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Immune Response (Cortisol, TNFA, HMGB1) in Trained and Untrained Adolescent after 12 Minutes Run Exercise

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Abstract: This study aims to explain the immune response (cortisol, TNF Alpha, HMGB1) after 12 minutes of moderate-intensity aerobic exercise. This exercise is a modification of Cooper's 12-minute running test that can be done by running 20 meters for 12 minutes according to an individual's ability with moderate intensity 60-80% maximum pulse rate. The research was conducted on 15 trained and 15 untrained students at SMAN 1 Banjarbaru. The sampling technique was the purposive sampling method. The value of VO2 max was measured on the first day with Multistage Fitness Test. Cortisol, TNF Alfa, HMGB1 was measured by taking blood test. Data analysis used the Mann-Whitney test. The results showed that the average VO2 max, cortisol, and HMGB1 scores of students with basketball achievement were higher than those of untrained students. TNF alpha level that did not increase was higher. The data analysis showed the difference in measurements between trained and untrained students ($p < 0.05$) on VO2 max, Cortisol, and TNF Alpha. There was no significant difference in HMGB1 ($p > 0.05$). There is a positive correlation between Cortisol and TNF Alpha, Cortisol and HMGB1, HMGB1 and TNF Alpha in adolescents. It can be concluded that trained adolescents' Cortisol levels increased higher. Trained adolescents' TNF alpha levels were lower. It was not proven that trained students' HMGB1 levels differed from those of untrained ones. In adolescents, there is an increase in cortisol levels followed by an increase in TNF Alpha levels, an increase in cortisol levels followed by an increase in HMGB1 levels, and an increase in HMGB1 levels followed by an increase in TNF Alpha levels. Moderate-intensity aerobic exercise (12 minutes of running) can be recommended as simple exercises to maintain fitness and boost the immune system or exercise health during a pandemic.

Keywords: immune response, 12 minutes of running, trained adolescents, untrained adolescents.

受过训练和未经训练的青少年跑步12分钟后的免疫反应（皮质醇、肿瘤坏死因子α、HMGB1）

摘要:

本研究旨在解释中等强度有氧运动12分钟后的免疫反应（皮质醇、肿瘤坏死因子α、HMGB1）。这个练习是对库柏12分钟跑步测试的修改，可以根据个人的能力以中等强度60-80%的最大脉搏率跑20米12分钟来完成。该研究是在斯曼1班加巴鲁对15名受过培训和15名未受过培训的学生进行的。抽样技术是有目的的抽样方法。在第一天使用多阶段体能测试测量最大摄氧量的值。皮质醇、肿瘤坏死因子阿尔法、HMGB1是通过血液测试来测量的。数据分析使用曼惠特尼检验。结果表明，有篮球成绩的学生的平均伏O2最大限度、皮质醇和HMGB1分数高于未

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受过训练的学生。没有增加的肿瘤坏死因子α水平更高。数据分析显示了受过训练和未受过训练的学生之间在最大摄氧量、皮质醇和肿瘤坏死因子α方面的测量差异(磷<0.05)。HMGB1没有显着差异（磷>0.05）。青少年皮质醇与肿瘤坏死因子α、皮质醇与HMGB1、HMGB1与肿瘤坏死因子α呈正相关。可以得出结论，受过训练的青少年的皮质醇水平升高得更高。受过训练的青少年的肿瘤坏死因子α水平较低。没有证明受过训练的学生的HMGB1水平与未受过训练的学生不同。在青少年中，皮质醇水平升高后肿瘤坏死因子α 水平升高，皮质醇水平升高后 HMGB1水平升高，HMGB1水平升高后肿瘤坏死因子α水平升高。中等强度的有氧运动（跑步12分钟）可以被推荐为简单的运动，以在大流行期间保持健康和增强免疫系统或锻炼健康。

关键词： 免疫反应，12 分钟跑步，受过训练的青少年，未受过训练的青少年.

