



Available online at:

<https://ejournal.upi.edu/index.php/penjas/article/view/25547>DOI: <https://doi.org/10.17509/jpjo.v5i2.25547>

## Exercise Serum Alters Genes Related Mitochondria in Cardiomyocyte Culture Cell

Ronny Lesmana<sup>1,2\*</sup>, Wibowo Budi Prasetyo<sup>3</sup>, Hamidie Ronald Daniel Ray<sup>4</sup>, Vita Murniati Tarawan<sup>1</sup>, Hanna Goenawan<sup>1,2</sup>, Iwan Setiawan<sup>1,2</sup>, Yuni Susanti Pratiwi<sup>1,2</sup>, Nova Sylviana, Juliati<sup>1,2</sup>, Unang Supratman<sup>5</sup>

<sup>1</sup>Physiology Division, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran

<sup>2</sup>Physiology Molecular Laboratory, Biological Activity Division, Central Laboratory, Universitas Padjadjaran

<sup>3</sup>Undergraduate Program Medical Doctor, Faculty of Medicine, Universitas Padjadjaran,

<sup>4</sup>Departement of Sport Science, Faculty of Sport and Health Education, Universitas Pendidikan Indonesia,

<sup>5</sup>Departement of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor

### Article Info

#### Article History :

Received June 2020

Revised July 2020

Accepted August 2020

Available online September 2020

#### Keywords :

cardiomyocyte cell line, mitochondrial homeostasis, exercise serum

### Abstract

Exercise-induced heart muscle adaptation is important for physiological process after exercise. This adaptation will ensure basal mitochondrial homeostasis and as a part of the mitochondria quality control. This process is reflected by the equal level of biogenesis stimulation as well as the selective degradation of old and undesirable mitochondria through fusion or fission cycle and Mitophagy. There is limited information about genetic regulation stimulated by training in cardiomyocytes. We believe that there is a specific myokines or protein release in the serum that initiates cardiac muscle adaptation process. In the present study, twelve male wistar rats were appointed to two groups: sedentary control group and aerobic-intensity group (AE, 15m/minute). Rats were trained for running with a specific protocol as follows: 30 minutes/day with a 5 times/week interval conducted for 8 weeks. On the last day, the serum from the control and exercise groups were taken via retro-orbital sinus. Then, 3.105 H9C2 cells (Rat cardiomyocytes cell line) were cultured and incubated by this serum for 24 hours. After the treatment, cells were extracted by using trisure for RNA purification and followed by reverse transcriptase PCR. Our data showed that the expression of the Pgc-1 $\alpha$ , Mfn1, Mfn2, Opa1, Drp1, Pink, and Parkin genes were altered and modulated. Specifically, Mfn1, Mfn2, and Opa1 gene expression levels significantly increased. Interestingly, we did not find a significant modulation for Pgc-1 $\alpha$ , Drp1, Pink, and Parkin. Taken together, serum of exercise rats might contain myokines or a specific protein released during training, which altered mitochondrial genes expression in cardiomyocytes culture cell. We believe that the myokines release in the serum had a contribution in the cardiacmyocyte adaptation.

## INTRODUCTION

Health is a state of a complete physical, mental, and social well-being and not merely the absence of disease or infirmity. Various efforts will be made to improve or maintain physical health, one of them is by exercising with a certain intensity (Han, Neuffer, & Pilegaard, 2019). Exercise is a solitary intense episode of body or strong muscular movement that requires a vitality consumption over the resting level, within a duration, intensity, and modality. Physical exercise comprises of aerobic and anaerobic physical exercises. Aerobic physical exercise is physical exercise identified with oxygen for vitality extraction as adenosine triphosphate (ATP) from amino acids, sugars, and unsaturated fats utilized for physical exercise, while anaerobic physical exercise is an exercise that does not require oxygen in the process of forming energy sources, where the ATP formation is from fermentation and glycolysis (Patel, Alkhawam, Madanieh, Shah, Kosmas, & Vittorio, 2017).

American School of Sports Medication (ACSM) characterizes oxygen consuming activity (aerobic exercise) as any action utilizing a huge muscle group that can be kept up constantly. Running, treadmill, bike, and swimming are some examples of aerobic physical exercise. European Society of Cardiology heart failure guidelines recommend aerobic exercise to improve the performance capacity and cardiovascular endurance (Ponikowski et al., 2016). Taking the aerobic physical exercise in a moderate intensity for 30 minutes/day, 150 minutes/seven day, or in vigorous intensity or blend of both for 75 minutes/seven day is recommended for adults (Gunadi et al., 2020). Physical exercise results in physiological changes in almost all body systems, including improving metabolic and cardiovascular status. The positive impacts of activity on the cardiovascular system are well documented (Gunadi et al., 2020; Piepoli et al., 2016).

Cardiovascular performance during exercise depends on cardiac muscle performance which needs an extra-extraordinary support amount of ATP. This will keep the cardiac muscle function normally and generate force every minute of every day over a lifetime. This demand is increased especially during physical exercise. The Myocardium is supported by mitochondria, cell organelles that work as the main energy source of

ATP synthesis: bioenergetics, metabolism (Oxidative Phosphorylation) in cells during aerobic respiration, well known as "powerhouse of the cell" (Murphy et al., 2016). The higher the heart's performance, the higher the heart rate and oxygen consumption (Maximum Oxygen Consumption/VO<sub>2</sub>Max) (Murphy et al., 2016). During exercise, mitochondria, which have a special function, are transporting calcium and controlling quality to improve the quality of the heart pump so that oxygen is distributed more and faster throughout the body (Murphy et al., 2016; Katch, McArdle, & Katch, 2011).

