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Physical Exercises By Giving Glutathione for Changes of Leukocytes, Neutrophils, and Lymphocytes Amounts

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Abstract

Physical exercise changes the antibody endurance of each person. This study aims to determine in depth changes in the number of leukocytes, neutrophils, and lymphocytes using physical exercise after being given glutathione. The method used experimentally using a posttest randomized control group design. The study was conducted in three groups where each group consisting of men and women who are in the age range of 20-30 years. Each group consisted of 10 people (n), K1 was given a placebo, K2 was given glutathione 54 mg / kg BW, K3 given 108 mg / kg BW. All treatments are given intraperitoneally twice a day until the third day. Maximum physical exercise is swimming. On the third day after 3 hours of treatment (placebo or glutathione), then participants take a leukocyte profile measurement. The findings show that the number of leukocytes: K2> K1; P = 0.005; K2 - K3; P = 0.168; - K3; P 0.105, neutrophils: K1, K2 and K3 are not different, even though K2 is highest, lymphocyte: K2> K1; P = 0.003, K1 - K3 and K2 - K3 is not different even though K2 is highest. The conclusion of this study is the number of leukocytes and lymphocytes treated with glutathione 54 mg / kg BW (K2) is significantly higher than K1 after strenuous physical exercise. Thus, the content of glutathione must be balanced with the body's needs so that the body is healthy and is able to do the heavy activities.

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INTRODUCTION

Every physical activity will increase free radicals, the higher of the intensity is more the free radicals produced, it is to neutralize excessive free radicals are necessary to consume antioxidants or antioxidant substances. Although the body itself can produce antioxidants, the amount is limited (Halliwell, 2001; McBride, Kraemer, Triplett-McBride, & Sebastianelli, 1998). Free radicals that occur in heavy physical activity and excessive, especially in several heavy and long-term sports (10K running, boxing, soccer, badminton, triathlon) can reduce the profile of leukocytes and the immune system (Halliwell, 2001; McBride et al., 1998; Metin, Gümüstas, Uslu, Belce, & Kayserilioglu, 2003). Thus, the athletes are susceptible to infectious diseases that may be carried by the audience, resulting in a decrease in appearance in the next match (Kowatari et al., 2001). Pre-match glutathione is expected to reduce the decrease in leukocyte profile and to neutralize excessive free radicals (Morozov, Tsyplenkov, Golberg, & Kalinski, 2006; Cases et al., 2005; Sureda et al., 2008; James et al., 2005).

Therefore, everyone needs exercise to be able to produce the amounts of antioxidants that meet the body's needs. Besides, spiritual health conditions such as stress management can also affect. So, exercise induces oxidative stress in most individuals, it can cause reductive stress or stress that can be ignored in a large number of people (Margaritelis et al., 2014). Regular physical exercise is highly recommended to achieve a healthy lifestyle (Marmett, Böek Carvalho, Pires Dorneles, Barcos Nunes, & Ramos Rhoden, 2020). While one physical exercise session can represent a challenge for cell homeostasis, a repetitive physical exercise session will increase immune surveillance and immunocompetence (Scheffer & Latini, 2020).

In some previous research that studied about giving off the maximal burden during physical exercise or in severe and prolonged fatigue can cause suppression of immune function through changes in lymphocytes that become less or less (Castel, 1993). Maximum work can trigger an imbalance between the production of free radicals and the body's antioxidant defence system known as oxidative stress (Leeuwenburgh & Heinecke, 2001). According to Ji (1999), during maximum work, oxygen consumption throughout the body increases to 20 times and oxygen consumption in muscles increases 100 times. This increase will result in increased production of free radicals that can cause lymphocyte cell damage (Pendersen & Toft, 2000)

To reduce the risk of free radical formation after exercising, one way is to consume antioxidants before doing maximum work. One of the recommended antioxidants is glutathione because the glutathione contained in the body is low that it is not enough to cope if large amounts of free radicals are formed in the body, as occurs in performance sports performers (Simioni et al., 2018)