1. Introduction

WHO states that in 2019, most people around the world are less physically active, thus endangering their health in the future, so WHO recommends carrying out physical exercise, especially moderate intensity, for 150 minutes a week for someone aged 18-64 years [1]. Regular exercise decreases the risk of developing various diseases and increases life expectancy [2, 3]. One form of physical exercise is a game of basketball. Although not as popular as football, basketball is popular among Indonesians [4]. Basketball uses alternating aerobic-anaerobic metabolism, although anaerobic metabolism is predominant.

Aerobic exercise is an activity that utilizes the continuous and normal contractions of large muscle groups for some time and utilizes aerobic metabolism as a source of energy. One of the components of aerobic exercise is intensity. Moderate-intensity aerobic exercise, an activity that consumes energy comparable to brisk walking, is the aerobic exercise most frequently studied [5]. Physical exercise is related to a person’s ability to perform activities through its effect on VO2 max. 12-minute running was used as a way to measure a person’s VO2 max value. In addition, exercise is a stressor that stimulates the neuroendocrine system and immune response. 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. Physical exercise drastically affects the number of leukocytes circulating in the peripheral blood. Leukocytosis that accompanies acute exercise is a transient phenomenon because the number and composition of the leukocytes usually return to normal values within 6-24 hours after an exercise session. Leukocytosis that occurs in physical exercise is mostly caused by the activation of neutrophils and lymphocytes and a small proportion of monocytes. 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.

2. Methods

Cross-sectional research was conducted on the population of all adolescents at Senior High School 1 (SMAN 1) Banjarbaru. The sample in this study was divided into two; namely, the sample was taken from 15 basketball-trained students and 15 untrained basketball students. The sample was cooperative and had filled out a consent form to be the subject of the study. The purposive sampling method was used to take samples according to the inclusion criteria, namely (a) male, (b) aged 15-18 years, (c) physically healthy, meaning that at the time of the study, the proband was not sick or infected, had no history of heart or lung disease, and allergies, (d) cooperative, research subjects could be invited to work together to carry out research procedures, (e) not smoking, (f) not consuming drugs that affect the number of leukocytes, monocytes, and neutrophils at least two days before blood collection, (g) having a normal body mass index (BMI) (20-25). The sample of basketball-trained adolescents is students of SMAN 1 Banjarbaru, members of a basketball sports club, who routinely practice basketball 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 while doing the exercises and cannot complete the exercise.

The research was conducted for three days. VO2 max measurements were carried out on the first day using the

MFT (Multi stage fitness test). The subject was rested on the second day. Blood samples were taken for analysis of levels of cortisol, Tnf alpha, and HMGB1 were checked on the third day. After running twelve minutes of moderate-intensity aerobic exercise, a 5 cc blood sample was taken from the brachial vein. Before training, the research subjects will have their maximum pulse rate (MHR) calculated using the Tanaka formula. After knowing MHR, research subjects will use pulse oxymetry and warm-up to run in groups of 3 people per group until they reach the MHR target of 70-79%. After reaching the target of 70-79% MHR, the subject will continue to run for twelve minutes at the same rhythm. Blood sampling and subject analysis were carried out by trained personnel from the Prodia Banjarmasin laboratory. Trained health workers always accompany training sessions to prevent life-threatening things from occurring. The Mann-Whitney test was used for VO2 max, cortisol, TNF Alpha, whereas the unpaired Student T-test for HMGB1.

3. Results

Sample characteristics included age, oxygen saturation, body mass index, pulse, height, weight, systolic and diastolic blood pressure. The average sample was 17 years. The mean value of pulse rate, systolic and diastolic blood pressure, body weight, body mass index of students trained in basketball was lower than students who were not trained in basketball. Pulse rate, systolic and diastolic blood pressure, body mass index, oxygen saturation were within normal limits. Table 1 shows that students trained in basketball were fitter than students who were not trained in basketball.

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 results

Marker	Trained Basketball Students (n = 15)		Untrained Basketball Students (n = 15)	
	Mean	SD	Mean	SD
VO_2 max	37,49	26,1	28,85	18,8
Cortisol	1440,1	1863.1	26,3	16.1
TNF Alpha	532,0	667,2	963,7	1144,8
HMGB1	2180,6	166.4	2168,9	295.8

The mean VO_2 max, cortisol, and HMGB1 concentrations of basketball-trained students were higher than untrained. In contrast, the untrained TNF alpha score was higher than the untrained.