Exercise induces the occurrence of remodelling of mitochondrial cardiac in the heart muscle that indicates a positive effect of physical exercise. To achieve heart muscle adaptation, it is necessary to save basal mitochondrial homeostasis and secure the heart under pressure conditions, known as Mitochondria Quality Control via stimulates biogenesis of mitochondria as well as the expulsion of old and undesirable mitochondria through mitochondrial dynamics (Fusion – Fission Cycle) and Mitophagy. It is comparable to supplanting low eco-friendliness old vehicles for high eco-friendliness new vehicles to clean the environment. Each stage of Mitochondria quality control has a coactivator as a trigger for the process (Yan, Lira, & Greene, 2012). It starts from biogenesis with Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (Pgc-1 $\alpha$ ), which is indeed able to activate the nuclear gene and mitochondria needed for the synthesis of organelles. After biogenesis occurs, it will enter the fusion-fission cycle stage. Starting from the results of biogenesis fusion with other mitochondria which are mediated by mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2) for the fusion in the outer membrane and mitochondrial dynamin like GTPase (Opa1) for fusion in the inner membrane. After fusion, mitochondria increase in size. To maximize their function, fission occurs in the old and unhealthy parts mediated by dynamin-1-like protein (Drp1). Finally, in the old and unhealthy mitochondrial section occurs mitophagy mediated by Pink-Parkin signalling pathway. Pgc-1 $\alpha$ , Mfn1, Mfn2, Opa1, Drp1, Pink, and Parkin serve as a marker to study Mitochondria Quality Control (Sun & Finkel, 2015; Gottlieb & Thomas, 2017; Youle & van der Bliek, 2012).

However, there are limited studies regarding

"Autophagic Adjustments to Long Haul Constant Exercise in Cardiovascular Muscle" (Tam et al, 2015). The differences of adaptation physiology in physical exercise with various intensities to the process of mitochondria quality control, like Mitophagy and Fusion, are still unknown. This study was aimed at understanding the mitochondria gene modulation induced by serum of aerobic training in cardiomyocyte (H9C2 cell line).

## METHODS

### Animals

Eight-week-old male Wistar rats (*Rattus Norvegicus*) were gotten from the Animal Rearing Focal point of PT Bio Farma in Cisarua, Indonesia. The habitat was kept up in a darklight cycle (12 hours of light cycle and 12 hours of dim cycle) and temperature (22°C). The rats were nourished a pellet rat diet, not obligatory, and had free access to water. Following one week of acclimatization period, 12 male rats were partitioned into two groups, sedentary control group and exercise aerobic group. Every single trial strategy followed the guide for the care and use of lab creatures and was affirmed by the Health Research Ethics Committee of the Faculty of Medicine Universitas Padjadjaran No 1355/UN6.KEP/EC/2019

### Treadmill Exercise Protocol

Rats were divided into sedentary control group (SC) and training group (TG). In SC group, rats were placed only in a treadmill without work out. In TG

group, rats were habituated for one weeks. The rats then were exercised 30 min/day duration. By using treadmill, the rats were trained for running for 15m/minutes, 5 days/week, for eight weeks. After eight weeks of training programs, serum from rats from control and exercise groups were taken via the retro-orbital sinus. Rats were conditioned as comfortable as possible. The microhemato pipet was etched on the medial canthus of the eye towards the foramen opticus, then rotated until it injured the plexus. The blood was collected in a tube for the purpose of taking blood serum.

### Cardiomyocyte (H9C2) Cell Line

Adherent H9C2 Cell lines (rat cardiomyocytes; ATCC, Manassas, VA, USA) were cultured and grown with Dulbecco's Modified Eagle Medium (DMEM, PAA Laboratories, Pasching, Austria), and supplemented with 10% fetal bovine serum, (Life Technologies, Carlsbad, CA, USA), 100 U/mL penicillin and 100 µg/mL streptomycin (all from Invitrogen, Carlsbad, CA, USA). Cells were maintained at 37°C in a humidified incubator with an atmosphere of 95% air and 5% CO<sub>2</sub>. H9C2 cells were plated on the 24 plate well, cultured for 2 to 3 days, and used for experiments at approximately 80% confluence was achieved. Cells were rinsed with PBS solution, then released with trypsin and EDTA solution (Life Technologies). Then, non-aerobic exercise serum, aerobic exercise serum 10%, and aerobic exercise serum 20% were added. The incubation lasted for 12 – 16 hours.

