Analysis of Kelch-Like Ech-Associated Protein (*Keap 1*) *Receptors* in Improving Physical Fitness ($VO2_{max}$) of Indonesian Hajj Healthcare Personnel Candidates

Ismail[1]*, Alfi Syahar Yakub, Muhammad Basri
Nursing Department, Health Polytechnic of Makassar, Indonesia
*Email : ismailskep@poltekkes-mks.ac.id

ABSTRACT

Genetic factors affect physical fitness ($VO2_{max}$) by 20-30%, since they are related to the biogenesis of mitochondria and the nuclear genome of human genes. The regulation of mitochondrial biogenesis in muscle cells is activated by marker NRF 2, which is KEAP 1 levels signalled by muscle contraction. The aims of this study was to identify the effect of Kelch-Like Ech-Associated Protein 1 receptor on $VO2_{max}$ levels in PKHI candidates. There were 30 PKHI Personnel candidates involved, whose blood samples were taken by 3 ml before and after exercise to identify KEAP 1 levels. Furthermore, $VO2_{max}$ was also measured using the Multistage Fitness Test (MFT) technique using the Bleep Test method before and after the intervention. After that, an intervention was provided by asking them to run 1,600 meters 3 times a week for 16 times. After an interval of 1 week, $VO2_{max}$ was remeasured without any intervention. The data obtained were further analyzed using a statistical test of Paired Independent sample test to know the effect of NRF2 gene expression on $VO2_{max}$levels. In addition, Mann Whitney Test was also performed aiming to see the trend of $VO2_{max}$retention changes post-exercise 1 and post-exercise 2 (SPSS 21, Chicago Inc.). EEwas able to change KEAP 1 levels pre-and post-exercise by 0.16 (pg/dL) even though the changes were not significant. In addition, $VO2_{max}$also increased at an average of 15.50 ml/kg/mt even though the changes were not significantly either even after one week of EE period.

Keywords: *KEAP 1 Receptors, Physical Fitness, $VO2_{max}$, PKHI*

INTRODUCTION

A Hajj Healthcare Personnel is required to have both general and specific physical fitness. Individual physical fitness standardization can be generally measured by the level of maximal oxygen useed ($VO2_{max}$). $VO2_{max}$is one of the indicators of physical fitness. In addition, the increase of $VO2_{max}$can decrease mortality rates (Goodwin, Headley and Pescatello, 2009; Radak *et al.*, 2019)

$VO2_{max}$is the maximal level of individual ability in using oxygen in liters/minute or milliliter/minute/kg of body weight (Pettersen, Fredriksen and Ingjer, 2001) In this case, the oxygen is processed by the body when the person performs intense activities(Munro and Treberg, 2017; Woods *et al.*, 2020). $VO2_{max}$score is determined more by the ability of the cardiovascular system to deliver oxygen than by the ability of the muscles to use it. This is proven by the fact that each individual has basically the same mitochondrial content but has a significantly different $VO2_{max}$score. On the other hand, individuals who have the same $VO2_{max}$have different levels of mitochondrial enzymes (Jones and Carter, 2000).

Several studies stated that mitochondria have an important function in improving physical fitness, (Broskey *et al.*, 2014) one of the receptors that trigger this is the Keap 1 receptor (Lu *et al.*, 2016; Bellezza *et al.*, 2018; Adelusi *et al.*, 2020). This receptor binds to NRF2 protein in the cytosol to activate target genes of anti-oxidant response elements (AREs) (Giudice, Arra and Turco, 2010; Sandberg *et al.*, 2014; Ramezani, Nahad and Faghihloo, 2018) which will stimulate mitochondrial biogenesis in order to increase the number, size, and shape of mitochondria in skeletal muscle cells during endurance exercise (Seo *et al.*, 2016; Theilen, Kunkel and Tyagi, 2017)

Mitochondria has a role to regulate signal transduction and dynamic mitochondrial morphology changes. As a new regulatory gene for mitochondrial morphology (Picard *et al.*, 2013; J Tronstad *et al.*, 2014), Keap 1 activates the mitochondrial reticulum so that a cell can survive, particularly skeletal muscle cells in humans, which is essential for aerobic metabolism in order to produce ATP as a source of muscle energy in endurance exercise activities (Ji, Kang and Zhang, 2016; Di Meo, Napolitano and Venditti, 2019; Kasai *et al.*, 2020)

