The Effectiveness of Vitamin C as an Antioxidant Against the Pulse of Recovery After Physical Activity in the New Normal

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Abstract. Physical activities in new normal led to changes in body temperature as well as the amount of oxygen consumption, pulse and changes in chemical compounds in the body. Vitamin C can prevent tissue damage by reducing the production of oxidants which have a protective effect against muscle injury. This study aims to determine the effect of vitamin C as an antioxidant on pulse recovery after physical activity on hockey athletes of PON South Sulawesi in the new normal. This type of research is an experimental with a randomized group design pretest and posttest design. The Subjects of this study were 30 South Sulawesi PON hockey athletes and the data was analyzed with T test. The result of this research showed that the group which was given Vitamin C is greater than the group that was only given unsweetened syrup with comparison 48,60 and 39,60.Thus, can be concluded that the provision of vitamin C has better effects on pulse recovery compared to the group that was only given unsweetened syrup.

Keywords: Recovery, Physical activity, Pulse, New normal.

1 Introduction

During the Covid 19 pandemic, it is not new anymore that the implementation of any program is carried out independently, including physical activity (exercise). Since the WHO declared global Covid-19 emergency in January 30, 2020 [1]. Public physical activity during the COVID-19 pandemic in several countries has decreased, so that many people have gained weight and degraded of mental health [2,3] In total, the average time spent on physical activity has decreased dramatically, from 540 minutes/ week (before the pandemic) to 105 minutes/ week (during the pandemic), resulting 435 minutes in total average [4] In the new normal, we need to prepare and develop ourselves to carry out a healthy lifestyle in order that our immune system is maintained, one way is by doing an exercise [5]. Several studies have shown that physical exercise increases the level of cytokine production mediated through the TLR (toll-like receptor) signaling pathway during microbial infection could increase host resistance to invasive pathogens [6,7]. Exercise is one of the physical activities with a specific purpose, including it could increase the efficiency of the body's work or what is commonly referred as (increasing physical fitness). Exercise is also a movement of the body for a certain period of time[8]. Exercise affects energy balance and leptin response, type I IFN responsiveness, muscle PDC activation, further increasing serum anti-influenza virus-specific IgG2c antibody percentage and

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CD8 + T cells in BAL; all possible mechanisms are important in host protection and infection [6,9].

Hockey is an exercise or a sport which is always competed in the highest multi-events in the world of the Olympics, Asian Games, SEA Games, and other championships including the National Sports Week or originally called Pekan Olahraga Nasional (PON). Therefore, hockey is a sport that requires a lot of energy, so that athletes are required to have a good level of physical condition to achieve optimal performance. A good hockey player needs to develop physical fitness, especially in this new normal. Physical activity requires 9 support systems that needed energy so that movements are performed by muscles[10], physical activity that is carried out regularly, with a certain period of time will improve one's physiological condition. Where the perpetrator of every physical activity in sports training results in changes in the anatomical, biochemical, psychological, and physiological conditions. Thus, exercise can train the body to improve the functional abilities of the physic [11]. An additional benefit of exercise is an increase in the antioxidant defense system and a reduction in oxidative stress [12,13]. However, if our bodies do physical activity with high intensity, the body will become weak and the ability to move within normal limits is reduced, in addition, besides the cooperation and cohesiveness of hockey athletes, they must also have excellent physical condition, especially endurance so that athletes do not get tired easily. Exercise will cause a response in the form of an increase in pulse rate, so that an increase in blood lactic acid levels and pulse are often used as indicators of intensity in exercising [7,14,15].

The frequency of heart rate and stroke content will increase 95% as long as a person does maximum exercise. Thus, the body is said to be experiencing fatigue[8] further involvement of intense (continuous) training without adequate recovery will lead to accumulated fatigue. Some heart rate monitors can be used to predict maximal oxygen absorption (VO2Max), so as to identify changes in fitness during exercise and early signs of overtraining [16]. Poor physical condition can be improved by increasing endurance and fast recovery [17,18] therefore athletes must have good physical condition in order to recover quickly [19] endurance has an influence on the body's ability to perform recovery. Maximum physical activity can trigger an imbalance between the production of free radicals and the body's antioxidant defense system known as oxidative stress [13,20].