3.1. VO_2 Max

The mean VO_2 max of basketball-trained students was 37.49 ± 26.1 ml/kg/minute higher than the untrained students, namely 28.85 ± 18.8 ml/kg/minute. With the Mann-Whitney test, the VO_2 max value obtained was a statistically significant difference in the VO_2 max value after 12 minutes of moderate-intensity running training for basketball-trained students and untrained p = 0.000 ($p < 0.05$). Spearman's rho correlation test showed a significant correlation p = 0.000, with a very strong positive correlation (0.870 **) between VO_2 max and whether adolescents were trained in basketball. The more trained a teenager is, the greater the VO_2 max value.

3.2. Cortisol Concentration

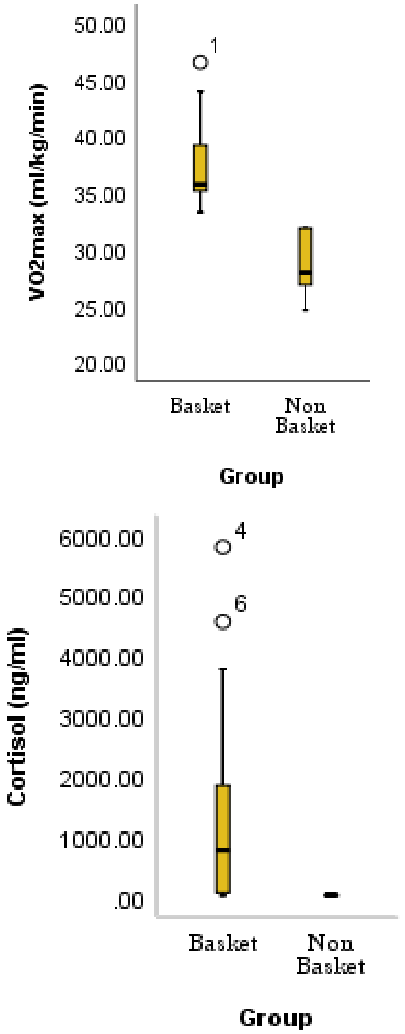
The average cortisol concentration of basketball-trained students was greater than that of untrained students. In basketball-trained students, the mean blood cortisol concentration was 1,440.1 ng/ml higher than in the untrained, 26.3 ng/ml. In this study, statistically significant results were obtained using the Mann-Whitney test. After twelve minutes of running with moderate-intensity training in basketball-trained and untrained students, there was a significant difference in cortisol levels (p = 0.000 ($p <0.05$). Spearman's rho correlation test showed a significant correlation p = 0.000, with a very strong positive correlation (0.720 **) between cortisol and whether adolescents were trained in basketball. The more trained a teenager is, the greater cortisol levels will be.

3.3. TNF Alpha Concentration

The mean TNF Alpha concentration of basketball-trained students was lower than that of untrained students. The mean TNF Alpha concentration for basketball-trained students was 532 pg/ml and untrained 963.7 pg/ml. Mann Whitney test showed a significant difference between the two after 12 minutes of moderate-intensity training for basketball-trained and untrained students p = 0.000 ($p <0.05$). There was no significant correlation between Spearman's rho test, p = 0.077, with a weak negative correlation (-0.327) between TNF Alpha and whether adolescents were trained or not in basketball. The more trained a teenager will have lower TNF Alpha levels.

3.4. Concentration of HMGB1

The 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 no significant difference in HMGB1 levels after 12 minutes of moderate-intensity exercise in basketball-trained and untrained students p = 0.895 (p> 0.05). There is no significant correlation between Pearson's correlation test p = 0.895, with a very weak positive correlation (0.025) between HMGB1 and whether or not teenagers are in basketball. The more trained a respondent is, the greater the HMGB1 level will be.