Previous researchers have found that endurance exercise for about 6 hours can significantly increase the levels and expression of Keap 1 cytosolic protein and NRF2 mRNA mediated by anti-oxidant (ARE) genes. Furthermore, after intensive exercise (IE), the activation changes of Keap 1-NRF2-ARE is different from endurance exercise (EE) with a higher response (Beyfuss *et al.*, 2018; Gallego-Selles *et al.*, 2020; Mason *et al.*, 2020)

Conflict of Interest

None

Ethical clearance

This research is bound to the requirement from the Health Research Ethics Commission of the Hasanuddin University Faculty of Medicine Makassar. Therefore permission from committee and conditions was meet and approved by the panel under the ethic category "Human" approval number 373/UN4.6.4.5.31/PP36/2019.

Tables and Figures

Table 1. Demographic characteristics of research subjects

Subject Characteristics	N	Percentage (%)
Age (year)		
30 – 35	17	56,7
36 – 39	13	43,3
Gender		
Male	17	56,7
Female	13	43,3
Height (cm)		
150 – 160	12	40
>160	18	60
Weight (kg		
45 – 65	17	56,7
66 – 85	13	43,3
Body mass index (kg/mg2)		
<18.4 (thin)	1	3,3
18,5 – 24.9 (normal)	18	60,0
25.0 – 29.9 (overweigh)	10	33,4
30.0 – 39.9 (fat)	1	3.3
Execise habits		
Regular	13	43.3
Irregular	17	56.7
Smoking habits		
Yes	1	3,3
No	29	96,7

Based on table 5.1, it was found that the majority of the sexes were male, namely 17 people and the rest were female; the mean age of the subjects was 35 with a standard deviation of 2.94; mean height 161.83 cm, with a standard deviation of 7.30; mean body weight 63.67 with a standard deviation of 9.37; a mean of 24.20 with a standard deviation of 2.80.

Table. 2 Description of the Results of Measurement of Physical Fitness Level ($VO2_{max}$) of PKHI candidates

Variabel (n=30)	Mean±SD*)	Mean Difference
VO2max**) Pre exercise	29.30±5.29	4.75±2.29
VO2max**) Post exercise 1	34.05±5.14	
		0,90±4.92
VO2max**) Post exercise 2	33.15±4.70	

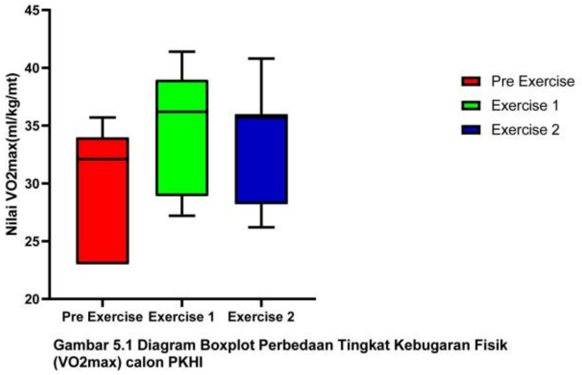
Keterangan : *SD=Standar Deviation; ** (ml/kg/min)

Based on table 2 above, the level of physical fitness ($VO2_{max}$) of the subjects during pre-exercise was at the average of 29.30 ml/kg/min with a standard deviation of 5.29; while during the post-exercise was at the average of 34.05 ml/kg/min with a standard deviation of 5.14. Meanwhile, after the post-exercise 2, the level of physical fitness ($VO2_{max}$) was at the average of 33.15 ml/kg/min with a standard deviation of 4.70

Table 3. Comparison of the average levels of Keap 1 based on the level of physical fitness ($VO2_{max}$)

Marker	Total n(60)	Groups		Dif Mean	P Value
		Pre exercise (30)	Post exercise (30)		
Keap 1 (pg/mL)	5,569± 531,095	4,665± 2,914	8,279±2,212	3,614	0,001*
VO2 max (ml/kg/mt)	32.27± 1.98	29.30±5.29	34.05±5.14	4.75 ±2.29	0,037*

Figure 1. Boxplot Diagram of Differences in Physical Fitness Levels ($VO2_{max}$) of PKHI Candidates



MATERIAL AND METHOD

Experimental design

This research was applied using a quasi-experimental research design through a pre-test and post-test with only control design. The population involved in this study was 70 Indonesian Hajj Healthcare Personnel candidates. Among these populations, 30 subjects were selected by consecutive sampling.

