

DIFFERENCES OF VO₂ MAX VALUE IN ADOLESCENTS AND CORTISOL LEVELS, COUNT OF LEUKOCYTES, MONOCYTES, AND NEUTROPHYL AFTER 12 MINUTES OF MODERATE AEROBIC EXERCISE

Huldani¹, Ahmad Husairi², Abdullah Zuhair³,
Muhammad Rafagih³, Zhasifa Khoirunnisa Suwanto³, Wisnu Wiryawan³,
Muhammad Zaini³

¹Department of Physiology, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia.

²Department of Anatomy, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia.

³Student of Medical Education, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia.

Email : huldani@gmail.com

ABSTRACT

This study aims to mention the difference in VO₂ max value in adolescents and the difference in leukocyte, monocytes, and neutrophil levels after 12 minutes of moderate aerobic exercise. The study was conducted using a cross-sectional study in 15 basketball players & 15 non-basketball students at SMAN 1 Banjarbaru. The sampling technique was carried out using purposive sampling method. Measurement of VO₂ max value was carried out on the first day using the MFT (Multistage Fitness Test / Bleep Test) and measurement of leukocyte levels was carried out on the third day using blood collection after the patient did 12 minutes of moderate aerobic exercise. Data analysis used the Mann-Whitney test for VO₂ max values & unpaired t test for the number of leucocytes, monocytes, neutrophils & cortisol levels. The results of the data analysis showed that there were still significant differences in the VO₂ max value in basketball & non-basketball players ($p > 0.05$), monocytes ($p > 0.05$), & neutrophils ($p > 0.05$) and there were significant differences in levels. cortisol ($p < 0.01$) after 12 minutes of moderate aerobic exercise in basketball and non-basketball students. The conclusion of this study is that 12 minutes of moderate aerobic exercise resulted in a higher increase in cortisol levels in basketball players compared to non-basketball players and the average value of leukocytes, monocytes, and neutrophils in basketball players was lower than non-basketball players. This is because the release of cortisol is a form of adaptation to the body's stress due to training, and becomes anti-inflammatory as a result reduces the number of leukocytes, monocytes and neutrophils of basketball players. 12 minutes of aerobic exercise can be used as a method to increase the body's immune system and explain the homeostasis process in adolescents in a good way.

1. INTRODUCTION

In 2019, the WHO stated that most of the global population tends to be passive in physical activities, thus putting their health at risk in the future, therefore advising them to exercise weekly, especially at moderate intensity, for 2 hours 30 minutes for people aged 18-64 years. Regular exercise is considered capable of increasing life expectancy and reduce the risk of the development of diseases.^{1,2,3,4,5,6} Basketball sport is one among so many type of exercise which has gained a huge attention among the Indonesian.^{7,8,9} Despite the prominent utilization of anerobic metabolism in basketball game, aerobic metabolism is also used alternately with anaerobic metabolism.^{10,11}

Aerobic exercise is a physical activity which utilizes aerobic metabolism as its energy source and uses continuous and regular contraction of large muscle groups over a period of time.^{11,12,13,14} Intensity is a part in aerobic exercise.¹¹ Moderate intensity aerobic exercise is the most studied aerobic exercise and utilizes comparable amount of energy to brisk walking.¹⁴

Exercise can affect VO₂ max therefore exercise is related to a person's fitness level.¹⁵ To value the VO₂ max, a 12 minute run is often used in the procedure.^{16,17,18} In addition, exercise is also related to the immune system

and neuroendocrine system.¹¹ The connection between exercise and the immune system was first investigated by David Nieman who observed, in his research, a lack of reports of acute respiratory infection complaints in individuals who routinely do moderate intensity of physical exercise.^{8,19}

The number of white blood cells in the blood can be affected by exercise. Exercise can cause a temporary increase of white blood cells count returning to normal within 6-24 hours. Neutrophil and lymphocyte activation plays a major role in this phenomenon, however, monocyte activation also plays a minor role.²⁰