Table 1. Primers Sequences

Gene Symbol	Primer Sequence	Product Size (bp)	Annealing (°C)	Input PCR template (NCBI Primer Blast)
<i>Pgc-1α</i>	F 5'-GTGCAGCCAAGACTCTGTAT-3' R 5'-CTCGAATATGTTTCGCGGGCT-3'	214	60°C	NM_031347.1
<i>Mfn1</i>	F 5'-TGACTTGGACTACTCGTGCG-3' R 5'-GTGGCCATTTCTTGCTGGAC-3'	133	55°C	NM_138976.1
<i>Mfn2</i>	F 5'-TCAGTAGCCAATCTGGACCT-3' R 5'-TCTCTGGATGTAGGCCCCC-3'	277	60°C	NM_130894.4
<i>Opa1</i>	F 5'-CTGGGATCTGCTGTTGGAGG-3' R 5'-GGTGTACCCGAGTGAAGA-3'	251	60°C	NM_133585.3
<i>Drp1</i>	F 5'-CGCTGATCCCGTTCATCAAT-3' R 5'-ACTCCATTTTCTTCTCCTGTTGT-3'	247	60°C	NM_053655.3
<i>Pink1</i>	F 5'-TGCAATGCCGCTGTGTATGA-3' R 5'-TCTGCTCCCTTTGAGACGAC-3'	113	60°C	NM_001106694.1
<i>Parkin</i>	F 5'-CAGTATGGTGCCGAGGAGTG-3' R 5'-CCGGTATGCCTGAGAAGTCG-3'	228	60°C	NM_020093.

## RNA Extraction and Semi-Quantitative PCR

Total RNA was isolated from H9C2 Cell lysate utilizing TRIzol reagent (Thermo Fisher Logical, Uppsala, Sweden). Total RNA in H9C2 Cell was quantified utilizing multimode microplate Peruser at 268/280 nm absorbance spectrophotometry (M200 Star, Tecan, Morrisville, NC). Semi quantitative PCR was performed utilizing the One Step RT PCR Pack (Qiagen, Valencia, CA). Semi quantitative quality articulation levels were standardized utilizing rats GAPDH. Primer sequences utilized for rat gene expression are recorded in Table 1. The band of PCR items was captured with and analyzed utilizing ImageJ programming (NIH).

## Statistical Analysis

All measurements were registered utilizing SPSS 20.0 programming. Results are presented as the mean  $\pm$  standard error of mean (mean  $\pm$  SEM). Mean contrasts between groups were analyzed with One Way ANOVA and Tukey post hoc test (for information with typical dissemination) or Kruskal Wallis and Mann Whitney (for information without ordinary distribution), with Significance is indicated as: \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , and \*\*\*\* $p \leq 0.0001$ .

## RESULT

### The Effect of Aerobic Exercise on Quantification of H9C2 Cell Line / Cardiomyocyte

The Quantification of H9C2 Cell line change was measured after culture incubation (adding non exercise serum or Exercise Serum). The result was the image of the Quantification of H9C2 Cell Line in magnification 4x (Figure 1C) and in magnification 20x (Figure 1D). The Quantification of Exercise Serum 10% ( $156.88 \pm 7.17$ ) and Exercise Serum 20% Group ( $159.18 \pm 7.17$ ) were not significantly different with the control group ( $137.68 \pm 5.14$ ) and the Non Exercise Serum ( $147.89 \pm 7.24$ ) in Figure 1A. In Zoom 20x, the Quantification of Exercise Serum 10 % ( $141.17 \pm 0.45$ ) and Exercise Serum 20% Group ( $158.53 \pm 9.09$ ) were not significantly different with the control group ( $118.58 \pm 1.36$ ) and the Non Exercise Serum ( $119.83 \pm 1.03$ ) after the 8 week experimental period (Figure 1B)

### Aerobic Exercise Serum Pgc-1 $\alpha$ , Mfn1, Mfn2, Opa1, Drp1, Pink1, and Parkin mRNA Expression in H9C2 Cell Line

Mitochondria biogenesis gene (Pgc-1 $\alpha$ ), Mitochondrial Fusion gene (Mfn1, Mfn2, Opa1), Mitochondrial Fission gene (Drp1), and Mitophagy gene (Pink1, Parkin) Pgc-1 $\alpha$ , Mfn1, Mfn2, Opa1, Drp1, Pink1, and Parkin (Figure 2A) expressions in H9C2 cell line/cardiomyocyte of rat were measured and normalized by using GAPDH. The Aerobic exercise serum did not significantly stimulate the Mitochondrial biogenesis, because of the Pgc-1 $\alpha$  gene expression. Drp1 gene expression was not significantly increasing. Also, Aerobic exercise serum did not stimulate the Mitophagy, because the Pink1 gene expression and Parkin gene expression did not significantly increase compared to the control (FBS) group and Non-Exercise group. On the other side, aerobic exercise serum only significantly increase the Mfn1 gene expression, Mfn2 gene expression, and Opa1 gene expression compared to the control (FBS) group and Non-Exercise group. This research found that, the Aerobic exercise serum might only significantly stimulate the mitochondrial fusion.

