophils and Monocytes. In contrast, the value of cortisol not trained basketball is lower so that the anti-inflammatory function is not strong enough to suppress the inflammatory reaction as indicated by a greater increase in the concentration of TNF alpha.

Sports and physical activity can be a source of stress for the body and have an impact on other body systems and have the potential to cause homeostatic disorders (Mastorakos et. al., 2005).

Previous research has consistently shown that exercise with an intensity of more than 60% VO_{2max} stimulates the release of higher cortisol concentrations in adults. Studies show that every teenager has the same body response to an increase in the HPA axis reaction and an increase in the cortisol response after exercise. In adolescents aged 15 to 16 years who exercise for 12 minutes with an intensity of 70-85% of the maximum pulse, there will be a striking increase in cortisol levels compared to the group that only does moderate intensity exercise (50-65% maximum pulse rate) (Budde et. al., 2015).

Exercise with VO_{2max} intensity of 60% (pre intervention 12.3 ± 4.1 and post intervention 20.1 ± 6.0) and 80% (pre intervention 12.9 ± 6.3 and post intervention) resulted in an increase in cortisol levels that was significantly greater than sessions with 40% intensity of exercise (Pre intervention 12.2 ± 4.3 and post intervention 10.8 ± 5.4). This means that cortisol levels in plasma increase in moderate and high intensity exercise. Conversely, exercise with light intensity did not show a significant increase in cortisol levels but decreased (Hill et. al., 2008).

Keyan et al. study of 62 healthy participants, 31 of whom did 10 minutes of intense exercise and the remaining 31 people did a leisurely walk. The cortisol levels in saliva were examined, the result was a significant increase in cortisol levels after intense exercise compared to before exercise. (Zhang, et. al., 2019).

The cortisol concentration will continue to increase with increasing exercise intensity and duration (Duclos and Tabarin, 2016). Different exercises have different effects on the hormonal system, a greater response is shown in strength training. Cortisol levels will increase according to the level of stimulation given. High intensity exercise will cause increased activity of stress hormones such as cortisol, ACTH, and catecholamines, which inhibit protein synthesis and trigger protein degradation leading to breakdown of skeletal muscle protein (Corazza et. al., 2014).

The level of cortisol secretion is influenced by the circadian cycle. Serum cortisol secretion begins to increase at midnight, peaking in the morning. Apart from that, it is possible that this increase is due to other factors that can increase cortisol secretion, namely psychological stress which also triggers the release of cortisol (Haslinda et. al., 2017).

Increased cortisol levels after acute physical exercise have been shown in several studies (Sato et. al., 2016; Muscella et. al., 2019) Increased cortisol levels are strongly influenced by the intensity of physical exercise and the subject's physical exercise habits. Hackney et. al. research showed that cortisol levels increased with increasing training load given to subjects aged 16-21 years (Hackney et. al., 2011). In addition, the study of Sato et. al., showed differences in the increase in cortisol levels in athletes and non-athletes according to the intensity of the physical exercise given. Increased levels of cortisol provide an anti-inflammatory effect by emphasizing the expression of pro-inflammatory cytokines, thereby inhibiting the increase in the number of leukocytes (Coutinho and Chapman, 2011; Ince et. al., 2019).

The release of cortisol is a form of body adaptation to stress due to exercise, this can be seen from the cortisol levels in blood plasma, as found in the results of this study. Cortisol causes an increase in fat content in the blood due to increased amino acids and fat mobilization, which, if excessive, can interfere with tissue structure and function, and even cell death (Tkacova et. al., 2012).

IL6

The results of this study showed that there was no significant difference in IL-6 levels after doing physical exercise in the form of running for 12 minutes. The study by Mallard et al. Showed the same result. Their group of research subjects who received physical exercise intervention did not show any significant changes in levels of IL-6, TNF alpha, and IL-10 (Mallard et. al., 2017). It is said that exercise is indeed associated with a decrease in levels of pro-inflammatory factors including TNF and an increase in anti-inflammatory factors including interleukins, but this happens if physical exercise is carried out regularly and regularly (de Lemos et. al., 2012). In addition, exercise also has the ability to maintain levels of inflammatory biomarkers through the body's physiological repair response including homeostatic mechanisms (Hollekim et. al., 2014).

In contrast to the research by Kinoshita et. al on 5 athletes with spinal cord injuries who play basketball in wheelchairs following basketball game activities. Blood samples are taken approximately 1 hour before the players warm up and immediately after finishing the game. The results showed that IL6 and plasma monocytes were significantly increased after play compared to before heating. There is a significant correlation between the increased level of IL-6 and the accumulated time playing basketball (Kinoshita et. al., 2013).