During maximum physical activity, oxygen consumption throughout the body increases even up to 20 times, whereas oxygen consumption in muscle fibers is estimated to increase 100-fold [21] this increase in oxygen results in increased production of free radicals which can cause cell damage [15] defense of endogenous antioxidants and exogenous antioxidants to ward off free radicals. Antioxidants are substances that can delay, prevent or eliminate free radicals [22]. Coconut sugar has benefits, namely as an anti-bacterial, anti-inflammatory and antioxidant, where the antioxidant content in coconut sugar, one of which is Vitamin C [23]. The concentration of Vitamin C in the body that experiences oxidative stress is lower than the level of vitamin C in the normal body [24].

Physical activity given to the hockey athletes of PON South Sulawesi in this new normal, resulted in changes in body temperature and the amount of oxygen consumption, pulse and changes in chemical compounds in the body. Vitamin C as an antioxidant can reduce the production of oxidants so that it can reduce levels of free radicals in the body, prevent tissue damage, protective effect against muscle injury. With this, this study aims to determine the effect of giving vitamin C as an antioxidant on pulse recovery after physical activity on hockey athletes of PON South Sulawesi in the new normal.

2 Method

This research used an experimental research design by using randomized group pre-test and post-test design. This method is used to determine the effect of one or more variables on other variables. According to Arikunto, Experimental research is research that is intended to determine whether there is a result of something that is imposed on the subject inquired. The subjects in this study were South Sulawesi's hockey male athletes of PON, have a weight between 50-70 kg, have an age between 18-26 years, have a normal body mass index (BMI) and blood pressure, are not undergoing medical or postoperative treatment 6 months before the study, are not seriously injured, does not consume energy drinks, does not do any physical activity before the test, did not have a history of cardiovascular disease. The total of samples was 30 divided randomly into 2 groups. The first group was the control group (A1) and the second group was the treatment group (A2), each group consisting of 15 people. Retrieval of research data in the form of a pulse was held at the UNM Hockey Field. The researcher used a stopwatch to count pulse. Pulse measurement would be done 2 times, the first before treatment (pretest), then 15 minutes after consuming vitamin C (posttest). In this study, the measurement of physical activity was carried out (push-ups, sit-ups, and harvard step tests) where the sample would perform the physical activity in 60 seconds (1 minute). Kolmogorov Smirnov was used to test the normality of the data. The test criteria, if the statistical significance of the calculated value is greater than $\alpha = 0.05$ (5%), the data distribution is normal. To calculate the data homogeneity test, the Levene Test formula was used. The test criteria, if the statistical significance of the calculated value is greater than $\alpha = 0.05$ (5%), the data variance is homogeneous. The data analysis technique used is the T-test using the SPSS 25 program.

3 Results and Discussion

3.1 The characteristics of research subjects

Table 1. Descriptive statistics of sample (average : age, height, weight, IMT)

	N	Minimum	Maximum	Mean
Age	30	18	26	19.67
Weight	30	52	70	59.33
Height	30	144	170	161.90
Body Mass index	30	17	24	18.89
Valid N (listwise)	30			

The characteristics of research subjects include: age, weight, height, and body mass index, in treatment 1 and 2. Characteristics of research subjects before receiving treatment could be seen in Table 1 above. Table 1 showed that the mean age of the subjects was 19.67 years, with an average weight and height of 59.33 kg and 161.90 cm. The Body Mass Index (BMI) of the subjects averaged 18.89 kg/ m^2.

Table 2. Descriptive statistics of pulse.

Variabel	Mean	Median	Std. Deviation	Minimum	Maximum
Pretest Vitamin C	118. 87	119.00	2.588	114	123
Posttest Vitamin C	70.33	71.00	5.460	62	80
Pretest Unsweetened Syrup	112.67	113.00	2.410	109	117
Posttest Unsweetened Syrup	73.07	73.00	3.195	117	78

Meanwhile, the pulse during restimg or the pulse before doing physical activity was carried out in this study (push-ups, sit-ups, and harvard step test). Treatment 1 was given Vitamin C and treatment 2 was given with unsweetened syrup. Each of them has a mean of 118.00 times / minute and 112.67 times / minute. It could be seen in Table 2.