**Table 2.** The analysed result of of Pgc-1 $\alpha$ , Mfn1, Mfn2, Opa1, Drp1, Pink1, and Parkin gene expression were altered by Aerobic

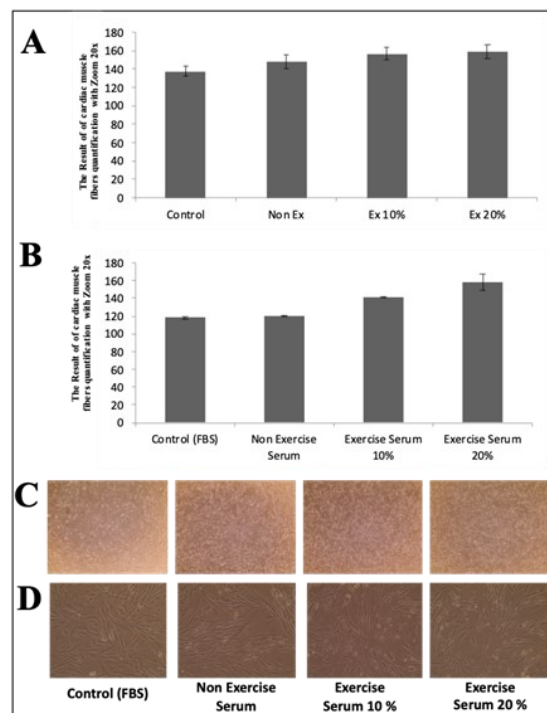
Gene Symbol	FBS (Control)	Non Exercise Serum	Exercise Serum 10%	Exercise Serum 20%
<i>Drp 1</i>	1.063 $\pm$ 0.19	0.935 $\pm$ 0.20	2.275 $\pm$ 0.31	1.969 $\pm$ 0.50
<i>Mfn1</i>	1.300 $\pm$ 0.07	1.078 $\pm$ 0.09	2.410 $\pm$ 0.16	2.271 $\pm$ 0.41
<i>Mfn2</i>	1.178 $\pm$ 0.10	0.989 $\pm$ 0.11	2.012 $\pm$ 0.05	1.927 $\pm$ 0.23
<i>Opa1</i>	0.977 $\pm$ 0.08	0.876 $\pm$ 0.15	2.141 $\pm$ 0.25	1.772 $\pm$ 0.46
<i>Pink1</i>	1.126 $\pm$ 0.23	1.076 $\pm$ 0.28	2.129 $\pm$ 0.25	1.726 $\pm$ 0.58
<i>Parkin</i>	1.163 $\pm$ 0.21	0.809 $\pm$ 0.15	1.255 $\pm$ 0.08	1.512 $\pm$ 0.24
<i>Pgc-1<math>\alpha</math></i>	1.019 $\pm$ 0.08	0.868 $\pm$ 0.03	1.295 $\pm$ 0.25	1.838 $\pm$ 0.93

## DISCUSSION

The effects of aerobic exercise are in the form of increasing the ability to exercise in a longer duration. Physiological changes due to endurance training include the body's ability to supply ATP through an aerobic process. The adaptation depends on the training sta-

tus and genetics of the individual concerned. Individuals who are new to exercise or deconditioning can experience a substantial increase in performance. The progressions in mitochondrial structure and capacity reflect changes in myocyte structure, and, in this manner, ought to be viewed as a fundamental part of myocyte differentiation. Interestingly, cardiac muscle fibers contain two particular mitochondrial populations (i.e. subsarcolemmal and intermyofibrillar). While the intermyofibrillar mitochondria keeps up moderately high respiratory limit, the subsarcolemmal mitochondria adjust more promptly to exercise training (Yan, Lira, & Greene, 2012). We did not observe any significant myotube structure changes (Figure 1). However, the importance in exercise-induced adjustment is not only to build the quantity of organelles, but also to improve the capacity/the effectiveness of the mitochondrial organization, results from expanded paces of mitochondrial biogenesis, and the productive expulsion of useless/harmed mitochondria through mitochondrial dynamics (Fusion-Fission Cycle) and Mitophagy, known as Mitochondria quality control (Figure 2) (Chen, Liu, & Dorn, 2011).

Pgc-1 $\alpha$  is required for the synthesis of mitochondria organelles. Pgc-1 $\alpha$ -instigated translation factors, Pgc-1 $\alpha$ , is presently considered the "chief controller" of mitochondrial biogenesis (Kang & Li, 2012). It shows the need for Pgc-1 $\alpha$  for typical incited metabolic adjustment, including mitochondrial biogenesis (Yan, Lira, & Greene, 2012). The consistently working muscles, for example the heart, have more mitochondrial movement and substance than sporadically working muscles. The muscles associated with ordinary and continued physical movement can expand their mitochondrial action and increment execution, which indicated an expansion in muscle cytochrome c fixation, just as expanded exercises of key mitochondrial catalysts and OXPHOS (oxidative phosphorylation) (Jornayvaz & Shulman, 2010). However, our result indicated that Pgc-1 $\alpha$  mRNA expression was not stimulated by aerobic exercise serum (Figure 2B). There is another factor that should be considered on mitochondrial biogenesis stimulation. We suggest that, for further research, it is necessary to detect the protein triggering an increase in PGC - 1 $\alpha$  gene expression, generally controlled by upstream