Exercise Protocol

Before the intervention (pre-exercise), the physical fitness level ($VO2_{max}$) of 30 PKHI Personnel candidates was measured using the Multistage Fitness Test (MFT) technique using the Bleep Test method (level and feedback). Another physical fitness test ($VO2_{max}$) was conducted again after the intervention was given in the forms of running 1600 meters 3 times a week with a frequency of 16 times (post-exercise 1) and 1 week later without intervention (post-exercise 2).

Measurements of Keap1

The measurement of Keap1 serum employed gel separator tube, sample cup, centrifuge, Elisa Kit; serum, ELISA reader (Reader 270, Biomeriaux), Keap1 reagent kit (LSBio), and freezer for storing the samples and reagents. In this case, Reader (Reader 270, Biomeriaux) and Freezer were used for ELISA, while 1 Keap1 reagent kit (LSBio) was used for the ELISA materials. (Beyfuss *et al.*, 2018; X. Wang *et al.*, 2020).

Measurements of $VO2_{max}$

The level and feedback values from the Bleep test were then converted into a $VO2_{max}$table based on the level and feedback obtained, then the $VO2_{max}$values were grouped into levels including special, excellent, good, medium, poor, and very poor (Purba *et al.*, 2021)

Statistical Analysis

Statistical analysis was performed using SPSS Software (version 21. Inc. Chicago, IL USA). Wilcoxon Sign Rank Test was also used to prove the effect of endurance exercise on the level of physical fitness ($VO2_{max}$) in PKHI candidates in addition to the Mann Whitney Test which was employed to prove changes in retention physical fitness ($VO2_{max}$) during post-exercise.

RESULT AND DISCUSSION

The Keap-1 Serum Levels and physical fitness level ($VO2_{max}$) of Indonesian Hajj Healthcare Personnel were analyzed during pre- and post-exercise. After Keap-1 the serum levels and the level of physical fitness ($VO2_{max}$) were measured, the data obtained were then tested using statistical analysis tests including normality test; Paired Sample Test, Wilcoxon sign rank test, and Mann Whitney Test.

Effect of Keap 1 Serum Levels on physical fitness level ($VO2_{max}$) before and after exercise

Based on the research results, it was revealed that the mean score of Keap-1 serum levels during pre-exercise was 4.665 ± 2.914, while during post-exercise was 8.279 ± 2.212. Thus, the mean difference obtained was 3.614, while the p-value was 0.001. Concerning the physical fitness level ($VO2_{max}$), the mean score before the exercise obtained 29.30± 5.29, while after the exercise obtained 34.05±5.14. Therefore, the mean difference obtained was 4.75±2.29 with p-value of 0.000. Furthermore, the mean score (Keap 1 with $VO2_{max}$) during the pre-exercise obtained was 32.27±1.98, while during the post-exercise was 34.05±5.14, with a mean value of 1.78 ±5.52 and p-value of 0.037.

Changes in physical fitness level ($VO2_{max}$) retention between post-exercise I and post-exercise II.

Table 2 Changes in physical fitness level ($VO2_{max}$) retention between before and after the exercise

Based on the results obtained from Mann Whitney Test statistical test, p-value obtained was 0.216 > 0.05, thus HO is accepted, while he is rejected. This indicates that there were no changes in physical fitness retention ($VO2_{max}$) between post-exercise 1 and post-exercise 2. Therefore, it can be concluded that there was no significant changes in the level of physical fitness ($VO2_{max}$) of Indonesian Hajj Healthcare Personnel candidates 1 (one) week after regular endurance exercise.