3.2 Pulse recovery

Table 3. Test of normality, *kolmogorov*-smirnov

Variabel	Statistic	df	Sig
Pretest Vitamin C	.187	15	.165
Posttest Vitamin C	.132	15	.200
Pretest Unsweetened Syrup	.110	15	.200
Posttest Unsweetened Syrup	.141	15	.200

To fulfill the statistical test that would be used, the normality test of the data from the measurement of the speed of pulse recovery after treatment is carried out first. The normality test used the Kolmogorov-Smirnov test, which showed that in treatment 1, by giving vitamin C as much as 250 cc from 500 grams of dissolved vitamin C, before doing physical activity (push ups, sit ups, and harvard step tests) the value p> 0.05, while in treatment 2, by giving 250 cc of unsweetened syrup, p value> 0.05. The data could be seen in Table 3 . Furthermore, based on the homogeneity test table below, it can be seen in Table 4 using Levene Statistic analysis, obtained a sig value of P> 0.05. So it can be concluded that the data has a homogeneous variance.

Table 4. Homogeneity test, *Levana Statistic.*

Variabel	**Levena Statistic**	**Df1**	**Df2**	**Sig**
Pulse Rate Pretest	.067	1	28	.797
Pulse Posttest	4.036	1	28	.054

Thus, the pulse recovery data after doing physical activity (push-ups, sit-ups, and harvard step tests) had a homogeneous and normal distribution. Based on this normality test, the statistical test used to compare the effect of vitamin C and unsweetened syrup on pulse recovery after physical activity (push-ups, sit-ups, and harvard step tests) was a parametric test, paired t test. test). To compare the effect of giving Vitamin C and unsweetened syrup on pulse recovery after

physical activity (push-ups, sit-ups, and harvard step tests), a parametric statistical test was applied which was the paired T-test, the results were showed in the table. 5 and Table 6.

3.5 T-Test (Paired Sampel T-Test)

Table 5. The effect of giving Vitamin C and unsweetened syrup on recovery of the pulse.

	Std. Deviation	t	df	Sig. (2-tailed)
Pair 1- Pretesst-postttest Vitamin C	6.022	31.213	14	.000
Pair 1- Pretesst-postttest Unsweetened Syrup	4.323	35.480	14	.000

Based on the paired sample T-test table in Table 5. Pair 1 was given vitamin C and Paired 2 was given unsweetened syrup using the paired sample t-test above, it could be seen in the table above, the sig value is above 0,000. So it can be concluded that there was an effect of giving Vitamin C as an antioxidant on pulse recovery, as well as giving unsweetened syrup.

Table 6. Differences in the effect of vitamin C and unsweetened syrup.

Variabel	N	Mean
Difference in Vitamin C	15	48.60
Differences in Unsweetened syrup	15	39.60

Based on the results of the analysis using paired t-test, using the mean difference technique in each group. The results obtained were that if the average group given vitamin C as an antioxidant was greater the result was 48.60 than the group given unsweetened syrup was 39.60, the data can be seen in Table 6 above. The difference in pulse recovery in the two treatment subjects was statistically significant, with a value of $p = 0.000$ ($p < 0.05$). The subjects of this study were 30 hockey athletes of PON, the subjects were selected and determined after fulfilling the research criteria set by the researcher. The mean age of the subjects was 19.67 years. The average weight of the subjects was 59.33 kg, while the mean height of the subjects was 161.90 cm. The mean body mass index (BMI) of the subjects was 18.89 kg / m^2 which gave a description of a person's nutritional status. The average resting pulse rate in both treatments was treatment 1, has given Vitamin C and treatment 2 hs given unsweetened syrup, each of which had a mean of 118.00 times/minute and 112.67 times/minute. To determine the distribution of research subjects before treatment, a normality test was carried out using the Kolomogorov-Smirnov. The variables tested included resting pulse rates for treatment 1 and 2. The statistical test results for normality showed a normal distribution ($p > 0.05$) for all variables.