**Figure 1.** Alteration of mitochondrial quality control gene expression in H9C2 Cell line (Cardiomyocyte) of wistar rats by Aerobic Exercise A. The Result of of cardiac muscle fibers quantification with magnification 4x. B. The Result of of cardiac muscle fibers quantification with magnification 20x. C. H9C2 Cell Line / Cardiomyocyte with magnification 4x. D. H9C2 Cell Line /

kinase protein signaling pathways. The two essential kinase protein engaged with the guideline of Pgc-1 $\alpha$  in muscles are AMPK and mitogen-actuated kinase protein (p38 $\gamma$  MAPK). At any rate, two AMPK phosphorylation locales had been recognized in Pgc-1 $\alpha$ . AMPK initiated Pgc-1 $\alpha$  as well as advanced Pgc-1 $\alpha$  gene translation and controlled the expression and action of Pgc-1 $\alpha$ . It has additionally been demonstrated that AMPK incited the statement of mitochondrial qualities through Pgc-1 $\alpha$ . AMPK has been known as the "fundamental metabolic switch" for intense guideline of energy metabolism and exercise-incited by work out. Intense exercise appears to initiate AMPK through phosphorylation in Thr172. While single activities bring about expanded AMPK movement in the muscles, long-term exercise prompts an expansion in AMPK protein content. Both intense exercise and a long-haul preparation were not ready to expand the substance of AMPK $\alpha$  protein in the muscles of Pgc-1 $\alpha$ -lacking rat. It shows that Pgc-1 $\alpha$  is not exclusively reliant on AMPK,

yet, at any rate, the AMPK  $\alpha$  subunit relies upon Pgc-1 $\alpha$  (Yan, Lira, & Greene, 2012).

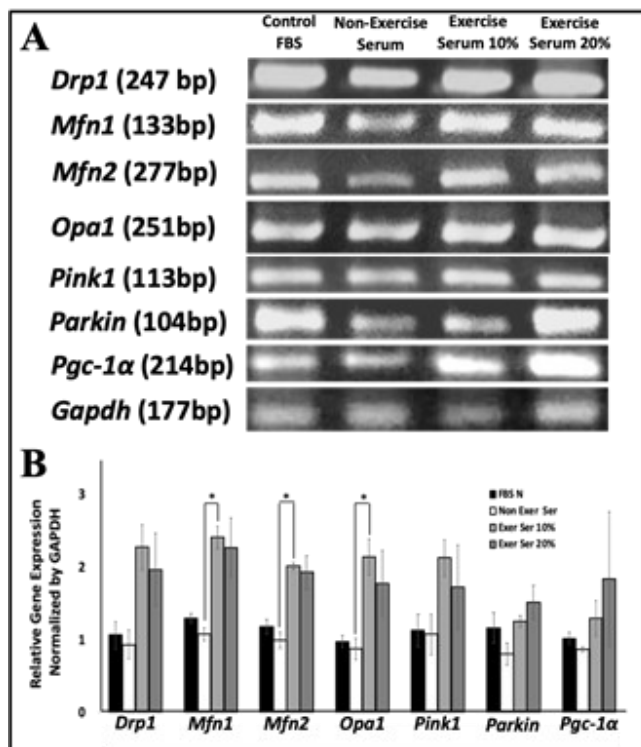
Aerobic exercise serum directs both mitochondrial fusion and fission forms and enables healthy and metabolically dynamic cells to shape interconnected mitochondrial tissues to share components (protein, substrate, mitochondrial DNA (mtDNA) and removal of dysfunctional regions (Song, Mihara, Chen, Scorrano, & Dorn, 2015). Fission machines and mitochondrial fusion are regulated by proteolysis and posttranslational modification (Youle & van der Blik, 2012). The process is regulated by the enzyme GTP-hydrolyzing (GTPase) such as Mfn1 (mitofusin 1), Mfn2 (mitofusin 2), Opa1, and Drp (Tarawan, Gunadi, Subekti, Widowati, & Goenawan, 2019). It have been appeared to dynamically build the protein substance of Mfn1, Mfn2 mRNA 24 h post-exercise simultaneous with expanded in COX IV mRNA (Ding et al., 2010). Mitochondrial fusion includes mitofusin 1 and 2 (Mfn1 and Mfn2), which controls the fusion of outer mitochondrial membranes encapsulating intermembrane inner and space membranes, and type 1 optical atrophy (Opa1), which controls mitochondrial inner membrane fusion containing membranes Complex binding oxidative phosphorylation enzymes and most soluble electron transport proteins such as cytochrome c (Mishra & Chan, 2014; Friedman & Nunnari, 2014). Opa1 is additionally significant for keeping up the crystalline structure (Iglewski, Hill, Lavandero, & Rothermel, 2010). Mitofusins and Opa1 usually work together to jointly combine both mitochondrial membranes (Murphy et al., 2016). Thus, which promotes mitochondrial fusion into dynamics, tubular complexes that maximize the efficiency of oxidative phosphorylation during stress are below the critical threshold by stimulating complementation between mitochondria (Youle & van der Blik, 2012).

Our data reveal a potential that aerobic exercise serum may modulate mitochondria fusion activity in the adaptation process after training in cardiac muscles. The mitochondrial fusion gene expression in cardiac muscle is presented in (Figure 2B). These findings are in agreement with those of previous studies that have demonstrated increased mitochondria fusion gene expression after aerobic treadmill training in cardiac muscles. These previous research recommend that Pgc-