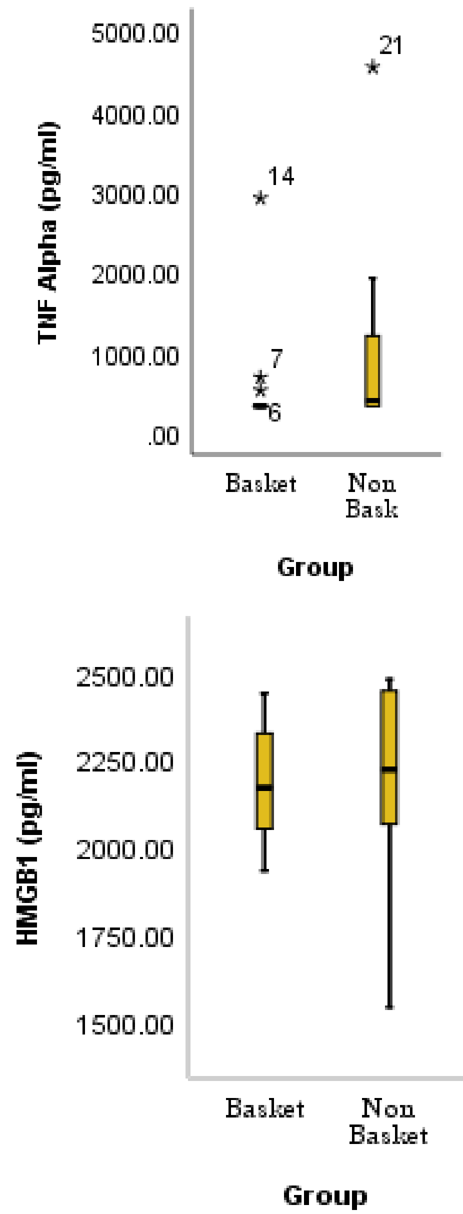


Fig. 1 Boxplot graph of VO2 max Value, Cortisol, TNF Alpha, and HMGB1 concentration

4. Discussion

The statistical tests in this study showed significant differences in VO_2 max, cortisol, and TNF alpha levels between basketball-trained and untrained students. While another aspect, namely HMGB1, there was no statistically significant difference between students trained in basketball and those who were not trained.

Physical activity causes changes in the body's physiological functions, both temporary changes (response) and permanent changes [6]. This also occurs in people who do sports; there is a physiological response to maintain the body's homeostasis. An 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 the release of heat as compensation characterized by sweating, an increase in the amount of energy needed, and the cardiovascular there is an increase in heart rate due to pumping blood to supply more to all organs. The occurrence of physiological response in the musculoskeletal system as an adaptation to physical exercise occurs by involving many molecular pathways that play a role in regulating muscle contraction and biosynthetic pathways for ATP formation, such as AMP-activated protein kinase (AMPK), Sirtuins (SIRTs), mitogen-activated protein kinases (MAPKs), and oxygen sensor prolyl hydroxylases (PHDs) [7-8]. All these changes require a good balance so that the work of the organs and body condition is not disturbed; that is called homeostasis. Thus, if the homeostatic response fails, it will reduce their physical performance and performance in athletes.

The VO2 max score of basketball-trained adolescents was higher than the untrained. This result 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 at moderate intensity increased VO2 Max values compared to those who did not exercise in adolescents ($p = 0.000$). The study explains that this is likely due to moderate intensity physical exercise on cardiac output, where physical exercise causes an increase in cardiac output due to an increase in stroke volume.

A research conducted in 2011 described two factors that causes an increase in VO_2 max, namely central factors and peripheral factors. Centrally, the increase in VO_2 max was due to increased stroke volume and a slight increase in maximal heart rate. Peripherally, the increase in VO_2 max occurs due to increased arterial-venous oxygen differences that are influenced by oxygen transport to active muscle fibers, local enzyme adaptation, and mitochondrial density. Peripheral VO_2 max increase occurs in sprint interval training [9].

According to [10], in healthy subjects aged 60-71 years, 53 men and 57 women and a control group did not follow any exercise. The VO_2 max value was measured before and after aerobic exercise in the form of treadmill walking and running for 9-12 months. The results showed an improvement in the VO_2 value after a series of exercises in the male and female groups by 21 and 19%, respectively. It was provided that both elderly women and men can obtain VO_2 max improvement during routine exercise stimuli. Statistically, this study showed that the improvement in VO_2 max level does not depend on age, gender, VO_2 max value before undergoing exercise, or the intensity of the exercise undertaken.