Relationship between Keap-1 Serum Levels and physical fitness level ($VO2_{max}$)

Based on the linear regression test, the baseline of $VO2_{max}$constant was 12.858 ml/kg/min, with a linear value of Keap-1 serum level was 0.59. Therefore, in this case, the researcher successfully obtained a new finding (Novelty) that every increase of Keap-1 serum level by 0.59ΔCT will be followed by an increase of 1 ml $VO2_{max}$(ml/kg/min).

Several factors can affect physical fitness ($VO2_{max}$), one of them is the Keap-1 serum level, which is related to the objective of this research of determining the role of Keap-1 serum levels in improving the physical fitness ($VO2_{max}$) of Indonesian Hajj Healthcare personnel candidates after getting endurance exercise of running for 3 times a week with a frequency of 16 endurance exercises. Detailed results are discussed as follows:

Physical fitness level ($VO2_{max}$)

The results showed that before the endurance exercise, the average level of physical fitness ($VO2_{max}$) of Indonesian Hajj Healthcare Personnel (±SD) was 29.30±5.29, so it is categorized as poor. This result is supported by the previous research that physical inactivity damages health (Kohl 3rd *et al.*, 2012). In contrast, decreased health conditions related to age will decrease physical fitness ($VO2_{max}$) significantly (up to 50%) in individuals who engage in endurance exercise training compared to inactive people (Booth, Roberts and Laye, 2012).

After undergoing regular endurance exercise which is by running 1600 meters 3 times a week for 16 times, another measurement test was carried out, obtaining a significant increase of the average level of physical fitness ($VO2_{max}$) to 34.05±5.14, so it is categorized as medium. Furthermore, a week after the exercise (post exercise-2), another measurement of physical fitness level ($VO2_{max}$) was carried out and the results did not change drastically, which is 33.15 ± 4.70, which is still categorized as medium. Therefore it can be summed up that before conducting the endurance exercise, the average level of physical fitness ($VO2_{max}$) obtained was 29.30±5.29, while after conducting the endurance exercise, there was an increase to 34.05±5.14 which showed an average increase of 4.75 ml/kg/min.

These results are supported by the results of previous research that someone who trains at least 3 times a week get good results because his endurance will begin to decrease after 48 hours if he does not undergo any other physical endurance exercise (Kohl III and Cook, 2013)

Similar studies also have revealed that 65-74 years old man can increase their $VO2_{max}$by about 18% after performing regular exercise for 6 months. (Dondzila, 2013)

After performing endurance exercise, physical fitness ($VO2_{max}$) increased because the body adapts physiologically by absorbing oxygen, and distributing it to muscles which experience fatigue. The speed of the body during the recovery process significantly depends on the $VO2_{max}$capacity, the better the $VO2_{max}$capacity, the faster the body recovers. The results of this study were supported by a study that has been conducted previously on Ulm student, Giessen Tubingen German under the age of 40 who found a significant $VO2_{max}$difference between those who were untrained (37.2 ± 7.8) and those who were trained (67.2. ±8.9) with a mean difference of 30 (mg/kg/mt) (Smith *et al.*, 2020)

Another study also discovered similar results, in which an increase in physical fitness ($VO2_{max}$) was found in the Han population in China before and after exercise, which was from 3.44±0.38 (ml/kg/min) to 3.49±0.40 (ml/kg/min) with an average increase of 0.05 ml/kg/min (He *et al.*, 2008)

In order to become an Indonesian Hajj Healthcare Personnel, the candidate must have good physical fitness ($VO2_{max}$), since it is one of the important elements that a PKHI candidate must possess when carrying out their duties. During carrying out an intensive physical activity of 1 x 24 hours to serve Hajj pilgrims candidates, the personnel must apply aerobic energy system. This system requires a lot of oxygen continuously, distributed to muscle fibers as aerobic metabolism material along with carbohydrates, fats, proteins, and minerals to be converted into energy (ATP) (Benardot, 2020). With good physical fitness ability ($VO2_{max}$), PKHI candidates will be able to maintain their physical condition while carrying out their duties to serve Allah guests.