In this study the subject was given 2 different treatments. The first treatment was given 250 cc of Vitamin C and the two different treatment subjects was given 250 cc of unsweetened syrup. Before being given vitamin C and unsweetened syrup, the resting pulse rate was measured first. Thirty minutes later, the subjects of the research carried out physical activities (push-ups, sit-ups, and Harvard step tests) while still paying attention to health protocols, the physical activity was modified for 3 minutes, which was divided into 3 stages with each stage being 1 minute. After training, the measurement of the pulse recovery to the resting pulse is measured. Based on data analysis on the average rate of pulse recovery in the two types of treatment, it was found that treatment 1, which was given vitamin C as much as 250 cc, 30 minutes before doing physical activity (push-ups, sit ups, and harvard step tests) had a pulse recovery pulse better than treatment 2, which was given 250 cc of unsweetened syrup.

Exercise causes excessive sweating, the content in it is a variety of electrolytes and other substances, which vary from individual to person and for each individual will also differ depending on the condition [25]. Research shows that loss of fluids equivalent to 2% of body mass can cause decreased performance and loss of fluids by 5-6% of body weight will increase the pulse rate[26]the work of the heart will automatically increase.To maintain stable blood flow in supplying oxygen and energy fuel to muscles, giving effective fluids will minimize changes in the pulse so that will delay fatigue and shorten the length of the pulse recovery period [27,28]. The main response that occurs during physical activity is an increase in body metabolism. All energy supply systems are involved in this response with a relative proportion of contribution, depending on the intensity and duration of activity [25] while physical exercise/ exercise also induces an increase in oxygen uptake associated with a 10 to 20-fold increase in cellular metabolism and intensive production of radicals oxygen [13,18].

When doing physical activity or exercising, it will result in an increase in pulse which is caused by reduced oxygen consumption in the body. Free radicals formed can react with macro molecules in cells such as DNA and proteins or with membrane lipids, causing damage to cell function [29]. If the number of free radicals exceeds the body's ability to cope with it, a condition called oxidative stress can arise [15,26,30]. The presence of oxygen radicals as a result of the use of oxygen by these cells can be overcome by an antioxidant system that is both enzymatic and nonenzymatic contained in body or those from outside the body (exogenous)[22,23,31]. As an antioxidant, Vitamin C has the ability to be 2 times higher than α-carotene and 10 times better than α-tocopherol. Antioxidants will protect important cellular biomolecules, including lipids, proteins and DNA thereby creating an initial defense against free radicals in the body[24,29,32].

Thus, giving vitamin C will increase the body's ability to ward off free radicals that arise as a result of an increase in cellular metabolism during physical activity or exercise. To find out the comparison of the two types of treatment in restoring the return of the exercise pulse to the resting pulse, it is seen through the paired t test. Based on the results of data analysis, it can be seen that the average pulse recovery in treatment 1 is given 250 cc of vitamin C which has been dissolved in water after physical activity (push-ups, sit ups, and Harvard step tests) for 3 minutes and treatment 2, given 250 cc of unsweetened syrup before doing the same physical activity is significant, which was seen as $p = 0.000$ ($p < 0.05$).

4 Conclusion

There were significant differences. The results of data analysis showed that the group which was given Vitamin C is greater than the group that was only given unsweetened syrup with

comparison 48,60 and 39,60. Thus, can be concluded that the provision of vitamin C has better effects on pulse recovery compared to the group that was only given unsweetened syrup to hockey athletes of PON South Sulawesi after physical activity in the new normal era.

References

[1] Harapan H, Itoh N, Yufika A, Winardi W, Keam S, Te H, et al. Coronavirus disease 2019 (COVID-19): A literature review. J Infect Public Health 2020;13:667–73. https://doi.org/10.1016/j.jiph.2020.03.019.

[2] Eirale AC, Bisciotti G, Corsini A, Baudot C, Saillant G, Chalabi H. Medical recommendations for home-confined footballers ' training during the COVID-19 pandemic : from evidence to practical application 2020:203–7.

[3] Boschilia B, Cristina L, Moraes L, Junior WM, Cristina L, Moraes L. Football and COVID-19 : the effects of the pandemic on training and performance of South American and Brazilian referees and performance of South American and Brazilian referees. Soccer Soc 2020;00:1–8. https://doi.org/10.1080/14660970.2020.1829597.