Exercise is capable of affecting the hipotalamic-pituitary axis response which causes the release of the corticotropin hormone (CRH) from the hypothalamus to the cells of the anterior pituitary, resulting in the release of adrenocorticotrophic hormone (ACTH) into the bloodstream. ACTH in the blood then stimulates the release glucocorticoids (cortisol) from the adrenal cortex.¹⁵

2. METHOD

This study used a cross sectional method. The population is all students of Sekolah Menengah Atas Negeri 1 (SMAN 1) Banjarbaru. The sample was selected using criteria and divided into two, a sample of 15 basketball players and 15 non-basketball players. The sample selection criteria were done using purposive sampling method using inclusion criteria, such as men, aged 15-18 years, physically healthy at the time of the study, and had no history of heart disease, lung disease and allergies, research subjects cooperative in research, didn't smoke, not taking drugs that affect the number of leukocytes, monocytes, and neutrophils at least 2 days before the event, body mass index (BMI) in the normal value range (20-25). The sample of basketball players is students of SMAN 1 Banjarbaru who are members of the basketball club and have been playing basketball for at least one year, three times a week for one hour per training. The study will be stopped if the subject experiences signs of fatigue during exercise so that he is unable to complete the exercise. After being selected the subject is then asked to cooperate to fill out the consent form to become the research subject.

The study lasted 3 days. On the first day, VO_2 max measurements were done using the MFT (Multistage fitness test). The second day the subject was rested. On the third day, the research subjects had their maximum pulse rate calculated using the Tanaka formula, then the research subjects would use oxymetry and warm up in the form of running in groups consisting of 3 people per group until the 70-79% pulse rate target was achieved. After reaching the target, subjects were asked to keep running for 12 minutes with a rhythm as a form of 12 minutes of moderate intensity aerobic exercise. Then the blood was drawn to analyze the number of leukocytes, monocytes, neutrophils, and cortisol levels. The subject's blood was drawn as much as 5 cc in the brachial vein after 12 minutes of moderate intensity aerobic exercise. Blood sampling and analysis of the subjects were done by personnel from the Prodia laboratory. Health workers are prepared to prevent unwanted things.

3. RESULTS AND DISCUSSION

Sample characteristics were based on age, pulse rate, body mass index, body weight, systolic and diastolic blood pressure.

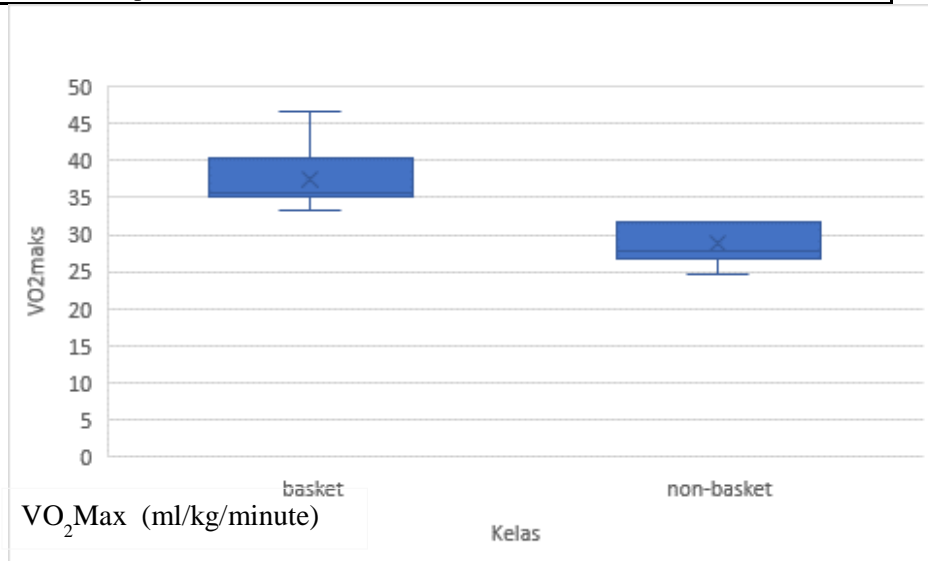
Table1. Characteristics of research subjects