The average increase of $VO2_{max}$value after performing the exercise was 4.75±5.13. This is in accordance with the results of research conducted by Fikenzer et al that endurance physical exercise that was conducted 3-4 times a week for 24-44 weeks with 40-55 minutes per session can increase $VO2_{max}$capacity by 65 – 80% (Fikenzer *et al.*, 2018).

During the research observations, no specific physical exercise was found other than the recommended one, which is running 1600 meters 3 times a week for 16 times to increase physical fitness endurance ($VO2_{max}$). In addition, several factors that affect the endurance of physical fitness ($VO2_{max}$) include food, rest and living habits, and the environment. Nutritious food affects individual physical fitness endurance ($VO2_{max}$), thus with a balanced nutritional intake, energy will be met adequately. (Amaro-Gahete *et al.*, 2019; Festiawan *et al.*, 2021)

$VO2_{max}$is the maximal level of individual ability in using oxygen optimally (Persky and Eudy, 2015; Jones, 2016). In this case, the oxygen is further processed by the human body when carrying out intense activities, so that the higher $VO2_{max}$, the better physical endurance (Kaidah, Kasab and Ridhoni, 2020). Physiologically, every cell needs oxygen to convert food energy into ATP (Adenosine

Triphosphate) which is ready to be used for work, each cell that consumes the least oxygen is the muscle in a resting state. During endurance physical activity, contracting skeletal muscle cells require a lot of ATP. As a result, the muscles used in exercise require more oxygen and produce CO_2 through respiratory function (Chen *et al.*, 2015; Kaidah, Kasab and Ridhoni, 2020). Along with carbohydrates, fats, and proteins, as well as minerals, oxygen is continuously needed by the body through the capacity of the aerobic energy system, that are channeled to muscle fibers as the basic material of aerobic metabolism. These elements were then converted into energy ready to be used in the body in the form of Adenosine Triphosphate (ATP) which limited in number. For this reason, it is necessary to resynthesize ATP through aerobic and anaerobic metabolism so that sufficient energy can be used for continuous activities (Salin *et al.*, 2015; Koch *et al.*, 2021).

The mechanism of ATP production through the aerobic system occurs in the mitochondria and the aerobic system requires oxygen to produce ATP, so that maximum oxygen consumption ($VO2_{max}$) also affects the work of this system. $VO2_{max}$is processed by the body during intense activities. The value of $VO2_{max}$is one of the main predictors of endurance performance (Plank and Dean, 2014; Manoj *et al.*, 2019).

Aerobic ability ($VO2_{max}$) is the greatest aerobic exercise ability that a person has (Ranković *et al.*, 2010; Nabi, Rafiq and Qayoom, 2015). This is determined by the amount of acid (O_2) that can be supplied by the heart, respiration, and hemo-hydro-lymphatic or O_2 transport, CO_2 and nutrients every minute (Morton and Billat, 2000; James *et al.*, 2017; Brechbuhl *et al.*, 2018).

The metabolic processes of these three energy sources can occur as long as the oxygen (O_2) is obtained through the respiratory process (Dalsgaard *et al.*, 2014; Nelson, 2016). The anaerobic activity requires energy quickly. This activity obtains energy through the hydrolysis of phosphocreatine (PCr) and anaerobic glycolysis of glucose (Wells, Selvadurai and Tein, 2009). This anaerobic energy metabolism process can run without oxygen (O_2) (Barclay, 2017). Anaerobic energy metabolism can produce ATP faster than aerobic energy metabolism. Therefore, in the case of physical exercise which requires high energy in a short time, the anaerobic energy metabolism process can provide ATP quickly but only for ± 90 seconds. Although the process occurs quickly, this anaerobic energy metabolism only produces fewer ATP molecules compared to aerobic energy metabolism (2 ATP vs 36 ATP per 1 glucose molecule) (Mergenthaler *et al.*, 2013; Karaman and Khawaja, 2015).