Research was conducted on 34 physically healthy children aged 10-18 years who participated in strength training [11]. The results showed an increase in VO_2 max values after training. This study revealed no significant difference in VO_2 max levels based on age. In compliance with [12], no significant difference in VO_2 max values between athletes and non-athletes after exercising. Meanwhile, as stated in [13], greater VO_2 max improvement can be obtained with exercise for more than 20 weeks and intensity of around 60% and 70% of VO_2 max.

4.1. Cortisol

This study indicated a significant difference in cortisol levels after 12 minutes of physical exercise running between trained basketball and not ($P = 0.000$). The basketball-trained cortisol value (1440.1 ng/μL) was higher than the untrained (26.3 ng/μL); this may be a homeostatic process not due to a stressor. Moreover, the average value of HMGB1 is also bigger. Therefore, cortisol here functions as an anti-inflammatory and suppresses pro-inflammatory cell cytokines such as IL6, TNF Alpha, Leukocytes, Neutrophils, and Monocytes. In contrast, untrained cortisol values were lower; therefore, the anti-inflammatory function was not strong enough to suppress the inflammatory reaction, as indicated by a greater increase in the TNF alpha concentration. Sports and physical activity can be a source of stress for the body and impact other body systems and have the potential to cause homeostatic disorders.

Previous research has consistently shown that exercise with an intensity of more than 60% VO2 max stimulates the release of higher cortisol concentrations in adults [14]. Studies show that every teenager has the same body response to an increase in the HPA axis reaction and 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 (maximum 50-65% pulse rate).

Exercise with VO2 max 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. significantly greater than the session with 40% exercise intensity (pre-intervention 12.2 ± 4.3 and post-intervention 10.8 ± 5.4). That is, 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.

Cortisol concentration will increase with increasing intensity and duration of exercise [15]. 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 an increase in the activity of stress hormones such as cortisol, ACTH, and catecholamines, which inhibit protein synthesis and trigger protein degradation resulting in the breakdown of skeletal muscle protein.

The level of cortisol secretion is influenced by the circadian cycle. Serum cortisol secretion begins to increase at midnight, peaking in the morning. In addition, this increase may be determined by other factors that can increase cortisol secretion, namely psychological stress, which also triggers the release of cortisol [16].

After acute physical exercise, the increase in cortisol levels has been proven in several studies [17, 18]. The increase in cortisol levels is strongly influenced by the intensity of physical exercise and the subject's physical exercise habits. Hackney et al.'s research showed that cortisol levels increased with increasing training load given to subjects aged 16-21 years. In addition, the research [17] showed differences in the increase in cortisol levels in athletes and non-athletes according to the intensity of the physical exercise given. Increased cortisol levels provide an anti-inflammatory effect by emphasizing the expression of proinflammatory cytokines, thereby inhibiting the increase in the number of leukocytes [19].

The release of cortisol is one 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 an increase in amino acids and fat mobilization, which, if excessive, can disrupt tissue structure and function and even cell death.

Beserra et al. conducted a review of five articles and seven analyzes to look at post-exercise cortisol levels in 463 respondents with major depressive disorder. Respondents did the exercises with different frequencies, ways of measuring cortisol, and control groups. The results found that physical exercise caused a decrease in cortisol levels. However, this decrease in cortisol depends on the type, frequency, and measurement of cortisol taken. It is known that the decrease in cortisol levels can occur effectively after doing aerobic exercise with a frequency of 5 times per week [20].

On the other hand, the study by Wang et al. [21] showed the opposite result with the research above. The study aimed to assess the comparability of the effectiveness of aerobic exercise and resistance training in improving cognitive function in 42 young adults who were asked to do Stroop exercises after undergoing aerobic exercise, resistance training, and sedentary conditions a few days later. The saliva samples taken were at baseline conditions before exercise, immediately after exercise, and 30 minutes afterward. The results showed that cortisol levels increased after exercise, compared with cortisol levels during the sedentary phase and baseline levels. Cortisol levels immediately after exercise were also higher than 30 minutes afterward. From this, it is concluded that an increase in cortisol levels can occur after exercise but is influenced by the type and intensity of exercise.