[4] Xiang M, Zhang Z, Kuwahara K. Impact of COVID-19 pandemic on children and adolescents' lifestyle behavior larger than expected. Prog Cardiovasc Dis 2020;63:531–2. https://doi.org/10.1016/j.pcad.2020.04.013.

[5] Hammami A, Harrabi B, Mohr M, Krustrup P. Managing Sport and Leisure Physical activity and coronavirus disease 2019 (COVID-19): specific recommendations for home- based physical training. Manag Sport Leis 2020;0:1–6. https://doi.org/10.1080/23750472.2020.1757494.

[6] Zheng Q, Cui G, Chen J, Gao H, Wei Y, Uede T, et al. Regular Exercise Enhances the Immune Response Against Microbial Antigens Through Up-Regulation of Toll-like Receptor Signaling Pathways. Cell Physiol Biochem 2015;37:735–46. https://doi.org/10.1159/000430391.

[7] Rusmania N. No Title空間像再生型立体映像の 研究動向. Nhk技研 2015;151:10–7. https://doi.org/10.1145/3132847.3132886.

[8] Subrata E, Hakim AA. PENGARUH PEMULIHAN AKTIF JOGGING TERHADAP TINGKAT DENYUT NADI SETELAH AKTIVITAS SUBMAKSIMAL PADA SISWA SMKN 1 DRIYOREJO KABUPATEN GRESIK Enes Subrata S1 Ilmu Keolahragaan , Fakultas Ilmu Olahraga , Universitas Negeri Surabaya Abdul Aziz Hakim. Kesehat Olahraga 2019;07:313–8.

[9] Warren KJ, Olson MM, Thompson NJ, Cahill ML, Wyatt TA, Yoon KJ, et al. Exercise improves host response to influenza viral infection in obese and non-obese mice through different mechanisms. PLoS One 2015;10:1–27. https://doi.org/10.1371/journal.pone.0129713.

[10] Arovah NI. STATUS KEGEMUKAN, POLA MAKAN, TINGKAT AKTIVITAS FISIK DAN PENYAKIT DEGENERATIF DOSEN DAN KARYAWAN UNIVERSITAS NEGERI YOGYAKARTA. vol. 0. 2012.

[11] Isna M, Wibisana N, Kusumawardhana B, Pratama DS. IDENTIFIKASI AKTIVITAS FISIK MAHASISWA PROGRAM STUDI PENDIDIKAN JASMANI KESEHATAN DAN REKREASI (PJKR) SELAMA PANDEMIC COVID-19 2020:211–9.

[12] Luzi L, Radaelli MG. Influenza and obesity: its odd relationship and the lessons for COVID-19 pandemic. Acta Diabetol 2020;57:759–64. https://doi.org/10.1007/s00592-020-01522-8.

[13] Elalfy MS, Adly AAM, Attia AAM, Ibrahim FA, Mohammed AS, Sayed AM. ORIGINAL ARTICLE EFFECT OF ANTIOXIDANT THERAPY ON HEPATIC FIBROSIS AND LIVER IRON CONCENTRATIONS IN β -THALASSEMIA MAJOR PATIENTS 2013;37:257–76. https://doi.org/10.3109/03630269.2013.778866.

[14] Pramono BA, Sifaq A, Bulqini A. Efek Posisi Tubuh Setelah Berlatih Terhadap Masa Pemulihan. JSES J Sport Exerc Sci 2018;1:25. https://doi.org/10.26740/jses.v1n1.p25-29.

[15] Heza FN, Wahono BS, Festiawan R, Pendidikan P, Fakultas J, Universitas IK. Jurnal Pendidikan Kesehatan Rekreasi Antioksidan Untuk Olahraga Kesehatan PENDAHULUAN Latihan yang dilakukan secara teratur dan terukur mampu meningkatkan berbagai komponen kebugaran