1 $\alpha$  plays a Significant capacity in controlling the expression of the machinery for in any event the mitochondrial fusion process in muscle under the states of activity (Yan, Lira, & Greene, 2012). Interestingly, Pgc-1 $\alpha$  was not significantly increasing. PGC1 $\alpha$  might not be the only factor for mitochondrial fusion. Previous research shows that there are other factors. Mfn1 and Mfn2 proteins have greater than 70% sequence similarity and share much of the same functional domain organization. Mitofusin is highly abundant in heart and skeletal muscle and is reportedly expressed at low levels in numerous other human tissues. Hence, when mild exercise is triggered, Mitochondrial fusion occurs earlier (Seo, Joseph, Dutta, Hwang, Aris, & Leeuwenburgh, 2010). The equal procedure of mitochondrial fusion, in which two mitochondria circuits create a bigger organelle, requires the coordination of the external and internal mitochondrial membranes (Filadi, Pendim, & Pizzo, 2018). Prior to fusion, bending of the outer membranes is advanced by the phospholipase D-subordinate hydrolysis of cardiolipin (Ranieri et al., 2013). In spite of the fact, its regulation and the collection of specific accessory proteins are probably going to be profoundly setting ward (Palmer, Osellame, Stojanovski, & Ryan, 2011). Machines engaged with mitochondrial dynamic require the cooperation of a few proteins. Quickly, healthy mitochondria keep up the electrochemical internal layer angle,  $\Delta\psi_m$ , which drives ATP creation by a complex electron transport (Generation of ATP: Bioenergy and Metabolism).

The mitochondrial fission is significant for developing and separating cells to fill them with a sufficient number of mitochondria (Youle & van der Blik, 2012). For fission, a procedure whereby a solitary mitochondrion squeezes the inner membranes and outers to frame 2 mitochondrial daughter, dynamic-linked peptide 1 (Drp1), appears to assume a significant role, a homolog of a decent dynamin (Murphy et al., 2016; Sun & Finkel, 2015). The activity of Drp1 is regulated by phosphorylation in several locations on proteins. This study showed a not significant alteration of Drp1, Pink1, and Parkin in cardiac muscle (H9C2 Cell Line) compared to those in control groups (Figure 2). Our findings are different from previous studies which showed a significant increase in mitochondria fission gene expression after aerobic treadmill training

in cardiac muscle. The Drp1, Pink1, and Parkin gene expressions were higher in Aerobic Exercise Serum 10% and Aerobic Exercise Serum 20% compared to that in control and non exercise serum.



**Figure 1.** Alteration of mitochondrial quality control gene expression in H9C2 Cell line (Cardiomyocyte) of wistar rats by Aerobic Exercise. A. Pgc-1 $\alpha$ , Mfn1, Mfn2, Opa1, Drp1, Pink1, and Parkin expression in control, non exercise and Aerobic exercise. B. Relative Pgc-1 $\alpha$ , Mfn1, Mfn2, Opa1, Drp1, Pink1, and Parkin expression levels normalized by GAPDH expression level in H9C2 Cell line (Cardiomyocyte) of wistar rats. \*p  $\leq$  0.05. Analysed with ANOVA post hoc tukey

Based on the previous research, Drp1 does not work alone. It includes additional proteins. For example, mitochondrial fission proteins (FIS1) are expected to enlist and control the general fission procedure. It is important to recruit Drp 1 to Mitochondria, Mid49, Mid51, and mff51, often in locations where mitochondria come into contact with the endoplasmic reticulum (Youle & van der Bliek, 2012). Many factors affect fission, one physique also requires several accessory proteins, including 18 kDa mitochondrial protein (MTP18), endophilin B1 (also known as Bif-1), ganglioside induction of protein-

related differentiation 1 (GDAP1), and protein related death 3 (DAP3) (Seo, Joseph, Dutta, Hwang, Aris, & Leeuwenburgh, 2010). Mitophagy is closely related to mitochondrial dynamism (ie, fission and fusion). It assumes a significant job in controlling the quality of cardiac mitophagy through mediated asymmetric Physiology by Drp 1 (Kageyama et al., 2014). These findings are in agreement with the result of Drp1 in this research that had demonstrated insignificant increase of mitochondria fission gene expression after aerobic treadmill training in cardiac muscles. The mitochondria fission and Mitophagy response only occur when Eldery mitochondria (aging) or damaged mitochondria cannot maintain normal  $\Delta\psi_m$ ; damaged mitochondria can completely eliminate  $\Delta\psi_m$  and bring about depolarization (Romanello et al., 2010; Lazarou et al., 2015; Narendra et al., 2010; Jin, Lazarou, Wang, Kane, Narendra, & Youle, 2010). Damaged mitochondria (and therefore depolarizations) will be immediately identified and removed through the Pink1-Parkin-interceded mitophagy, while health mitochondria will rejoin the mitochondria cell, potentially by joining other comparable mitochondria. The specific role for the mechanism of mitophagy which depends on Parkin versus independent Parkin or "alternative" in the healthy and diseased cardiac is only just in the beginning to be investigated (Kageyama et al., 2014; Song et al., 2015).

The effects of mitochondrial quality control could improve metabolic capacity and oxygen supply to cardiac muscles and may be a beneficial mechanism to improve health in cardiovascular disease. The limitation of this research is that the study had only been conducted in 8 weeks and it is possible that adaptation had occurred in earlier time period. Another point is hormone may take a role in stimulating mitochondrial quality control and we did not perform experiment using female rats. Therefore, it can eliminate the gender difference responses. It could be more interesting to study whether stress during treadmill plays a role in mitochondrial quality control alteration in rats. We also suspect that our research method is new in this study where cardiac culture cells (H9C2 Cell Line), which were reacted with treated rat blood serum, carried out in incubation, and taken RNA isolates from the reaction for poly chain reaction and electrophoresis, were used. Meanwhile, previous research isolated RNA directly

taken from the rat heart muscle. Taken together, serum of aerobic exercise could stimulate gene expression of Mitochondria fusion (Mfn1, Mfn2, Opa1) for Mitochondrial adaptation.