Characteristics (Average \pm SD)	Basketball group (basketball player Student n=15)	Kelompok Non Basket (basketball player Student n=15)
Age (years)	16.93 \pm 0.258	17.07 \pm 0.495
O ₂ Saturation (%)	97.27 \pm 2.576	96.93 \pm 3.731
Pulse per minute	84.20 \pm 11.384	92.93 \pm 12.981
Body mass index (kg/m ²)	21.65 \pm 2.104	21.68 \pm 5.911
Height (cm)	167.37 \pm 8.067	172.00 \pm 5.305
Body weight (kg)	61.13 \pm 10.034	64.33 \pm 18.289
Systolic blood pressure (mmHg)	127.53 \pm 14.282	132.20 \pm 13.078
Diastolic blood pressure (mmHg)	78.73 \pm 7,601	82.87 \pm 9.797

The characteristics of the research subject indicate the state of the research subject. Based on the data in table 1, we can conclude that the research subjects are in good health so that they are able to carry out the research procedures assigned to them.

Table 2. VO₂ max value

Variable	median (minimum-maximum)	p
Siswa pemain basket	35.75 (33.20-46.50)	<0.01
Siswa bukan pemain basket	27.90 (24.60-31.80)	

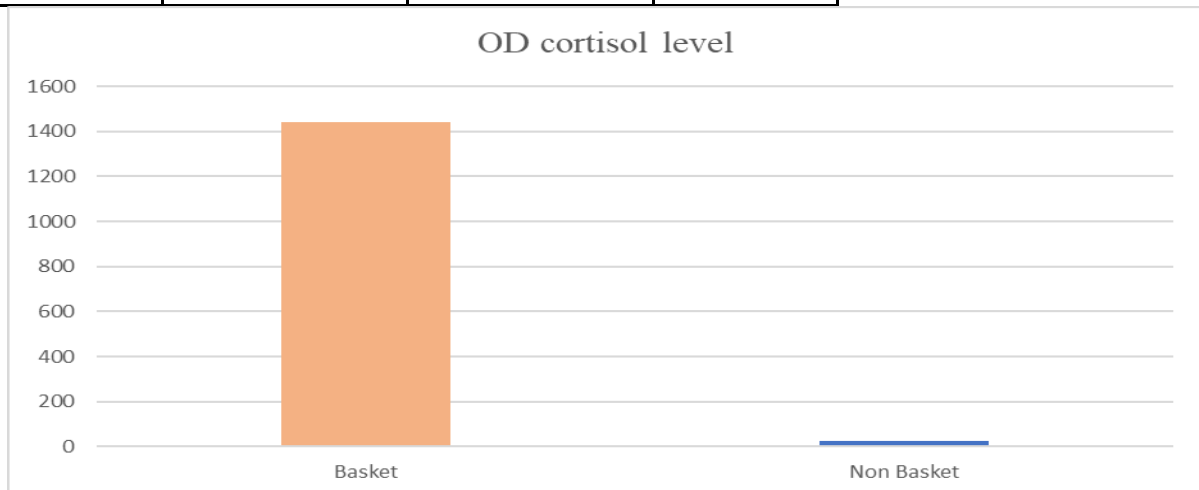


The VO₂ max value of basketball trained adolescents was higher than that of untrained basketball youth. Research by Buchan DS et al. It was found that physical exercise three times a week for seven weeks with moderate intensity increased VO₂ Max values compared to those who did not exercise in adolescents (p = 0.000). That this is probably due to the influence of moderate intensity physical exercise on cardiac output, where physical exercise results in an increase in cardiac output due to an increase in stroke volume.²¹

There are 2 factors, central and peripheral, increasing the VO₂ max value based on a study by Macpherson REK et al. Centrally, an increase in stroke volume and a little increase in the maximal heart rate can increase the VO₂ max value. Peripherally, an increase in the difference in arterial-venous oxygen, influenced by oxygen transport to active muscle fibers, local enzyme adaptation, and mitochondrial density, can increase the VO₂ max value. A peripheral increase in VO₂ max is observed in sprint interval training.²²

Table 3. Blood cortisol OD value

Variable	OD value	SD	P
Basket	1440.054	1863.176	<0.01
Non Basket	26.34353	16.10597	