jasmani , meningkatkan prestasi bagi atlet dan menurunkan risiko penyaki 2020;6:200–5.
[16] Pattanakuhar S, Phrommintikul A, Tantiworawit A, Srichairattanakool S, Chattipakorn SC. N-acetylcysteine restored heart rate variability and prevented serious adverse events in transfusion-dependent thalassemia patients: A double-blind single center randomized controlled trial. Int J Med Sci 2020;17:1147–55. https://doi.org/10.7150/ijms.45795.
[17] Kellmann M, Bertollo M, Bosquet L, Brink M, Coutts AJ, Duffield R, et al. Recovery and performance in sport: Consensus statement. Int J Sports Physiol Perform 2018;13:240–5. https://doi.org/10.1123/ijspp.2017-0759.
[18] Blohm K, Beidler J, Rosen P, Kressler J, Hong MY. Effect of acute watermelon juice supplementation on post-submaximal exercise heart rate recovery , blood lactate , blood pressure , blood glucose and muscle soreness in healthy non-athletic men and women. Int J Food Sci Nutr 2019;0:1–8. https://doi.org/10.1080/09637486.2019.1675604.
[19] Article R. Effect of recovery modalities on blood lactate clearance 2017:65–9. https://doi.org/10.4103/1319-6308.207577.
[20] Yanpanitch O, Hatairaktham S, Charoensakdi R, Panichkul N, Fucharoen S, Srichairatanakool S, et al. Treatment of ? -Thalassemia / Hemoglobin E with Antioxidant Cocktails Results in Decreased Oxidative Stress , Increased Hemoglobin Concentration , and Improvement of the Hypercoagulable State 2015;2015.
[21] Rachmilewitz EA, Weizer-stern O, Adamsky K, Amariglio N, Rechavi G, Breda L, et al. Role of Iron in Inducing Oxidative Stress in Thalassemia Can It Be Prevented by Inhibition of Absorption and by Antioxidants ? 2005;123:118–23. https://doi.org/10.1196/annals.1345.014.
[22] Erejuwa OO, Sulaiman SA, Ab Wahab MS. Honey: A novel antioxidant. Molecules 2012;17:4400–23. https://doi.org/10.3390/molecules17044400.
[23] G Vallianou N. Honey and its Anti-Inflammatory, Anti-Bacterial and Anti-Oxidant Properties. Gen Med Open Access 2014;02. https://doi.org/10.4172/2327-5146.1000132.
[24] Finkelstein FO, Juergensen P, Wang S, Santacroce S, Levine M, Kotanko P, et al. Hemoglobin and plasma Vitamin C levels in patients on peritoneal dialysis. Perit Dial Int 2011;31:74–9. https://doi.org/10.3747/pdi.2009.00154.
[25] Plowman SA, Smith DL. Exercise Physiology for Health Fitness 2008.
[26] Ayu I, Widiastuti E, Wiguna PA, Buanayuda GW. Efek Pemberian Jus Tomat terhadap Kecepatan Pemulihan Denyut Nadi pada Mahasiswa Medical Sports Club Fakultas Kedokteran Universitas Mataram 2017;6:24–7.
[27] Krisnawati D, Pradigdo SF, Kartini A. Efek Cairan Rehidrasi terhadap Denyut Nadi, Tekanan Darah dan Lama Periode Pemulihan. Media Ilmu Keolahragaan Indones 2011;1. https://doi.org/10.15294/miki.v1i2.2028.
[28] Games SS, With P, Player W. Copyright [a] 2016 National Strength and Conditioning Association 2016. https://doi.org/10.1519/JSC.0000000000001736.
[29] Manurung WP, Wibowo A. Pengaruh konsumsi semangka (citrullus vulgaris) untuk menurunkan tekanan darah pada penderita hipertensi. Majority 2016;5:102.
[30] Quindry J, Dumke C, Slivka D, Ruby B. Impact of extreme exercise at high altitude on oxidative stress in humans. J Physiol 2016;594:5093–104. https://doi.org/10.1113/JP270651.
[31] Wyckelsma VL, Venckunas T, Brazaitis M, Gastaldello S, Snieckus A, Eimantas N, et al. Vitamin c and e treatment blunts sprint interval training–induced changes in inflammatory mediator-, calcium-, and mitochondria-related signaling in recreationally active elderly humans. Antioxidants 2020;9:1–20. https://doi.org/10.3390/antiox9090879.
[32] Riso P, Visioli F, Erba D, Testolin G, Porrini M. Lycopene and vitamin C concentrations increase in plasma and lymphocytes after tomato intake. Effects on cellular antioxidant protection. Eur J Clin Nutr 2004;58:1350–8. https://doi.org/10.1038/sj.ejcn.1601974.