The aerobic energy metabolism process is also considered to be a clean process because this process does not only produce energy, but also carbon dioxide (CO_2) and water (H2O) (Lashgari, 2015; Bharathiraja *et al.*, 2018). This is different from the anaerobic metabolic process which produce CO_2, H_2O, and lactic acid (Kondaveeti and Min, 2015; Sikora *et al.*, 2017; Rusdiawan, Sholikhah and Prihatiningsih, 2020). The accumulated lactic acid in the body can inhibit muscle contraction and cause muscle pain (Astriyana, Doewes and Purnama, 2017; Russeng *et al.*, 2018). This is why powerful movements during physical exercise cannot be carried out continuously for a long time and must be interspersed with rest intervals (Irawan, 2007). Aerobic energy metabolism is a metabolic process that requires oxygen (O_2) so that ATP can be produced perfectly. During physical exercise, carbohydrates (blood glucose, muscle, and liver glycogen) and fat are stored in the form of triglycerides, which will contribute to the rate of aerobic energy production in the body. These two energy stores can provide different amounts of contribution depending on the intensity of the physical exercise performed (Nelson, 2016; Martin, Tielens and Mentel, 2020).

Endurance physical exercise makes a major contribution in helping the body's metabolism to stabilize in producing ATP to support continuous physical activity (Haman and Blondin, 2017; Mach and Fuster-Botella, 2017). This is based on the results of a study which discovered that endurance physical exercise was able to reduce triglyceride levels by 8%, cholesterol by 4%, and LDL by 5% as well as increase HDL levels by 4% (Fikenzer *et al.*, 2018).

Analysis of Keap-1 Serum Levels on the level of physical fitness ($VO2_{max}$)

In this study, it was found that the average Keap-1 serum level by reference gene normalization using the Livak method was 0.73±4.15. In this case, comparison was carried out between the Keap-1 serum level before and after exercise. The results were calculated using a machine, in which comparison was done between the normalized Keap-1 serum levels after exercise and the normalized values of Keap-1 serum levels before exercise, in which case the Keap-1 serum level was 4.15.

There are several considerations regarding the findings mentioned above, those are (1) the variability of the research subjects; 2) the different gene expression of each subject; 3) The subject's lifestyle, especially those related to exercise and smoking habits).

After statistical analysis of various test were conducted in accordance with the proposed hypothesis, it was concluded that there was an effect of Keap-1 serum levels on the physical fitness ($VO2_{max}$) of Indonesian Hajj Healthcare Personnel candidates.

A number of research projects support the findings obtained in the current research, including a previous research which was conducted in Cerritos College in California, United States that genetic factors can increase $VO2_{max}$by 20-30% (Cerrito).

Physical endurance exercise performs stimulation to mitochondrial biogenesis in order to increase Keap-1 serum levels. The function of Keap-1 is to regulate genes involved in mitochondrial function, affecting the respiratory capacity and levels of ATP production during exercise. In addition, Keap-1 serum levels also regulate mitochondrial transcription factor A (TFAM), cytochrome c, and heme protein biosynthesis, (Puigserver and Spiegelman) (Craig and Meyersfeld).

Endurance training also stimulates an increase in several components of the respiratory mitochondrial chain and enzymes involved in fatty acid oxidation. This change is caused by an increase in mitochondrial protein synthesis and results in high mitochondrial density and size in skeletal muscle (Zihong He et al.).

Some researchers have found that Keap-1 serum levels concentration has a function of regulating skeletal muscle contraction and relaxation during exercise. Keap-1 serum levels are induced as part of skeletal muscle adaptation during physical exercise (Bouchard); (MacIejewska-Karłowska et al.)

The increase of Keap-1 serum levels will decrease NADPH oxidase which then leads to the increase of anti-oxidant capacity which greatly supports exercise performance (Bowtell and Kelly).

Similar studies have revealed that increased Keap-1 serum levels activity is required to maintain homeostasis of mitochondrial function in muscle and to optimize specific mitochondrial proteins as a response against the endurance exercise (Bowtell and Kelly).

Other studies also discovered that systematic endurance physical exercise can increases the capacity of human muscles to produce oxidative energy, as demonstrated by the measurements of mitochondrial density in certain cells. However, the process by which systematic endurance training stimulates mitochondrial biogenesis is not fully understood (Baar, 2004).