Glutathione, also called Glutathione SulphHydril (GSH), is a protein that is naturally produced in the body that plays an important role in the immune system and cell regeneration, is antioxidant and anti-toxin. Glutathione is very necessary for the body, without Glutathione other antioxidants such as Vitamins C and E will not work optimally, which is why Glutathione is called a master-Antioxidant. Without Glutathione, lymphocytes cannot produce antibodies to fight bacterial and viral infections (Jimmy, 2010; Minich & Brown, 2019). Antioxidant compounds are also produced endogenously by the body such as glutathione (Halliwell, 1999; Evans, 2000). GSH functions in the immune system especially in the formation, division, purification and defence of T lymphocyte cells which are the leading defence mechanism of infection (Desai, Grolleau-Julius, & Yung, 2010; Gutman, 2002). Glutathione plays an important role in the formation of lymphocytes that occur in the formation of an effective immune response. The ability of lymphocytes to inhibit oxidation damage can be measured by the ability of these cells to recover intracellular GSH. The ability of lymphocytes to neutralize oxidative damage depends on intracellular GSH reserves that can respond better to antigenic stimuli.

The human body has units of the body's defence system to fight various kinds of infectious and toxic agents. This system is called leukocytes which consist of eosinophils, basophils, neutrophils, lymphocytes and monocytes. From several studies found that glutathione plays an important role in the formation of lymphocytes to protect the immune system against infectious and toxic agents and damage caused by the formation of free radicals due to maximal and prolonged work.

From previous studies, it is known that studies of physical exercise are affected by the presence of free radicals and food intake that affect the body's organs. However, these studies have not focused on physical exercise with the administration of glutathione which impacts on changes in the number of leukocytes, neutrophils, and lymphocytes. However, this research is important to know how big is the role of glutathione on leu-

kocyte profile in responding to the emergence of free radicals through maximal physical exercise and the duration of exercise using the object of examination in white rats (Rattus norvegicus strain wistar) given glutathione before it is won. So, the purpose of this study is to find out more about the effect of physical exercise by giving glutathione before exercise and the impact of changes that occur after exercise seen from the number of leukocytes, neutrophils, and lymphocytes.

METHODS

This study used the laboratory experimental method. The study design uses the randomized posttest only control group design. Laboratory experiments can usefully supplement established methods in innovation research and provide new empirical evidence by creating and analyzing counterfactual situations (Brüggemann & Bizer, 2015).

This study used 30 participants (@ 10 = n). The study was conducted in three groups where each group consisting of men and women who are in the age range of 20-30 years. Sample are divided into 3 groups; the control group or the first group (K1) were given a placebo for 3 days, it is given twice daily intraperitoneally, the second group (K2) were given glutathione 54 mg/kg BW in the same way, the third group (K3) were given glutathione 108 mg/kg BW. The variables measured were body weight, total leukocytes, neutrophils and lymphocytes. Then the statistical difference test is performed. The collected data is tested for normality. After that, the data analysis process uses variance analysis.

RESULTS AND DISCUSSION

Measuring the magnitude of the variable physical exercise was done to determine the initial conditions of experimental animals. Following are the results **Table 1** of weight measurements obtained from the three groups of research samples.

Table 1. The data of Body Weight

Group @ 10 (n)	Bodyweight/kg
Plasebo Group (K1)	45-50 kg
Glutathione Group 54 mg / kgBW (K2)	50-60 kg
Glutathione group 108 mg / kgBW (K3)	60-70 kg