Taken together, serum of exercise rats might contain myokines or specific protein which was released during training and it altered mitochondrial genes expression in cardiomyocytes culture cell. We believe that myokines release in the serum had a contribution in cardiacmyocyte adaptation.

## CONCLUSION

In summary, serums from aerobic exercise intensity Mfn1, Mfn2, Opa1 showed significant alteration gene expressions. However, Pgc-1 $\alpha$ , Drp1, Pink, and Parkin did not significantly alter levels in cardiac muscle (H9C2 cell line). The data demonstrate that aerobic exercise might play a role on mitochondrial quality control activity via mitochondrial Fusion (Increasing Size of Mitochondria) in cardiac muscle during training. Alteration of these gene expressions might contribute to cardiac physiological adaptation during training. It is important to understand biocellular adaptation in cardiac muscle.

## ACKNOWLEDGEMENT

We The authors thank to Susianti, M.Si and Canada Revalita, S.Si for the technical assistance; and Central Laboratory, Padjadjaran University that had supported this study.

## Conflict of Interest

The authors declare there is no conflict of interest.

## Funding Information

This study was supported by Penelitian Unggulan Dasar Perguruan Tinggi (PUPT) Grant from Ministry of Research Technology and Higher Education Republic Indonesia (to R. Lesmana)

## REFERENCES

- Chen, Y., Liu, Y. & Dorn, G. W. (2011). Mitochondrial fusion is essential for organelle function and cardiac homeostasis. *Circulation Research*, 109(12), pp. 1327-1331. DOI: 10.1161/circresaha.111.258723.
- Ding, H., Jiang, N., Liu, H., Liu, X., Liu, D., Zhao, F. et al. (2010). Response of mitochondrial fusion and fission protein gene expression to exercise in rat skeletal muscle. *Biochimica et biophysica acta*, 1800(3), pp. 250-256. DOI: 10.1016/j.bbagen.2009.08.007.
- Filadi, R., Pendin, D., & Pizzo, P. (2018). Mitofusin 2: from functions to disease. *Cell Death & Disease*, 9(3), pp. 1-13. DOI: 10.1038/s41419-017-0023-6.
- Friedman, J. R. & Nunnari, J. (2014). Mitochondrial form and function. *Nature*, 505(7483), pp. 335-343. DOI: 10.1038/nature12985.
- Gottlieb, R. A. & Thomas, A. (2017). Mitophagy and Mitochondrial Quality Control Mechanisms in the Heart. *Current pathobiology reports*, 5(2), pp. 161-169. DOI: 10.1007/s40139-017-0133-y.
- Gunadi, J. W., Tarawan, V. M., Daniel Ray, H. R., Wahyudianingsih, R., Lucretia, T., Tanuwijaya, F. et al. (2020). Different training intensities induced autophagy and histopathology appearances potentially associated with lipid metabolism in wistar rat liver. *Heliyon*, 6(5), pp. 1-12. DOI: 10.1016/j.heliyon.2020.e03874.
- Han, J., Neuffer, D., & Pilegaard, H. (2019). Exercise physiology: future opportunities and challenges. *Pflügers Archiv - European Journal of Physiology*, 471(3), pp. 381-384. DOI: 10.1007/s00424-019-02263-6.
- Iglewski, M., Hill, J. A., Lavandero, S., & Rothermel, B. A. (2010). Mitochondrial fission and autophagy in the normal and diseased heart. *Current hypertension reports*, 12(6), pp. 418-425. DOI: 10.1007/s11906-010-0147-x.
- Jin, S. M., Lazarou, M., Wang, C., Kane, L. A., Narendra, D. P., & Youle, R. J. (2010). Mitochondrial membrane potential regulates Pink1 import and proteolytic destabilization by PARL. *The Journal of cell biology*, 191(5), pp. 933-942. DOI: 10.1083/jcb.201008084.
- Jornayvaz, F. R. & Shulman, G. I. (2010). Regulation of mitochondrial biogenesis. *Essays in biochemistry*, 47, pp. 69-84. DOI: 10.1042/bse0470069.
- Kageyama, Y., Hoshijima, M., Seo, K., Bedja, D., Syta-Shah, P., Andrabi, S. A., ... Sesaki, H. (2014). Parkin-independent mitophagy requires Drp1 and maintains the integrity of mammalian heart and brain. *The EMBO journal*, 33(23), pp. 2798-2813. DOI: 10.15252/embj.201488658.
- Kang, C. & Li, J. L. (2012). Role of PGC-1 $\alpha$  signaling in skeletal muscle health and disease. *Annals*