In addition, another research project stated that endurance training stimulates mitochondrial biogenesis in skeletal muscle tissue, which leads to increased respiratory capacity and increased rate of ATP formation during exercise (Zihong He et al.).

Aerobic exercise affects the Keap-1 serum protein and mRNA amount, in the form of an increase in muscle Keap-1 serum protein levels at 12-18 hours after swimming. Peroxisome proliferative activated receptor gamma, coactivator 1 alpha (PPARGCIA) are assumed as a mediator to induce a two to threefold increase in Keap-1 serum protein levels. This means that PPARGCIA modulates oxidative capacity in muscle, via coactivation of Keap-1 serum levels of other mitochondrial proteins. PPARGCIA has the ability to induce a 10% shift in fast to slow muscle fibers. This shift is either a direct effect of PPARGCIA or a related indirect effect in cell metabolism (Baar K, 2004).

Endurance exercise induces the increase of respiratory muscle capacity, thus leads to the increase of this capacity to produce ATP and increase the muscle contraction efficiency (Tachtsis *et al.*, 2016; Verges, 2019). Endurance training is determined through maximal oxygen uptake ($VO2_{max}$) (Montero and Díaz-Cañestro, 2016; Knuiman *et al.*, 2019; Landgraff *et al.*, 2021). This exercise affects mitochondrial changes, but this adaptation is largely a coordinated genetic response of both nuclear and mitochondrial genes. Exercise can affect mitochondria or mitochondrial biogenesis mediated by transcription factors and transcription co-activator factors. Transcription factors attach or bind to the promoter region of mitochondrial genes and function to identify genes to be transcribed, while co-activators do not bind to DNA but instead bind to transcription factors and promote the formation of protein complexes (Islam, Edgett and Gurd, 2018; Kim, Kim and Hwang, 2019).

The gene involved in mitochondrial biogenesis is Peroxisome proliferator-activated receptor delta Peroxisome proliferator-activated receptor gamma,coactivator1alpha – Nuclear respiratory factor-Mitochondrial transcription factor A). The regulation of mitochondrial biogenesis in muscle cells is activated by the signal-induced Keap-1 serum level from muscle contraction. Keap-1 serum levels will interact with PPAR (peroxisome proliferator-activated receptor), regulate gene expression, and initiate mitochondrial transcription and translation (Pearce *et al.*, 2017; Pettit *et al.*, 2017; Barshad *et al.*, 2018).

Furthermore, the role of Keap-1 serum levels is as the main regulator of antioxidant enzymes and cellular stress resistance. Several studies have shown that Keap-1 serum level pathway plays a key role in regulating oxidative stress and the positive effects of physical exercise. Episodic increases in acute exercise-induced oxidative stress stimulate the activation of Keap-1 serum levels with regular exercise. This may lead to upregulation of endogenous antioxidant defenses and the ability to counteract the negative effects of oxidation (nucleic acids, proteins, and lipids)

(Done and Traustadóttir, 2016; Vargas-Mendoza *et al.*, 2019; Gao *et al.*, 2020; Kasai *et al.*, 2020).

Endurance exercise induces an increase in respiratory muscles, to produce ATP and increases skeletal muscle contraction. The determination of the assessment of endurance physical exercise begins with measuring physical fitness ($VO2_{max}$) and this exercise affects changes in mitochondria. Furthermore, changes in the number and size of mitochondria are affected by exercise factors (MacInnis and Gibala, 2017). (Psilander), (Cunningham).

Mitochondria employs carbohydrate, fat, and protein to produce energy in the form of ATP (adenosine triphosphate). The ATP is formed through the oxidative phosphorylation process, which requires oxygen and involves five respiratory chain enzyme complexes so that this energy can be used for different cellular processes such as muscle contraction. The more cells that need energy, the more mitochondria present. For example, in terms of cardiac muscle fibers that continuously needs ATP, these cardiac muscle fibers have more than 25% mitochondria,(Tyrrell *et al.*, 2016; Hoppeler, 2019; Oliveira and Hood, 2019), while the skeletal muscle in individuals with less activity (sedentary) have 3-6% mitochondria, while trained individuals have 12% mitochondria (F. Wang *et al.*, 2020).