4.2. TNF Alpha

The results of this study indicated a significant difference in the levels of TNF Alfa after 12 minutes of running physical exercise between trained basketball and not ($P = 0.000$).

Downregulation of monocytic TNF production during acute exercise was mediated by high epinephrine levels [22].

According to the study [23], covering 50 men (mean age 58.9 ± 9.9 years) and 50 women (mean age 50.9 ± 11.2 years), all observed cytokine concentrations after exercise (IL6, IL8, IL10, IL1 beta, and TNF alpha) increased from baseline values ($P <0.001$). Then the concentration decreased from day one to day two ($P <0.01$). Exercise induces an increase in cytokines, but these levels will decrease in the following days even though the exercise is still carried out with the same training intensity and load [23].

A study was conducted on 11 healthy males of middle school age, ranging from 14 to 18.5 years, after 1.5 hours of single wrestling exercise [24]. There was an increase in proinflammatory cytokines after exercise, namely IL6, TNF alpha and IL1 beta.

A study on six healthy male subjects who performed knee extensor exercises for 180 minutes [25] revealed that IL-6 and TNF-mRNA were both detected in the muscle sample at rest. IL-6 levels increased 100-fold during exercise, and there was no significant increase in arterial plasma TNF-alpha mRNA.

Direct muscle contraction stimulates the release of IL-6, 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) effectively reduces 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 [26].

Comparison of inflammatory response rates and muscle damage indexes between four popular elite-level team sports is reported in [27]. Thus, 72 elite male players from four sports: soccer, basketball, volleyball, and handball (18 in each), were asked to finish the match, while 18 non -athletes were tested as a control. Blood samples were drawn before, immediately, 13 and 37 hours after the match. The results found changes in TNFα levels in all types of sports, including basketball, in blood samples taken immediately after, 13 hours after, and 37 hours after a competitive match, compared to baseline values (measured in the morning about 8 hours before the match). Soccer players showed a 3–4-fold increase immediately after the largest inflammatory cytokine match, namely TNF alpha tumor. Volleyball players showed the smallest increase in inflammation and markers of muscle damage compared to the other three sports.

4.3. HMGB1

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

The human body has endogenous danger signals to prevent inflammatory responses secondary to releasing intracellular inflammatory factors into the extracellular area, namely the molecular pattern of associated damage (DAMP). High Mobility Group Box 1 (HMGB1) is a protein from DAMP which is a sign of muscle cell damage and causes the mobilization of immune cells to the site of trauma [7]. HMGB1 levels will return to their original concentration after 30 minutes of rest after exercising [28].

Overall, DAMP triggers the release of massive cytokines, including TNF-α, IL-6, and IFN. These mediators enhance the activation, proliferation, maturation, and recruitment of immune cells at the site of trauma, causing indirect activation of adaptive and innate immune cells such as DC or T cells [29].

HMGB1 exposure to human monocyte cultures will stimulate proinflammatory cytokines such as IL6, IL-8, TNF, and inflammatory protein macrophages-1 (MIP-1). The kinetic responses to the LPS mediated release of TNF and HMGB1 were very different. HMGB1 stimulated biphasic TNF release with the second wave is delayed, whereas LPS mediated TNF release occurred in the initial monophasic mode [30].

A study was conducted for three weeks with a high-intensity training program in 7 healthy young men [31]. Aerobic and endurance training is done for three consecutive days each week at the same time. Saliva and blood samples were collected Pre and Post, 30 minutes after exercise each week and 24 hours after exercise at the end of the session at week 3. The results showed plasma HMGB1 increased from Pre- to Post-exercise ($P < 0.05$).