Different BW test for the three groups: P = 0,598

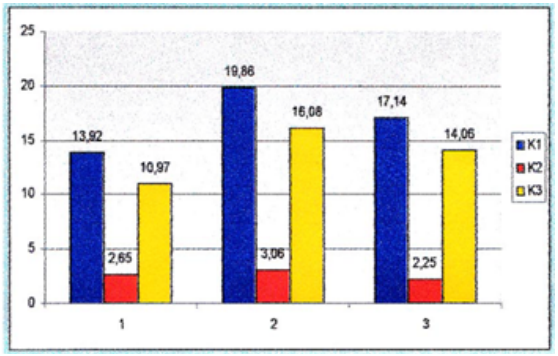
From the results **Table 1** of the weight difference test for each group showed that there is no significant difference. Then the total number of leukocytes, neutrophils and lymphocytes measured, the results are as follows **Table 2**:

Table 2. Descriptive data measuring the number of leukocytes, neutrophils and lymphocytes, immediately after physical activity.

Group @ 10 (n)	Leukocytes X ± SDx103/ mm3	Neutrophils X ± SDx103/ mm3	Lymphocy X ± SDx103/ mm3
Plasebo Group (K1)	13,92 ± 3,97	2,65 ± 1,39	10,97 ±3,53
Glutathione Group 54 mg / kgBW (1(2)	19,86 ± 4,Q1	3,06 ± 1,58	16,08 ±3,05
Glutathione group 108 mg / kgBW (K3)	17,14 ± 4,85	2,25 ± 1,21	14,06 ± 4,01

The results **Table 2** of data analysis can be concluded that:

1. Total leukocytes: K1 with K2 ; P = 0.005; K2 with K3: P = 0.168; K1 with K3: P = 0.105.
2. Neutrophils: K1, K2 and 1 (3 there is no significant difference, even though K2 is the highest.
3. Lymphocytes: Kl with K2: P = 0.003; K2 with K3: P = 0.059; Kt with K3: P = 0.210.



Graph 1. The difference of amount leukocytes, neutrophils, and lymphocyte after the group did the physical exercise

It is from giving 54 mg/kg BW of glutathione, the highest number of leukocytes and lymphocytes was very significant, while neutrophil (K2) was higher than (K1) or (K3) but it is not significant. While a dose of 108 mg/ kg does not increase the number of leukocytes, neutrophils and lymphocytes. It can be seen from the below **Graph 1**.

Mild to moderate physical activity is recommended for health and fitness. But in sports achievements or athletes performed heavy, overloaded and progressive sports (Halliwell, 2001a). Immediately after strenuous physical activity, including after a match, there will be a decrease in the profile of leukocytes (Kowatari et al., 2001; Metin et al., 2003; Cases et al., 2005). By providing antioxidants such as vitamins A, C and E are often not enough to neutralize the free radicals that occur. Thus, it is by giving glutathione, it can help reduce the decrease in leukocyte profile that occurs. It turns out that exogenous glutathione cannot be given orally, because it will be damaged by enzymes in the digestive tract (Cases et al., 2005). Some foods that are recommended for consumption because they contain glutathione, are not fast enough to increase glutathione in the body, because glutathione synthesis mainly occurs in Kati cells.

Giving glutathione intravenously has been done in research abroad turned out to be able to increase the profile of leukocytes, whereas in Indonesia is still very rarely done, because there has been no official report on the use of glutathione in athletes. The use of glutathione in Indonesia, especially in cosmetics as a skin whitener, it reduces skin melanin. In the results of this study showed that the administration of glutathione 54 mg/kg BW reduced the decrease in the number of leukocytes, lymphocytes were very significant, whereas neutrophil changes were not significant, maybe the number of samples was lacking. What is interesting is the provision of 108 mg/kg BW did not increase the number of leukocytes, neutrophils and lymphocytes. The possibility is the suppression of endogenous glutathione synthesis, with excessive antioxidant administration. So, it is recommended giving antioxidants should be by needs.

