THE ANALYSIS OF mRNA EXPRESSION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA COACTIVATOR 1-ALPHA (PGC-1α) GENE AND THE PHYSICAL FITNESS LEVELS (VO2max) IN THE CANDIDATES FOR INDONESIAN HAJJ HEALTH OFFICERS

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Abstract

Indonesian Hajj Health Officers (PKHI) are health officers instructed by the Minister of Health of the Republic of Indonesia to provide health services, development, and protection to Hajj pilgrims during the Hajj pilgrimage implementation, hence, a healthy physical condition in PKHI becomes one of the primary keys of success in providing health services. This study aimed at knowing the role of PGC-1α gene expression in increasing the physical fitness levels (VO2max) of the PKHI candidates. The data were collected from 30 subjects of PKHI consisting of 17 males and 13 females. The assessment of PGC-1α gene expression using mRNA and RT-PCR was done in the biomedical-biomolecular laboratory of Hasanuddin University Medical Research Center (HUM-RC), Makassar, and the measurement of VO2max of three 3 times using the Multistage Fitness Test (MFT) technique with Bleep Test method, namely before and after 1600 meter running workout with a duration of 20-30 minutes per session, 3 times a week with a frequency of 16 times. The result showed that the average scores of PGC-1α gene expression after physical exercise in males and females were (7.45±2.17 ΔΔCT) and (5.46±2.87 ΔΔCT) respectively. They were higher than the average scores before physical exercises in males and females, namely (3.46±0.72 ΔΔCT) and (3.38±0.57 ΔΔCT) respectively with the statistical significance level or p-value of less than 0.001. Further, the average scores of VO2max in males and females after physical exercises were 38.65±1.69 ml/kg/min and 28.98±1.30 ml/kg/min respectively, and they were better than average scores before physical exercises, namely 33.78±1.29 ml/kg/min and 24.82±0.37 ml/kg/min respectively (p-value <0.001). It concludes that mRNA expression of PGC-1α gene increases the level of physical fitness (VO2max) in PKHI candidates.

INTRODUCTION

Indonesian Hajj Health Officers (PKHI) are health officers instructed by the Minister of Health of the Republic of

Indonesia to provide health services, development, and protection to Hajj pilgrims during the Hajj pilgrimage implementation; having job competence from the aspect of knowledge, skills, and work attitude according to the

established standard is not enough, excellent physical endurance is also needed [1]. An Indonesian Hajj Health Officer is demanded to have three competencies in performing the duties and responsibilities in the health sector, namely 1) managerial competence [2], 2) skills [3], and 3) excellent physical competence. An excellent physical competence becomes one of the primary keys to providing health services to Indonesian Hajj pilgrims. It may happen because the number of Indonesian Hajj pilgrims is high and the duration of PKHI to work is around 39 days for TKHI/Indonesian Hajj Health Team (Departure Group Officers) and 76 days for PPIH/Hajj Pilgrimage Implementation Committee (Non-Departure Group Officers) [4]. The physical fitness of a Hajj health officer is required to be, in general, healthy and have specific physical fitness (special). The standard of individual physical fitness generally can be measured by maximal oxygen consumption (VO_{2max}). [5]. Based on the result of an investigation, the researcher only found 1 scientific publication related to VO_{2max} in PKHI [6]. The study only described the physical fitness levels (VO_{2max}) in PKHI. Further, the researchers of this study tried to develop the subject and the variable to be more complex, namely the role of gene expression, especially PGC-1α, in supporting the physical fitness (VO_{2max}) in PKHI. The researchers conducted preliminary research about the physical fitness levels (VO_{2max}) of the PKHI candidates. For the physical fitness levels (VO_{2max}) of the PKHI candidates, the Embarcation of Makassar in 2018, it was found that out of 30 PKHI candidates, 20 people (67%) had the physical fitness levels (VO_{2max}) in the moderate/poor category, only 10 people (33 %) of them were in the category of good [7]. The higher the VO_{2max} in a person, the endurance will be better. Endurance consists of two groups, namely cardiorespiratory endurance (general) that can be measured by the maximal oxygen consumption (VO_{2max}) [8] and muscular endurance (local) that is measured by the total and quality of skeletal muscle fiber through a muscle biopsy.