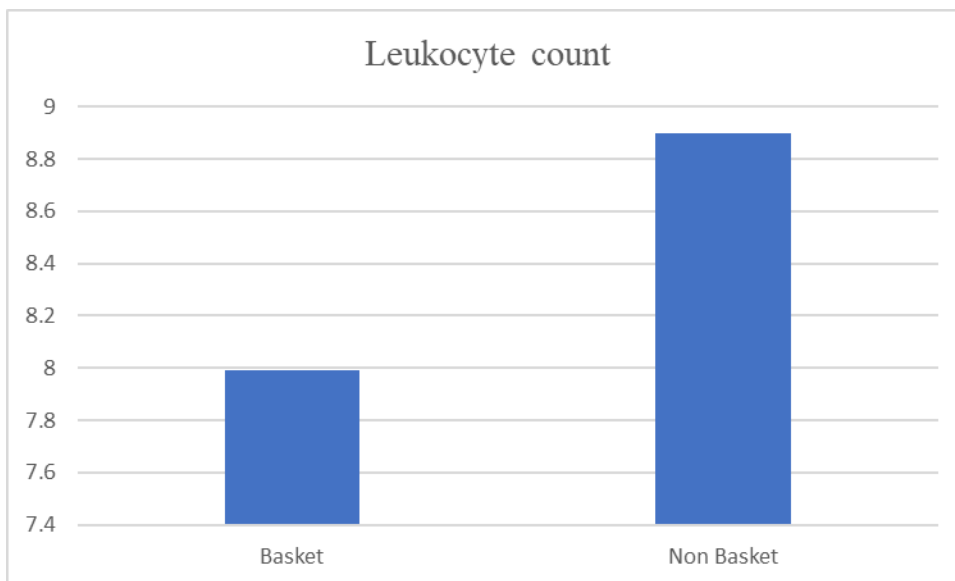
In the study, there was a statistically significant difference in cortisol levels after moderate intensity exercise among basketball players and non-basketball players ($p < 0.01$) (table 3). This shows that there is a significant difference in the cortisol value of basketball-trained adolescents compared to non-basketball-trained adolescents after 12 minutes of moderate aerobic running intervention. As in several previous studies that exercise 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 affect homeostasis.^{23,24}