Exercise with high intensity and a competitive nature is one of the causes of stress (stressor). But continuous exercise with the right dose will reduce the secretion of HPA Axis, low hypercortisol, activate proinflammatory cytokines IL-6, stimulate the secretion of growth hormone, prolactin and increase immunity by stimulating Th2. regular and continuous exercise will improve fitness (Sugiharto, 2012).

A study shows that giving glucocorticoids to patients with doses that resemble cortisol levels when someone is experiencing stress can have an acute effect in the form of suppressing the cytokine IL-6 which is more significant than giving glucocorticoid doses that exceed cortisol levels during stress (pharmacological doses) or not given at all or administration of etomidate which suppresses cortisol production. On the other hand, cortisol has a delayed effect which can cause an increase in the pro-inflammatory cytokine IL-6 (Yeager et. al., 2011).

Exercise such as walking, jogging, or running, can cause a short-term inflammatory response that triggers an increase in oxidative stress, an increase in serum cortisol. Regular exercise will reduce the concentration of CRP, IL-6 and TNF alpha and increase anti-inflammatory substances such as IL-4 and IL-10. A 12-week high-intensity aerobic exercise program will reduce the release of cytokines and monocytes in healthy young people (Golbidi and Laher, 2012; Svendsen et. al., 2014).

TNF Alfa

The results of this study indicated that there was a significant difference in the levels of TNF Alfa after doing physical exercise in the form of running 12 minutes between trained basketball and not (p = .000).

The study by Dimitrov et al. Demonstrated downregulation of monocytic TNF production during acute exercise mediated by high epinephrine levels (Dimitrov et. al., 2017). Terink et. al. research on 50 men (mean age 58.9 ± 9.9 years) and 50 women (mean age 50.9 ± 11.2 years). The result was that all cytokine concentrations observed after exercise (IL6, IL8, IL10, IL1 beta, and TNF alpha) increased from baseline values ($p < .001$). Then the concentration decreased from day one to day two ($p < .01$). Exercise induces an increase in cytokines, but these levels will decrease in the following days even though they continue to do exercises with the same training intensity and load (Terink et. al., 2018). Nemet et. al. conducted a study on 11 healthy male high school-age boys, ranging from 14 to 18.5 years, following 1.5 hours of single wrestling exercise. There was an increase in proinflammatory cytokines after exercise, namely IL6. TNF alpha and IL1 beta (Nemet et. al., 2002).

In contrast to the results of the study by Steensberg et al., six healthy male subjects were asked to do knee extensor exercises for 180 minutes. The result was that IL-6 and TNF-mRNA were both detectable in muscle samples at rest. IL-6 levels increased 100-fold during exercise, and there was no significant increase in arterial plasma TNF-alpha mRNA (Steensberg et. al., 2002).

Muscle contraction directly stimulates the release of IL-6, which is an anti-inflammatory cytokine, decreasing the production of TNF alpha and IL1 beta, in the acute phase and during cell proliferation. Moderate intensity exercise (MIT) is effective in reducing body fat, which can prevent fat cell damage and cell hypoxia, so that proinflammatory cytokines (IL6 and TNF) are reduced through increased adiponectin secretion and increased anti-inflammatory cytokines. (Huldani et al., 2020).

Leukocytes, neutrophils and monocytes

In this study, there was no statistically significant difference in the number of leukocytes for basketball players and non-basketball players, but it was found that the average number of leukocytes for basketball players was lower than for non-basketball players. This is in line with the research of Baffour-Awuah B et al. where there was no statistically significant difference in the number of leukocytes in athletes and non-athletes at rest ($p > .05$). From this study, it was found that the mean and standard deviation of the leukocyte count for athletes and non-athletes were 5.18 ± 1.29 and 5.81 ± 1.24, respectively. From these data, it can be seen that athletes have a lower number of leukocytes than those who are not athletes (Baffour et. al., 2017). This is in line with the research of Abdossaleh et. al. In his study, it was found that the results were not statistically significant on differences in the number of leukocytes after and before 60 minutes of moderate intensity physical exercise in male judo athletes ($p > .05$) (Abdossaleh et. al., 2014). In addition, in the study of Bartlett et al., It was found that moderate intensity exercise of 30-45 minutes, three times a week, for 10 weeks in adults did not cause significant changes in the number of leukocytes and monocytes between before and after exercise ($p > .05$.). (Bartlett et. al., 2017).