Several findings in the biomolecular sector showed that one of the genetic markers for endurance was Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) gene. It is the major regulator of mitochondrial biogenesis as the co-activator of transcription process in several genes that play a central role in mitochondrial regulation [9–12]. Mitochondrial biogenesis and nucleus expressed by one of the human genes, namely PGC-1α mRNA. It a human protein encoded by the PPARGC1A gene [13]. The mitochondrial biogenesis regulation in muscle cells was activated by PGC-1α induced by a signal of muscle contraction. PGC-1α would interact with PPAR (peroxisome proliferator-activated receptor) to regulate gene expression and initiate the transcription as well as mitochondrial translation [14, 15]. Physical exercise stimulated mRNA expression of PGC-1α gene; it had been reported by a study that physical exercise would trigger the activation of some molecules and enzymes, such as reactive oxygen species (ROS), calcium Ca^{2+}, which then stimulated the mRNA expression of PGC-1α gene through P38, MAPK, and AMPK receptors as the second messengers. Furthermore, PGC-1α stimulated the formation of mitochondrial biogenesis [16]. The findings showed that PGC-1α regulated the development of cell cycle through the modulation of CyclinD1, CyclinB1 by ATP, and ROS. This finding confirmed that PGC-1α played a role in coordinating the energy metabolism and the cell cycle [17].

The study related to the role of mRNA expression of PGC-1α gene against the physical fitness (VO_{2max}) in Indonesian Hajj Health Officers is vital to be conducted since, up until now, there is no similar study that shall be a reference for PKHI in gaining excellent physical fitness, which affects the improvement of health service quality.

MATERIALS AND METHODS

Research Design

This study was a quasi-experimental study with a pretest-posttest only control design [18]. The population in this study was 70 PKHIs using a consecutive sampling technique. The total sample in this study was 30 subjects consisting of 17 males and 13 females. The inclusion criteria for the respondents were (a) the respondents were nurses at the age of 30 – 39 years old, (b) passing the recruitment and selection process of the candidates for Indonesian Hajj Health Officers, (c) understanding the instruction of physical exercise program with Rockport method (d), willing to participate in every activity and requirement of the study. The exclusion criteria were (a) having coronary heart disease, diabetes mellitus, asthma, COPD, rheumatoid arthritis, Gout, hyperthyroidism, and mental disorders in the medical history; (b) having no approval by a physician to participate in the study due to the medical condition.

Exercise Protocol

Before Exercise Training

The first steps were performing the anamnesis and physical examination by a physician, in the form of personal data, physical activities, physical examination including height, weight, Heart Rate, Respiratory Rate, and Blood Pressure (BP). The second step was filling out the form of the feasibility of physical activities. Meanwhile, the third step was taking a blood sample of 3 ml put into a purple test tube containing EDTA. The blood sample was brought to the biological molecular laboratory of HUM-RC, Makassar, and stored in freezer storage. The fourth step was measuring the initial test of physical fitness (VO_{2max}) using the Multi Fitness Test (MFT) or the Bleep Test method in the Health Training Center (Balai Besar Pelatihan Kesehatan/BBPK), Makassar.

During Exercise Training

The session of 1600 meter running workout was done for 20-30 minutes, 3 times a week with a frequency of 16 times. The first step was choosing the schedule for exercises based on the requirements in the study. The second step was selecting the schedule choices as follows: Exercise I: Monday-Wednesday-Friday; Exercise II: Tuesday-Thursday-Saturday; and Exercise III: Wednesday-Friday-Sunday. The third step was documenting the endurance training in the form of a workout video.