A similar study was performed on 40 male Wistar rats (weight 220 ± 10.2 g) which were randomly divided into four groups: diabetic neuropathy, diabetic neuropathy + exercise, healthy + exercise, and healthy controls, diabetes was induced by injection of STZ (50 mg/kg) [32]. After confirming the development of diabetic neuropathy with a behavioral test, the exercise group received six weeks of continuous aerobic exercise at an average intensity of 15 m / min for 30 minutes on a treadmill. The serum HMGB1 level was measured by ELISA, and the concentration of malondialdehyde (MAD) and the action of superoxide dismutase (SOD) and catalase (CAT) enzymes in the spinal cord were determined by biochemical methods. Two-way ANOVA and Tukey's post hoc test were used for statistical analysis. The results showed that aerobic exercise significantly reduced HMGB1 protein levels and MDA concentrations and increased SOD and CAT enzyme activity compared to the diabetic neuropathy group ($p < 0.05$). Also, HMGB1 and MDA levels were increased, and SOD and CAT enzyme activity decreased in the diabetic neuropathy group ($p < 0.05$). Aerobic exercise is known to alter the protein HMGB1 and oxidative stress levels and increase the sensitivity of nociceptors to painful agents. It is recommended to use aerobic exercise as a non-prescriptive therapeutic intervention for diabetes patients to reduce neuropathic pain [32].

In another sports study, 34 and 36 nonprofessional female and male runners and half marathon finished each race; immediately, blood samples were collected 1-2 days before and 2-7 days after the race [33]. After the race HMGB1 serum concentration increased significantly by 1.5 times (half marathon; 3.13 ± 1.63 ng/mL to 4.78 ± 2.1 ng/mL) and 2.3 times (marathon; 2.58 ± 1.58 ng/mL to 6.02 ± 2.18 ng/mL), corresponding to an increase in circulation sRAGE for a half marathon but not a full marathon. During the recovery period, the concentration of HMGB1 and sRAGE increases back to the previous training level.

Not all HMGB1 respond positively to every exercise. In recent research, HMGB1 plasma remains below the detection limit (78 pg/mL) of testing after 1,200 km of plyometric training or cycling [34].

There was a decrease in serum HMGB1 at two weeks of Nordic walking exercise combined with vitamin D in elderly women; this suggests regular exercise can weaken the alarmin response in healthy adults [35].

5. Conclusion

This study showed that basketball-trained students had better VO2 max scores than untrained basketball students. Twelve minutes of moderate aerobic exercise resulted in higher cortisol levels in basketball-trained students than in untrained students, while TNF alpha levels were opposite. A very strong positive correlation between VO2 max and whether or not young people are trained in basketball. The more trained a teenager is, the greater the VO2 max value. There is a very strong positive correlation between cortisol and whether adolescents are trained in basketball. The more trained a teenager is, the greater cortisol levels will be. Twelve minutes of aerobic exercise can be used to boost the immune system and show that the homeostasis process in adolescents is working well.

The Covid-19 pandemic has forced everyone to move and stay at home. It is difficult to do some exercises outside, and not all types of sports can be done because they are vulnerable to transmits of Covid-19. One of the sports that can be done during the pandemic is a 12-minute run in the morning. The scientific novelty in this research is 12 minutes-run modified from Cooper's 12-minute running test; this can be done on a 20-meter running track for 12 minutes according to each ability, people can run or walk quickly according to their abilities with a target maximum pulse rate according to age (220- Age) with moderate intensity 60-80% of maximum pulse rate.

This research was conducted in July 2020 on adolescents at SMA 1 Banjarbaru, who did a 12-minute running test (modified of Cooper's 12-minute running test) with moderate intensity 60-80% of the maximum pulse rate; the results showed that in people who were trained in sports it did not cause system disorders. Immunity due to increased cortisol occurs not because of a response to inflammatory reactions but due to the influence of sympathetic nerves as a homeostatic response. Normal cortisol levels in untrained people indicate that loading with moderate-intensity aerobic exercise for 12 minutes of running does not cause an inflammatory reaction that causes cell damage. Moderate-intensity aerobic exercise for 12 minutes of running can be used as a weight-bearing exercise that improves fitness and immunity in both trained and untrained people.

The limitations of this study are:

1. This study did not measure Cortisol, TNF-α, and HMGB1 levels before and after exercise, so it could not assess the effect of exercise on Cortisol, TNF-α, and HMGB1 levels.
2. The number of samples in this study is limited.
3. No serial 0, 30, and 60-minute examinations were performed to see the recovery of the immune response.
4. Examination concerned only systemic inflammation markers (using serum and blood), so the local inflammatory response in muscle tissue (macrophages) cannot be evaluated.

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