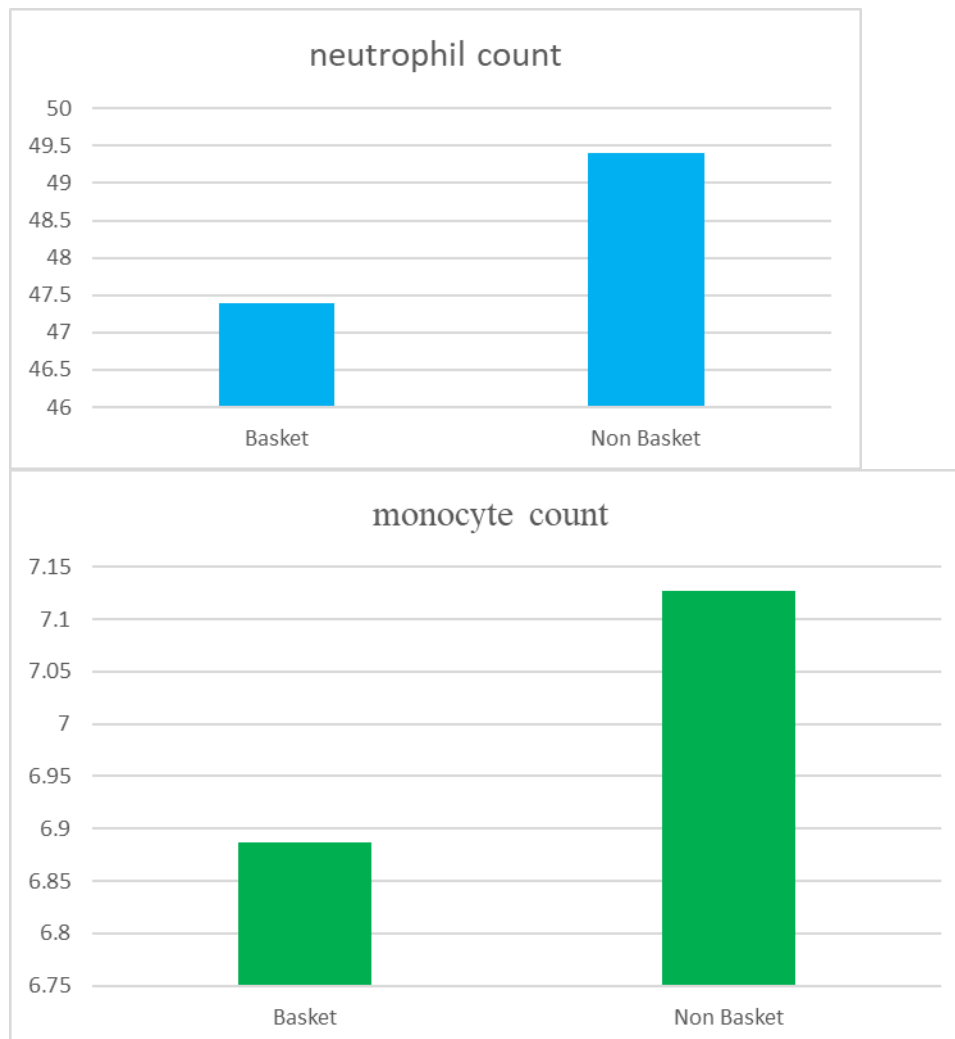
Exercise is capable of affecting the hypothalamic-pituitary axis response which causes the release of the corticotropin hormone (CRH) from the hypothalamus to the cells of the anterior pituitary, resulting in the release of adrenocorticotropic hormone (ACTH) into the bloodstream. ACTH in the blood then stimulates the release of glucocorticoids (cortisol) from the adrenal cortex.¹⁵

In this research's results, the cortisol levels of basketball player group was higher than the non-basketball group. The results showed a similarity with a study by Minetto et al which observed a higher salivary cortisol levels in competitive athletes than sedentary individuals.²⁵ A possible reason is that the basketball player group HPA axis has a better response to stress than the non basketball player group which is important for the basketball player group to be able to adapt to chronic stressful situation such as competition and daily exercise.²⁶

Table 4. leukocyte, neutrophil, monocyte count

	Variable	mean	SD	P
leukocyte	Basket	7.9933	2.04152	0.35
	Non Basket	8.9	2.79438	
neutrophil	Basket	47.4	9.02623	0.564
	Non Basket	49.4	9.70493	
monocyte	Basket	6.8867	1.56245	0.454
	Non Basket	7.1267	3.51069	





The results of this study were that the difference in the leukocyte counts, neutrophil counts, and monocyte counts were insignificant between basketball player group and non basketball player group after 12 minutes of moderate intensity aerobic physical exercise ($p > 0.05$) (table 4). It is also possible that this result is due to the delayed increase in neutrophils which is influenced by the suppressive effect of cortisol on the increase in neutrophils in the subject.

A similarity was observed in the study of Zar Abdossaleh et al., who obtained a statistically insignificant difference in the number of leukocytes before and after 60 minutes of moderate intensity physical exercise in male judo athletes ($p > 0.05$).²⁷ In addition, Bartlett DB et al., also observed insignificant changes in the leukocyte count between before and after 30-45 minutes of moderate intensity of physical exercise, three times a week, for 10 weeks in adults ($p > 0.05$).²⁸ Those two researches shared a similar finding with our research in which there were a insignificant effect of moderate intensity exercise to white blood cell count. Albeit those two researches, there were also studies which had a conflicting results with ours. Khosnam MS et al., Found that the mean white blood cell count of male athletes was significantly lower than that of non-athletic male after aerobic exercise.²⁹

This conflicting result may be due to the use of the Bruce protocol by Khosnam et al., as their exercise procedure in their study in which participants ran on a treadmill at a speed and dryness which increased slowly every 3 minutes until they were exhausted and unable to continue exercising.^{30,31} This protocol was clearly different from the training procedure we gave our study participants in which our samples weren't exerted to exhaustion. Therefore this difference is likely to cause a difference in exercise intensity between the Bruce protocol participants and our study participants.