After Exercise Training

The first step was measuring the initial test of physical fitness (VO_{2max}) by using the Multi Fitness Test (MFT) or Bleep Test I method in Asrama Haji Sudiang, Makassar. The second step was the recovery session for 1 hour after the physical fitness test. The third step was taking a blood sample of 3 ml put into a purple test tube containing EDTA. The blood sample was brought to the biological molecular laboratory of HUM-RC, Makassar, and stored in freezer storage.

VO_{2max} Measurement

The measurement of physical fitness levels (VO_{2max}) was done two times (before and after exercises) by using the Multistage Fitness Test (MFT) technique and Bleep Test method before and after exercises. The result was converted into a table of VO_{2max} based on the achievement levels and feedback collected from the method. Then, the VO_{2max} values were classified into several categories, namely excellent, very good, good, fair, poor, and very poor [19].

Measurement of the mRNA Expression of PGC-1α Gene

To measure the mRNA expression of PGC-1α gene before and immediately after the exercise training, the researchers took the blood sample of 3 ml of the PKHI candidates and performed the relative RT-PCR examination calculated by real-time PCR machine CFX96 touch series (Bio-Rad Laboratories, Inc) in $\Delta\Delta^{CT}$ (delta cycler threshold) unit. The result was calculated using the formula of relative RT – PCR (the expression value of PGC-1α Δ^{CT} after exercise training – the expression value of PGC-1α Δ^{CT} before exercise training = the expression value of PGC-1α $\Delta\Delta^{CT}$)[20].

Real-time PCR

RT-PCR analyzed the human RNA in the RNA sample of PGC-1α as a target gene, and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was selected as the control gene (housekeeping gene). The sample was preserved in nuclease-free water (Norgen biotech, Inc), and RNA was extracted using RNA isolation kit "RNAprep pure kit for blood" (Tiangen Biotech, Beijing). cDNA was synthesized using a cDNA kit, namely "iScript cDNA synthesis kit" (Bio-Rad Laboratories, Inc) and stored at a temperature of -20°C and it was analyzed using RT-PCR. It was operated in CFX96 touch (Bio-Rad Laboratories, Inc) using a qPCR master mix "Ssofastevagreen supermix" (Bio-Rad Laboratories, Inc). The pair of specific gene primer is described as follows:

Forward primer:
5'- CACTTACAAGCCAAACCAACAAC-3'
Reverse primer:
5'-CAATAGTCTTGTTCTCAAATGGGGA-3' [21]

Real-time qPCR Quantification

Firstly, the total CT of the target gene (PGC-1α) was deducted from the total CT of the endogenous reference gene (GAPDH); [ΔCT = CT (target) - CT (reference)]. Further, the total value of ΔCT PGC-1α after being deducted from the total value of ΔCT PGC-1α before exercise training; [$\Delta\Delta CT$ = total value of ΔCT PGC-1α after exercise training – the total value of ΔCT PGC-1α before exercise training). The finding was reported as fold-changes using the $\Delta\Delta CT$ method, calculated as 2 $\Delta\Delta CT$ Primer detecting B-Actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) selected as the endogenous reference gene [20, 22].

Statistical Analysis

To ensure whether the expression of PGC-1α gene before and after exercise training increased significantly, the researchers conducted a statistical test using SPSS (version 21. Inc. Chicago, IL USA) and Wilcoxon signed-rank test. To know the difference between the PGC-1α gene expression and VO_{2max}, the researchers conducted the Mann-Whitney test. Furthermore, to analyze the contribution of the PGC-1α gene expression in increasing the physical fitness levels (VO_{2max}), the researchers conducted Spearman's correlation coefficient and ANOVA for linear regression test.

Ethical Clearance

This study obtained ethical clearance from the Ethical Committee of Faculty of Medicine, Universitas Hasanuddin, Makassar, number 373/UN 4.6.4.5.31/PP36/2019.

RESULTS

Subjects' Demographic Characteristics

The subjects' demographic characteristics in the study consisted of age at a range of 30 – 39 years old, gender dominated by males, the height of more than 160 cm, weight at a range of 45 – 85 kg, body mass index generally at a range of 18.5 – 24.9 (normal), exercise habit mostly in the category

of irregular, and smoking habit generally in the category of not smoking (Table 1).