Regular physical activity seems to be one of the most important contributors to preventing disease and improving health. Being physically active reduces the risk of developing chronic diseases. The immune system also plays an important role in the development of atherosclerosis, thus making white blood cells relevant to study when looking at molecular mechanisms caused by physical activity. The effects of exercise on the body are very diverse. Although the research designs, groups included, and types of exercise used in the studies included in this review vary, it seems reasonable to conclude that exercise affects cells of the immune system. Genes that are regulated after exercise are involved in inflammation, cellular communication, signal transduction, cellular protection, growth, and repair (Gjevestad, Holven, & Ulven, 2015). Intensive exercise is physiological stress which can encourage the interaction of neutrophils with muscle endothelial cells and transmigrate them into the tissues. The mechanism that drives this physiological inflammatory response is unknown.

CONCLUSION

All physical activity will produce free radicals. The body will form endogenous antioxidants to neutralize. The higher intensity and the longer of the physical activity produced the higher the production of free radicals if antioxidants are not enough in the body's production, the free radicals will give abnormalities to the body's cells; cell membranes, organelle and can occur in the nucleus. It is including the decline in leukocyte activity and the amount. By giving exogenous antioxidants, glutathione, among others can reduce the decrease in the profile of leukocytes, if given excessively, it will give unexpected results. Thus, giving glutathione must be adjusted to the conditions of the body's needs and body weight in order to provide maximum benefit, so that people can do heavy activities well.

REFERENCES

Brüggemann, J., & Bizer, K. (2015, December 1). Laboratory experiments in innovation research: a methodological overview and a review of the current literature. Journal of Innovation and Entrepreneurship. SpringerOpen. https://doi.org/10.1186/s13731-016-0053-9

Cases, N., Aguiló, A., Tauler, P., Sureda, A., Llompart, I., Pons, A., & Tur, J. A. (2005). Differential response of plasma and immune cell's vitamin E levels to physical activity and antioxidant vitamin supplementation. European Journal of Clinical Nutrition, 59(6), 781–788. https://doi.org/10.1038/sj.ejcn.1602143

Castel. (1993). Exercise and Immune System. Sports of Physiology, 324–325.

Desai, A., Grolleau-Julius, A., & Yung, R. (2010). Leukocyte function in the ageing immune system. Journal of Leukocyte Biology, 87(6), 1001–1009. https://doi.org/10.1189/jlb.0809542

Evans, W. J. (2000). Vitamin E, vitamin C, and exercise. Am J Clin Nutr, 72, 647S-52S.

Gjevestad, G. O., Holven, K. B., & Ulven, S. M. (2015, July 26). Effects of Exercise on Gene Expression of Inflammatory Markers in Human Peripheral Blood Cells: A Systematic Review. Current Cardiovascular Risk Reports. Current Medicine Group LLC 1. https://doi.org/10.1007/s12170-015-0463-4

Gutman. (2002). Leukocyte and Immune System. Sports Medicine, 671-672.

Halliwell, B. (1999). Free Radicals in Biology and Medicine. New York: Oxford University Press.

Halliwell, B. (2001a). Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treat¬ment. Drags Aging, 18(9), 685-716.

Halliwell, B. (2001b, August 31). Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs and Aging. Adis International Ltd. https://doi.org/10.2165/00002512-200118090-00004

James, S. J., Slikker, W., Melnyk, S., New, E., Pogribna, M., & Jernigan, S. (2005). Thimerosal neurotoxicity is associated with glutathione depletion: protection with glutathione precursors. NeuroToxicology, 26(1), 1–8. https://doi.org/10.1016/j.neuro.2004.07.012

Ji, L. L. (1999). Antioxidants and oxidative stress in exercise. In Proceedings of the Society for Experimental Biology and Medicine (Vol. 222, pp. 283–292). Blackwell Publishing Inc. https://doi.org/10.1046/j.1525-1373.1999.d01-145.x

Jimmy. (2010). Immune System, Exercise and Oxidative Stress. Experimental Blood and Immune System., 131–132.