Another reason for the statistically insignificant result of this research is the increasing cortisol levels after an acute physical exercise as mentioned in several studies.³²⁻³⁵ The cortisol affects the increase in neutrophils as

reported in the study of VanBruggen et al.³² an anti-inflammatory effect resulted from the suppression of pro-inflammatory cytokine expression by the increasing cortisol levels restrains the increase in the number of leukocytes.^{36,37} This notion is supported by a results of a study which showed that giving a glucocorticoid dose that resembles cortisol levels under a stress is able to suppress IL-6 and this effect is more significant than giving excessive glucocorticoid doses or suppressing glucocorticoids.³⁸ This indicates that the increase in cortisol in response to a stress (physical exercise) can affect the immune system.

In addition, cortisol levels may also be related to the timing of the physical exercise. Glucocorticoid levels increase in the morning and decrease at night. The Increasing morning glucocorticoids levels give rise to an increasing expression of CXCR4 in CD8 + and CD4 + cells which cause a remobilization of CD8 + and CD4 + cells from blood to bone marrow ,in which a lot of CXCL 12 are expressed, which activates CXCR4 therefore decreasing the number of CD8 + and CD4 + cells.³⁹ This phenomenon possibly suppresses the increase in the number of leukocytes thus resulting in an insignificant different between the white blood cell count of basketball player group and non-basketball player group. This notion is supported by a research done by Albayrak C. D et al., in which they found that the cortisol levels of basketball players during pre-competition training was inversely proportional to their white blood cell counts ($p < 0.05$) and their CD4 + cell counts ($P < 0.01$).⁴⁰

Another possibility that causes insignificant difference in the WBC count between samples of basketball players and non-basketball players after 12 minutes of moderate intensity aerobic exercise is the white blood cell (WBC) migration. the interaction between WBC and post-capillary venous endothelial cells around the site of inflammation is the main process in WBC migration. This interaction will cause the leukocytes to be trapped in the endothelial cells, then adhere to and migrate into the site of inflammation from the lumen of blood vessel.^{41,42,43} This migration process was likely to cause accumulation of WBCs in the veins of the lower extremities due to the prominent utilization of lower extremity muscles in our 12 minutes of moderate intensity aerobic exercise. On the other hand, the blood sample used came from the cubital vein therefore causing a mismatch between the location of blood collection and the location of inflammation so that the blood drawn does not reflect the ongoing inflammatory process. This results in an insignificant difference between the white blood cell count of basketball players and non-basketball players.

4. CONCLUSION

Based on the results and discussions of this research, the researcher concluded that moderate intensity aerobic exercise for 12 minutes had insignificant effect on the differences in the leucocyte counts, neutrophils counts, and monocyte counts of basketball players and non-basketball players. the insignificant result is possibly caused by the migration process of WBC and the lack of training duration and intensity which are important in the formation of muscle injury therefore resulting in a similar result from a measurement of resting blood samples, as described in the study of Baffour-Awuah B et al.³⁵ In addition, increasing cortisol levels resulted from stress induced exercised may also impact the finding of this research thus resulting in insignificant different between the leucocyte counts, neutrophils counts, and monocyte counts of basketball players and non-basketball players due to the cortisol anti inflammatory effect.

Furthermore, the research also found that the basketball player group had a better fitness level than the non-basketball player group and a statistically different cortisol level between both group with basketball player group has higher cortisol levels than non basketball player group