Table 1. Distribution of Subjects' Characteristics (n=30)

Subjects' 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	40.0
>160	18	60.0
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 (overweight)	10	33.4
30.0 – 39.9 (obesity)	1	3.3
Exercise Habit		
Regular	13	43.3
Irregular	17	56.7
Smoking Habit		
Yes	1	3.3
No	29	96.7

The Role of the mRNA Expression of PGC-1α Gene

The contribution of the mRNA expression of PGC-1α gene to physical fitness (VO_{2max}) increased by 3.16 ΔCT on average after the subjects did the scheduled physical exercises. From the perspective of gender, the finding showed that PGC-1α gene expression in males was higher than in females of 3.99 ΔCT on average (Table 2).

Table 2. The PGC-1α Gene Expression and Physical Fitness Levels (VO_{2max}) before and after Exercises

Variables (n=30)	Before Mean±SD	After Mean±SD	Difference Mean±SD	p-value
PGC-1α ($ΔΔ^{CT}$)				
Male (17)	3.46±0.72	7.45±2.17	3.99±1.88	<0.001[a]
Female (13)	3.38±0.57	5.46±2.87	2.08±3.06	0.033[a]
VO_{2max} (ml/kg/min)				
Male (17)	33.78±1.29	38.65±1.69	4.88±1.49	<0.001[b]
Female (13)	24.82±0.37	28.98±1.30	4.16±0.84	<0.001[b]

Description: PGC-1α= Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; $ΔΔ^{CT}$ = Delta; Cycler Threshold; SD= standard deviation; [a]) Wilcoxon Signed-Rank Test; [b]) Mann-Whitney Test

Physical Fitness Levels (VO_{2max})

The result of the measurement of physical fitness levels (VO_{2max}) showed that the physical fitness levels (VO_{2max}) in males and females before physical exercises increased by 4.88 ml/kg/min vs 4.16 ml/kg/min respectively. Nevertheless, the physical fitness levels (VO_{2max}) in males

were higher than in females with a difference of 0.72 ml/kg/min (Table 2).

The Correlation between mRNA Expression of PGC-1α Gene and Physical Fitness Levels (VO₂ₘₐₓ)

The result of the correlation test showed that the PGC-1α gene expression in males and females was positively and strongly correlated with physical fitness levels (VO$_{2max}$) of (p<0.001; r=0.916) and (p<0.001; r=0.934) respectively. It showed that the higher the PGC-1α gene expression in a

person, the physical fitness levels (VO$_{2max}$) would also be higher.

The contributions of the mRNA expression of PGC-1α gene to the physical fitness levels (VO$_{2max}$) based on the result of the ANOVA test with the model summary, namely R-squared, in both males and females were 0.872 and 0.840 respectively or 87.2% and 84% respectively. Meanwhile, 12.8% and 16% of each consecutively were the contributions of other variables that were not included in this study (Table 3).

Table 3. The Correlation between PGC-1α Gene Expression and Physical Fitness Levels (VO2$_{max}$)

Variables	Male				Female			
	PGC-1α		VO$_{2max}$		PGC-1α		VO$_{2max}$	
	r	P	r	p	r	p	R	p
	R-Squared= 0.872				R-Squared= 0.840			
PGC-1α	**0.916**	**<0.001**			**0.934**	**<0.001**		
VO$_{2max}$			**0.916**	**<0.001**			**0.934**	**<0.001**
Age	-0.005	>0.984			0.083	>0.787		
Height	0.242	>0.349			-0.030	>0.921		
Weight	-0.502	<0.040			0.192	>0.529		
Body Mass Index	-0.146	>0.577			-0.089	>0.771		
Exercise Habit	-0.318	>0.213			-0.693	<0.009		
Smoking Habit	-0.272	>0.290						