- of the New York Academy of Sciences, 1271(1), pp. 110-117. DOI: 10.1111/j.1749-6632.2012.06738.x.
- Katch, V. L., McArdle, W. D., & Katch, F. I. (2011). *Essentials of Exercise Physiology*. Lippincott Williams & Wilkins.
- Lazarou, M., Sliter, D. A., Kane, L. A., Sarraf, S. A., Wang, C., Burman, J. L. et al. (2015). The ubiquitin kinase Pink1 recruits autophagy receptors to induce mitophagy. *Nature*, 524(7565), pp. 309-314. DOI: 10.1038/nature14893.
- Mishra, P. & Chan, D. C. (2014). Mitochondrial dynamics and inheritance during cell division, development and disease. *Nature reviews Molecular cell biology*, 15(10), pp. 634-646. 2014/09/23. DOI: 10.1038/nrm3877.
- Murphy, E., Ardehali, H., Balaban, R. S., DiLisa, F., Dorn, G. W. 2nd., Kitsis, R. N. et al. (2016). Mitochondrial Function, Biology, and Role in Disease. *Circulation Research*, 118(21), pp. 1960-1991. DOI: 10.1161/RES.000000000000104.
- Narendra, D. P., Jin, S. M., Tanaka, A., Suen, D.-F., Gautier, C. A., Shen, J. et al. (2010). Pink1 Is Selectively Stabilized on Impaired Mitochondria to Activate Parkin. *PLOS Biology*, 8(1), pp. 1-22. DOI: 10.1371/journal.pbio.1000298.
- Palmer, C. S., Osellame, L. D., Stojanovski, D., & Ryan, M. T. (2011). The regulation of mitochondrial morphology: intricate mechanisms and dynamic machinery. *Cellular signalling*, 23(10), pp. 1534-1545. DOI: 10.1016/j.cellsig.2011.05.021.
- Patel, H., Alkhawam, H., Madanieh, R., Shah, N., Kosmas, C. E., & Vittorio, T. J. (2017). Aerobic vs anaerobic exercise training effects on the cardiovascular system. *World Journal Cardiology*, 9(2), pp. 134-138. DOI: 10.4330/wjc.v9.i2.134.
- Piepoli, M. F., Hoes, A. W., Agewall, S., Albus, C., Brotons, C., Catapano, A. L. et al. (2016). European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *European Heart Journal*, 37(29), pp. 2315-2381. DOI: 10.1093/eurheartj/ehw106.
- Ponikowski, P., Voors, A. A., Anker, S. D., Bueno, H., Cleland, J. G. F., & Coast, A. J. S. (2016). ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *European Heart Journal*, 37(8), pp. 891-975. DOI: 10.1002/ehfj.592.
- Ranieri, M., Brajkovic, S., Riboldi, G., Ronchi, D., Rizzo, F., Bresolin, N. et al. (2013). Mitochondrial Fusion Proteins and Human Diseases *Neurology Research International*, 2013, pp. 1-11. DOI: 10.1155/2013/293893.
- Romanello, V., Guadagnin, E., Gomes, L., Roder, I., Sandri, C., Petersen, Y. et al. (2010). Mitochondrial fission and remodelling contributes to muscle atrophy. *The EMBO journal*, 29(10), pp. 1774-1785. DOI: 10.1038/emboj.2010.60.
- Seo, A. Y., Joseph, A., Dutta, D., Hwang, J. C. Y., Aris, J. P., & Leeuwenburgh, C. (2010). New insights into the role of mitochondria in aging: mitochondrial dynamics and more. *Journal of Cell Science*, 123(15), pp. 2533-2542. DOI:10.1242/jcs.070490.
- Song, M., Gong, G., Burelle, Y., Gustafsson, Å. B., Kitsis, R. N., Matkovich, S. J. et al. (2015). Interdependence of Parkin-Mediated Mitophagy and Mitochondrial Fission in Adult Rat Hearts. *Circulation Research*, 117(4), pp. 346-351. DOI: 10.1161/circresaha.117.306859.
- Song, M., Mihara, K., Chen, Y., Scorrano, L., & Dorn, G. W. (2015). Mitochondrial fission and fusion factors reciprocally orchestrate mitophagic culling in rat hearts and cultured fibroblasts. *Cell metabolism*, 21(2), 273-286. DOI: 10.1016/j.cmet.2014.12.011.
- Sun, N. & Finkel, T. (2015). Cardiac mitochondria: A surprise about size. *Journal of Molecular and Cellular Cardiology*, 82, pp. 213-215. DOI: 10.1016/j.yjmcc.2015.01.009.
- Tam, B. T., Pei, X. M., Yung, B. Y., Yip, S., Chan, L., Wong, C. et al. (2015). Autophagic Adaptations to Long-term Habitual Exercise in Cardiac Muscle. *International Journal of Sports Medicine*, 36(07), pp. 526-534. 11.03.2015. DOI: 10.1055/s-0034-1398494.
- Tarawan, V. M., Gunadi, J. W., Subekti, T. A. B., Widowati, W., & Goenawan, H. (2019). Effect of Acute Physical Exercise with Moderate Intensities on FGF23 Gene Expression in Wistar Rat Heart. *Majalah Kedokteran Bandung*, 51(4), pp. 221-225. DOI: 10.15395/mkb.v51n4.1844.
- Yan, Z., Lira, V. A., & Greene, N. P. (2012). Exercise training-induced regulation of mitochondrial quality. *Exercise and sport sciences reviews*, 40(3), pp. 159-164. DOI: 10.1097/JES.0b013e3182575599.
- Youle, R. J. & van der Bliek, A. M. (2012). Mitochondrial fission, fusion, and stress. *Science (New York, NY)*, 337(6098), pp. 1062-1065. DOI: 10.1126/science.1219855.