Kowatari, K., Umeda, T., Shimoyama, T., Nakaji, S., Yamamoto, Y., & Sugawara, K. (2001). Exercise training and energy restriction decrease neutrophil phagocytic activity in judoists. Medicine and Science in Sports and Exercise, 33(4), 519–524. https://doi.org/10.1097/00005768-200104000-00003

Leeuwenburgh, & Heinecke. (2001). Sports and Stress Oxidative. Med Sci Sports Med, 32, 158-159.

Margaritelis, N. V., Kyparos, A., Paschalis, V., Theodorou, A. A., Panayiotou, G., Zafeiridis, A., … Vrabas, I. S. (2014). Reductive stress after exercise: The issue of redox individuality. Redox Biology, 2(1), 520–528. https://doi.org/10.1016/j.redox.2014.02.003

Marmett, B., Böek Carvalho, R., Pires Dorneles, G., Barcos Nunes, R., & Ramos Rhoden, C. (2020). Should i stay or should i go: can air pollution reduce the health benefits of physical exercise? Medical Hypotheses, 144, 109993. https://doi.org/10.1016/j.mehy.2020.109993

McBride, J. M., Kraemer, W. J., Triplett-McBride, T., & Sebastianelli, W. (1998). Effect of resistance exercise on free radical production. Medicine and Science in Sports and Exercise, 30(1), 67–72. https://doi.org/10.1097/00005768-199801000-00010

Metin, G., Gümüstas, M. K., Uslu, E., Belce, A., & Kayserilioglu, A. (2003). Effect of Regular Training on Plasma Thiols, Malondialdehyde and Carnitine Concentrations in Young Soccer Players - PubMed. Chin J Physiol, 46(1). Retrieved from https://pubmed.ncbi.nlm.nih.gov/12817703/

Minich, D. M., & Brown, B. I. (2019). A Review of Dietary (Phyto) Nutrienys for Glutathione Support. Nutrients, 11(9).

Morozov, V. I., Tsyplenkov, P. V., Golberg, N. D., & Kalinski, M. I. (2006). The effects of high-intensity exercise on skeletal muscle neutrophil myeloperoxidase in untrained and trained rats. European Journal of Applied Physiology, 97(6), 716–722. https://doi.org/10.1007/s00421-006-0193-x

Nunes-Silva, A., Bernardes, P. T. T., Rezende, B. M., Lopes, F., Gomes, E. C., Marques, P. E., … Pinho, V. (2014). Treadmill Exercise Induces Neutrophil Recruitment into Muscle Tissue in a Reactive Oxygen Species-Dependent Manner. An Intravital Microscopy Study. PLoS ONE, 9(5), e96464. https://doi.org/10.1371/journal.pone.0096464

Pendersen, B. K., & Toft, A. D. (2000, August 1). Effects of exercise on lymphocytes and cytokines. British Journal of Sports Medicine. British Association of Sport and Excercise Medicine. https://doi.org/10.1136/bjsm.34.4.246

Scheffer, D. da L., & Latini, A. (2020, October 1). Exercise-induced immune system response: Anti-inflammatory status on peripheral and central organs. Biochimica et Biophysica Acta - Molecular Basis of Disease. Elsevier B.V. https://doi.org/10.1016/j.bbadis.2020.165823

Simioni, C., Zauli, G., Martelli, A. M., Vitale, M., Sacchetti, G., Gonelli, A., & Neri, L. M. (2018, March 30). Oxidative stress: Role of physical exercise and antioxidant nutraceuticals in adulthood and aging. Oncotarget. Impact Journals LLC. https://doi.org/10.18632/oncotarget.24729

Sureda, A., Tauler, P., Aguiló, A., Cases, N., Llompart, I., Tur, J. A., & Pons, A. (2008). Influence of an antioxidant vitamin-enriched drink on pre- and post-exercise lymphocyte antioxidant system. Annals of Nutrition & Metabolism, 52(3), 233–240. https://doi.org/10.1159/000140515.