Description: Pearson's Correlation Test, ANOVA for linear regression test

DISCUSSION

The mRNA Expression of PGC-1α Gene Increases after Physical Exercises

The finding showed that the PGC-1α gene expression increased. Several factors contributing to the increase were gender, age, and physical endurance training (sports). In this study, it was found that the average score of the mRNA expression of the PGC-1α gene increased after the male or female subjects participated in an exercise training program with a 5-week Rockport method. The result of further exploration showed that PGC-1α gene expression in males was higher than in females. Hence, it could be said that gender was one of the factors determining the PGC-1α gene expression. This finding was supported by a finding stating that PGC-1α gene expression in males was determined by two types of primordial germ cells, namely MSGs and FSGs; the MSGs type was found more dominant in males [23].

The age range of the research subjects was around 30 – 39 years old. It was found that PGC-1α gene expression had a prominent increase in the age range of 30 – 32 years. It was caused by the existence of the adaptation of mitochondrial cells in responding to the mechanism of the aging process in a person. The older the person is, the needs for calorie becomes more limited. On the other hand, the younger the person is, the calorie needed for the body to do physical activities, such as exercise training, is higher [24].

The findings of this study showed that there was an increase of PGC-1α gene expression after participating in an exercise training program with a 5-week Rockport method. Physical exercises would influence the total and the size of mitochondria (mitochondrial biogenesis). Regular physical exercises might have a 3 – 4 fold increase of the total and the size of mitochondria in the skeletal muscle cells compared to the total and the size without doing physical exercise [25]. PGC-1α is the major regulator of mitochondrial biogenesis, oxidative metabolism, and antioxidant protection [16, 26]. The effect of vigorous exercises triggered the PGC-1α gene expression that was the biomarker of mitochondrial biogenesis to increase the density of skeletal muscle fiber during exercise training [16]. A finding revealed that aerobic might significantly increase the gene expression, especially for PGC-1α.b and VO$_{2max}$. The gene induced the mitochondrial biogenesis in the skeletal muscle for increasing the physical fitness levels (VO$_{2max}$) [27]. Intensive exercises triggered the PGC-1α expression as a biomarker [28] to increase the function of mitochondria and to be able to increase the aerobic capacity (VO$_{2max}$) of ≥ 70% [29].

This finding was also in line with a study on the increase of the mRNA expression of PGC-1α gene reported by the previous researchers that the interval of exercises in the moderate intensity for 20 minutes, including 4 minutes of recovery session, might induce the PGC-1α gene that contributed to endurance training, such as mitochondrial

biogenesis [30]. PGC-1α gene is one of two genes triggering the increase of mitochondrial biogenesis volume, the increase of muscle cell capacity to produce ATP through oxidative phosphorylation [31]. Physical exercises produced an increase of adenosine triphosphate (ATP) and reactive oxygen species (ROS) production by the mitochondria of skeletal muscle in a person [32]. Mitochondrial biogenesis regulation in muscle cells was activated by PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha) induced by a signal of muscle contraction. PGC-1α would interact with PPAR (peroxisome proliferator-activated receptor) to regulate the gene expression and initiate the mitochondrial transcription and translation [14, 15].

The Physical Fitness Levels (VO$_{2max}$) Increase after Physical Exercises

The physical fitness levels (VO$_{2max}$) in male subjects were higher than in female subjects after participating in the physical exercise program with a 5-week Rockport method. The difference was caused by the aerobic capacity in males was higher than in females. Also, it was due to the females' age that was at a range of 35 – 39 years old. The decline of VO$_{2max}$ capacity in females was caused by the decline of the physiology of organ transportation and oxygen utilization as the people get older (aging). The result of the risk analysis, it was found that the risk of physical fitness based on cardiopulmonary (VO$_{2max}$) at the age of around 18–35 years had a risk of being fit 42 times higher than at the age of above 35–45 years. Someone who had good physical fitness had a higher VO$_{2max}$ value so that he/she was able to perform more intensive activities than the person who was not in good condition. This finding confirmed that the aerobic capacity of a PKHI through a physical endurance training is an important element in creating physical fitness that can be assessed through the maximum oxygen intake capacity (VO$_{2max}$). VO$_{2max}$ refers to the intensity of the aerobic process; the higher the intensity is, the higher the use of maximum oxygen uptake capacity [33].