Changes in the physical fitness ($VO2_{max}$) retention rate of PKHI candidates

Based on the results obtained, there was no decrease in the retention of physical fitness level ($VO2_{max}$) in PKHI candidates after undergoing endurance exercise. This shows that after performing an endurance exercise, the physical fitness levels ($VO2_{max}$) is able to remain constant for a week after exercise.

There are several factors affecting physical fitness ($VO2_{max}$). One of them is endurance exercise which is related to this study with the aim to determine the physical fitness level ($VO2_{max}$) before and after conducting a physical exercise intervention in the forms of running for 3 weeks for 16 times.

Physical exercise is a planned, structured, and repetitive physical activity aimed at improving or maintaining one or more components of physical fitness. Physical exercise is a physical activity that can improve physical health because it does not only involve the musculoskeletal system, but also other systems in the body, such as the cardiovascular system, respiratory system, endocrine system, nervous system, and so on (F. *et al.*, 2018; Rogers *et al.*, 2018).

Through physical exercise activities with dominant aerobic activity, energy metabolism to produce ATP (adenosine triphosphate) will run through aerobic glycolysis in the form of burning carbohydrates, fat, and a small part of the breakdown of protein stores in the body (Hackney, 2016; Ehrman, Kerrigan and Keteyian, 2017).

During physical exercise, various metabolisms occur in the organs of the body. The greater the metabolism in an organ, the greater the need for blood. This will be compensated by the heart with changes in the cardiovascular system in the form of increased cardiac output and redistribution of blood from inactive organs to active organs. This increase in cardiac output is accomplished by increasing stroke volume and heart rate. Stroke volume will increase as the venous return increases via the Frank-Starling mechanism and the contractility of the myocardium also increased as stimulated by sympathetic nerves. Increased heart rate during physical exercise occurs due to increased sympathetic activity and decreased parasympathetic activity in the sinoatrial (SA) node (Hackney, 2016; Ehrman, Kerrigan and Keteyian, 2017).

Regular physical exercise causes hypertrophy to both skeletal muscles and myocardium so that the heart chambers will also enlarge (Saghiv and Sagiv, 2020). This will cause an increase in the pumping capacity of the heart which leads to an increase in stroke volume. Although the heart of a trained person is larger than a normal person's, the cardiac output at rest is almost the same as a normal person's.(Wilson, Ellison and Cable, 2016; Cunningham, Spears and Care, 2019)

A person's cardiac output is equal to stroke volume multiplied by heart rate (Magder, 2016). Therefore, to obtain the same cardiac output as a normal person at an increase in stroke volume, the resting pulse rate in a trained person will be slower than in a normal person. This is what is commonly referred to as the efficiency of the heart by the myocardium (Lundby, Montero and Joyner, 2017). Changes in pulse rate are often used as the basis for physical fitness tests, where slight changes indicate good regulation of the circulatory system (Bennett *et al.*, 2016; Tanveer and Hasan, 2019).

CONCLUSION

Based on the results of research and discussion concerning Keap-1 serum levels with physical fitness ($VO2_{max}$), several conclusions are obtained. First, the physical fitness level ($VO2_{max}$) of Indonesian Hajj Healthcare Personnel candidates before and after the exercise was at an average of 4.75±2.29, so it is categorized as medium. In addition, there was a difference in the Keap-1 serum level and the physical fitness level ($VO2_{max}$) during before and after the exercise of 1.78±5.52 in the Indonesian Hajj Healthcare Personnel candidates. It is also proven that Keap-1 serum levels affects the physical fitness level ($VO2_{max}$) in the Indonesian Hajj Healthcare Personnel candidates. No changes were found in the retention level of physical fitness ($VO2_{max}$) of the Indonesian Hajj Healthcare Personnel candidates 1 (one) week after regular endurance exercise. Each increase in the Keap-1 serum level of 0.59 (pg/dL) will be followed by a 1 ml increase in $VO2_{max}$(ml/kg/min).

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