REFERENCES

1. Bennie JA, De Cocker K, Teychenne MJ, et al. The epidemiology of aerobic physical activity and muscle-strengthening activity guideline adherence among 383,928 U.S. adults. *Int J Behav Nutr Phys Act.* 2019;16(1):1–11.
2. Sand KL, Flatebo T, Andersen MB, et al. Effects of exercise on leukocytosis and blood hemostasis in 800 healthy young females and males. *World J Exp Med.* 2013;3(1):11.
3. Singh R, Pattisapu A, Emery MS. US physical activity guidelines: current state, impact and future directions. *Trends Cardiovasc Med.* 2019.
4. Kokkinos P. Physical activity, health benefits, and mortality risk. *ISRN Cardiol.* 2012;2012:1–14.
5. McKinney J, Lithwick DJ, Morrison BN, et al. The health benefits of physical activity and cardiorespiratory fitness. *B C Med J.* 2016;58(3):131–7.
6. Mallo Ferrer F. Physical activity and exercise [Internet]. 2nd ed. *Encyclopedia of Endocrine Diseases.* Elsevier Inc.; 2019. 436–441 p.

7. Nieman DC. Clinical implications of exercise immunology. *J Sport Heal Sci* [Internet]. 2012;1(1):12–7.
8. Piepoli MF, Hoes AW, Agewall S, et al. European Guidelines on cardiovascular disease prevention in clinical practice. *Eur Heart J*. 2016;37(29):2315–2381.
9. Singh Chahar P. Physiological basis of Growth and Development among Children and Adolescent in Relation to Physical Activity. *Am J Sport Sci Med*. 2014;2(5A):17–22.
10. Dananjaya. Perbedaan VO 2 maks atlet bola basket bimasakti nikko steel Malang dengan atlet sepak bola arema indonesia. Universitas Muhammadiyah Malanng; 2012
11. Gomes De Araujo G, Manchado-Gobatto FDB, Papoti M, et al. Anaerobic and aerobic performances in elite basketball players. *J Hum Kinet*. 2014;42(1):137–47.
12. Plowman SA, Smith DL. *Exercise physiology for health, fitness, and performance*. 4th ed. China: Lippincott Williams & Wilkins; 2014. 717 p.
13. WHO. *Global recommendation on physical activity for health*. Switzerland: WHO; 2010.
14. Patel H, Alkhawam H, Madanieh R, et al. Aerobic vs anaerobic exercise training effects on the cardiovascular system. *World J Cardiol*. 2017;9(2):134.
15. U.S. Department of Health and Human Services. *Physical activity guidelines for americans*. 2nd ed. Rodgers AB, editor. U.S. Department of Health and Human Services; 2013. 56–63 p.
16. Habibi E, Dehghan H, Moghiseh M, et al. Study of the relationship between the aerobic capacity (VO2 max) and the rating of perceived exertion based on the measurement of heart beat in the metal industries Esfahan. *J Educ Health Promot*. 2014;
17. Bandyopadhyay A. Validity of cooper’s 12-minute run test for estimation of maximum oxygen uptake in male university students. *Biol Sport*. 2015;32(1):59–63.
18. Penry J, Wilcox AR, Yun J. Validity and reliability analysis of cooper’s 12 minute run and the multistage shuttle run in healthy adults. *J Strenght Cond Res*. 2011;25(3):597–605.
19. Das B. Estimation of maximum oxygen uptake by evaluating cooper 12-min run test in female students of West Bengal, India. *J Hum Sport Exerc*. 2013;8(4):1008–14.
20. Simpson RJ, Lowder TW, Spielmann G, et al. Exercise and the aging immune system. *Ageing Res Rev* [Internet]. 2012;11(3):404–20.
21. Gleeson M, Bishop N, Walsh N. *Exercise immunology*. Exercise Immunology. New York: Routledge; 2013. 342 p.
22. Buchan DS, Ollis S, Young JD, et al. The effects of time and intensity of exercise on novel and established markers of CVD in adolescent youth. *Am J Hum Biol*. 2011;23(4):517–26.
23. MacPherson REK, Hazell TJ, Olver TD, et al. Run sprint interval training improves aerobic performance but not maximal cardiac output. *Med Sci Sports Exerc*. 2011;43(1):115–22.
24. Minetto MA, Lanfranco F, Baldi M, et al. Corticotroph axis sensitivity after exercise: Comparison between elite athletes and sedentary subjects. *J Endocrinol Invest* [Internet]. 2007 Mar 31;30(3):215–23.
25. Cevada T, Vasques PE, Moraes H, et al. Salivary cortisol levels in athletes and nonathletes: A systematic review. *Horm Metab Res*. 2014;46(13):905–10.
26. Abdossaleh Z, Fatemeh A, Frozan K, et al. Leukocytes subsets is differentially affected by exercise Intensity. *Int J Sport Stud* [Internet]. 2014;4(2):246–53.
27. Bartlett DB, Shepherd SO, Wilson OJ, et al. Neutrophil and monocyte bactericidal responses to 10 weeks of low-volume high-intensity interval or moderate-intensity continuous training in sedentary adults. *Oxid Med Cell Longev*. 2017;2017.
28. Baffour-Awuah B, Addai-Mensah O, Moses M, et al. Differences in haematological and biochemical parameters of athletes and non-athletes. *J Adv Med Med Res*. 2017;24(12):1–5.
29. Sadegh M, Khoshnam E, Abedi HA. The effect of aerobic physical exercise on immune system andhs- crp in male athlete and non-athletes. 2012;6(12):3023–7.
30. Hamlin M, Draper N, Blackwell G, et al. Determination of maximal oxygen uptake using the bruce or a novel athlete-led protocol in a mixed population. *J Hum Kinet*. 2012;31(1):97–104.
31. VanBruggen MD, Hackney AC, McMurray RG, et al. The relationship between serum and salivary cortisol levels in response to different intensities of exercise. *Int J Sports Physiol Perform*. 2011;6(3):396–407.
32. Muscella A, Vetrugno C, Spedicato M, et al. The effects of training on hormonal concentrations in young soccer players. *J Cell Physiol*. 2019;234(11):20685–93.
33. Budde H, Voelcker-Rehage C, Pietrassyk-Kendziorra S, et al. Steroid hormones in the saliva of adolescents after different exercise intensities and their influence on working memory in a school setting. *Psychoneuroendocrinology*. 2010;35(3):382–91.
34. Hackney AC, Viru M, VanBruggen M, et al. Comparison of the hormonal responses to exhaustive