Physical fitness levels (VO$_{2max}$) significantly increase in the subject who performs a regular physical exercise. It can be proven by a study on the effect of weekly exercise duration on VO$_{2max}$; the subjects were divided into 3 groups, namely group A (not performing exercises); group B (walking workout ±200 minutes/week; group C (walking workout >200 minutes/week and the scores were 1.735±467.5, 2.113±443.6, and 2.445±616.6 respectively. The data showed that the VO$_{2max}$ value in group B was higher than in group A and group C [34].

The Correlation of the mRNA Expression of PGC-1α Gene with Physical Fitness Levels (VO$_{2max}$)

The linear regression test proved that the contribution of PGC-1α gene expression to the increase of physical fitness

(VO$_{2max}$) in males was 87.2% and in females was 84%, while the 16% and 12.8% of each consecutively were the contribution of other variables that were not included in the study. One of the other variables was the regular exercise habit that was higher in males than in females.

The finding showed that PGC-1α gene expression was the Ser482 homozygotes. This genotype mediated the relationship between energy metabolism (ATP) and VO$_{2max}$ to increase cardiorespiratory fitness (CRF) when it was physically active. PGC-1α was a strong gene candidate for cardiorespiratory fitness (CRF). The PGC-1α mRNA gene was expressed primarily in the hyper-energy tissue that was mostly rich in mitochondria [35]. PGC-1α played a role to induce the coactivator that might increase the antioxidant enzyme expression and induce a number of mitochondria to produce ATP as a source of energy for the cells during the physical activities [36].

A study on the transcription modulation of mitochondrial biogenesis path in mice showed that the PGC-1α expression (mitochondrial biogenesis biomarker) and PPARβ positively correlated with aerobic capacity (VO$_{2max}$) but they were negatively correlated with the intensity of exercises. The underlying reasons that the complex responses of mitochondrial biogenesis path in muscle were fully modulated by the different intensity of exercises and molecular signals in a whole estimation of exercise intensity used in endurance training. The exercise intensity that maximized the mitochondrial biogenesis still needed to be determined [37]

The different finding was reported in a study that acute mRNA expression of PGC-1α was not correlated with the changes of VO$_{2max}$ in an individual after doing sprint interval training (SIT) for 2 or 6 weeks (p>0.05) due to the increase of mRNA PGC-1α after vigorous exercises followed by the increase of the mRNA expression of other mitochondrial genes [21].

The result of this study found new information that the mRNA expression of PGC-1α gene triggered the increase of VO$_{2max}$ through mitochondrial biogenesis after participating in the process of physical exercise with a 5-week Rockport method. Each increase of the value of PGC-1α gene expression of 3.99 ΔΔ^{CT} in males would contribute to the increase of VO$_{2max}$ of 4.88 ml/kg/min. Furthermore, each increase in the value of PGC-1α gene expression of 2.08 ΔΔ^{CT} in females would contribute to the increase of VO$_{2max}$ of 4.16 ml/kg/min.

CONCLUSION

PGC-1α gene expression triggers the increase of physical fitness (VO$_{2max}$) through mitochondrial biogenesis after participating in the physical exercise program with a 5-week Rockport method. Each increase of the value of PGC-1α gene expression of 3.99 ΔΔCT in males will contribute to the increase of VO$_{2max}$ of 4.88 ml/kg/min. Furthermore, each increase in the value of PGC-1α gene expression of 2.08

ΔΔCT in females will contribute to the increase of VO_{2max} of 4.16 ml/kg/min. These findings are beneficial for the development of biomedical and exercise science, especially gene expression in increasing physical fitness (VO_{2max}) in PKHI candidates.

It needs a further examination of the PGC-1α enzyme levels to assess the interaction of PGC-1α gene expression with PGC-1α enzyme levels against physical fitness (VO_{2max}).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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