The timing of physical exercise may also have an effect on the effects of glucocorticoids. Glucocorticoid levels increase in the morning and decrease at night (Besedovsky et. al., 2014). This is likely to have an effect on the anti-inflammatory effect of glucocorticoids, leading to suppression of the leukocyte count so that the difference in leukocyte counts between basketball-trained and basketball-trained students is not statistically significant. This opinion is supported by research conducted by Albayrak C. D et al., Where the amount of cortisol in basketball players during pre-competition training was inversely proportional to the number of leukocytes ($p < .05$) and the number of CD4 + cells ($p < .01$) (Albayrak et. al., 2013).

In contrast to the research conducted by Sadegh et. al., the results were contrary to the researchers. The results of this study were that there was a statistically significant difference in leucocytes between fifteen male athletes and fifteen male non-athletes after aerobic exercise ($p < .05$). In this study, it was found that the average leukocyte count for male athletes was lower than the leukocyte count for non-athletic men with an average of 5995 ± 186 and 6759 ± 230 for each sample ($p < .05$). In addition, it was also found an increase in the number of leukocytes between before and after aerobic exercise in both male athletes and non-athletes ($p = .014$) with the mean number and standard deviation of leukocytes pre-test and post-test respectively 5885 ± 103 and 5995 ± 186 in men. athletes and 6426 ± 320 and 6759 ± 230 in non-athletes (Sadegh et. al., 2012).

The conflicting results of this study are likely due to the difference in aerobic exercise procedures given to the study of Sadegh et. al., thus providing a different workout intensity. The study used the Bruce protocol where participants in the Bruce protocol ran on a treadmill at a speed of 2.7 km / h at a 10% inclination for 3 minutes then the speed and incline were increased every 3 minutes simultaneously until the participants felt tired and could not continue the exercise (Hamlin et. al., 2012). This protocol is different from the aerobic exercise that the researchers gave where the aerobic exercise that the researchers gave was moderate intensity aerobic exercise and did not cause the participants to be so tired that they could not continue the exercise. This may affect the difference in intensity between the physical exercise that the researchers gave and the Bruce protocol so that different results were found.

12 minutes of moderate intensity aerobic exercise did not have a statistically significant effect on the differences in the number of leucocytes, neutrophils, and monocytes of basketball players and non-basketball players. The possible cause is the duration and intensity of exercise that is not enough so that it is not enough to cause muscle injury in adolescents who are not basketball players. So that the results found may be similar to blood samples taken at rest (Baffour et. al., 2017). Other causes are due to a delayed increase in neutrophils and monocytes and the suppressive effect of cortisol. In several studies, the increase in neutrophils has varied, such as in the study of Sand et. al. The results showed an increase in leukocyte levels after 1 hour of exercise and 3 hours plus rest, especially an increase in neutrophils, and in the group of 5 minutes of high intensity exercise, an increase also occurred but with a different pattern. Likewise with the research of Gustafson et. al. where there is a difference in the increase in neutrophil-inducing cytokines immediately after exercise and 3 hours after exercise. Meanwhile, in the research of Jamuartas et. al. obtained a lower number of granulocytes before exercise than after exercise then increased more after 24

hours of rest and so on. This is also reported in Bartlett et. al. that exercise with moderate intensity 30-45 minutes and 3 times a week in adults has no effect on the number of monocytes with a value ($p > .05$) (Bartlett et. al., 2017).

Whereas in the study of Belviranli et al. Exercising for a week to prepare the subject for exercise, then the actual exercise was carried out, a gradual increase in leukocytes, especially neutrophils, compared to before exercise then continued to increase until 3 hours after exercise then decreased at 6 hours after exercise and then return to pre-training values (Mezzani et. al., 2013).

There is a theory known as the inverted J hypothesis, which states that moderate exercise and physical activity increase immunity and reduce the risk of developing disease (Woods et. al., 1999). Many studies have shown that groups who do moderate activity have a lower incidence of viral and bacterial infections. However, if exercise and physical activity are done excessively, it will reduce the ability of the body's immune system and have a higher risk of developing disease (Charansonney et. al., 2014; Milton et. al., 2014). Although in general there is an increase in body immunity, this is influenced by various factors such as age, gender, as well as the intensity, frequency and duration of exercise performed (Huldani et. al., 2020). Each subject has a different background of regularity and exercise habits, which means that the frequency of exercise for each subject is not the same, even though this is one of the factors that influence the achievement of sports-induced immune repair targets.

CONCLUSION

This study indicates that basketball-trained students have better VO_{2max} scores than untrained basketball students. The twelve minutes of moderate aerobic exercise resulted in a higher increase in cortisol levels in basketball-trained students than in untrained students, while TNF alpha levels were opposite, twelve minutes of aerobic exercise can be used as a method to increase the body's immune system and show the homeostasis process in adolescents is going well.

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