- incremental exercise in adolescent and young adult males. *Arq Bras Endocrinol Metabol.* 2011;55(3):213–8.
35. Sato K, Iemitsu M, Katayama K, et al. Responses of sex steroid hormones to different intensities of exercise in endurance athletes. *Exp Physiol.* 2016;101(1):168–75.
36. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol Cell Endocrinol [Internet].* 2011;335(1):2–13.
37. Ince LM, Weber J, Scheiermann C. Control of leukocyte trafficking by stress-associated hormones. *Front Immunol.* 2019;10(JAN):1–9.
38. Yeager MP, Pioli PA, Guyre PM. Cortisol exerts bi-phasic regulation of inflammation in humans. *Dose-Response.* 2011;9(3):332–47.
39. Besedovsky L, Born J, Lange T. Endogenous glucocorticoid receptor signaling drives rhythmic changes in human T-cell subset numbers and the expression of the chemokine receptor CXCR4. *FASEB J.* 2014;28(1):67–75.
40. Albayrak CD, Çiftçi S, Beylero M, et al. Association of immune parameters with stress hormone levels in elite sportsmen during the pre-competition. *Int J Hum Sci.* 2013;10(1):1412–20.
41. Nourshargh S, Alon R. Leukocyte Migration into Inflamed Tissues. *Immunity [Internet].* 2014;41(5):694–707.
42. Huldani, Achmad H, Arsyad A, Putra AP, Sukmana BI, Adiputro DL, et al. Differences in VO2 max based on age, gender, hemoglobin levels, and leukocyte counts in Hajj prospective pilgrims in Hulu Sungai Tengah Regency, South Kalimantan. *Syst Rev Pharm.* 2020;11(4):9–14. . doi:10.31838/srp.2020.4.03.
43. Huldani , Siti Kaidah, Julia Kasab, Fauziah, Muhammad Hasan Ridhoni, Wafa Ahdiya. VO2 Max in River Beach communities in hst district by age, gender, and mobile immunity (neutrofil and limfosite). *European Journal of Molecular & Clinical Medicine,* 2020; 7(8): 1126-1132