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Original article

Effect of different intensities aerobic exercise to cardiac angiogenesis regulation on Wistar rats

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Abstract

The adaptation response of myocardium angiogenesis stimulated by specific exercise intensities remains unclear. The aims of this study is to explore the effect of different intensities aerobic exercise to cardiac angiogenesis regulation via HIF-1 α , PGC-1 α , VEGF, and CD34⁺ in Wistar rats. Wistar rats were divided into control and exercise groups. Exercise groups were trained on a treadmill for 12 weeks, 30 min/day for 5 days with low, moderate, and high-intensity groups. The rats were sacrificed, and the myocardium was collected and preserved at -80°C until used. Cardiac protein samples were extracted and run for Western blotting using the specific antibodies: hypoxia-inducible factor (HIF)-1 α , Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α), vascular endothelial growth factor (VEGF), and Cluster of differentiation 34 (CD34⁺). Results showed that protein expression of HIF-1 α , PGC-1 α , VEGF, and CD34⁺ was increased significantly by different intensities in the exercise group compared to the control. A correlation statistics test showed that there was a strong correlation effect of HIF-1 α on VEGF protein expression in low (p=0.047) and high intensity exercise groups (p=0.009), but no effect was found in the moderate groups. In addition, there was a significant strong effect of PGC-1 α on VEGF protein expression in the moderate groups (p=0.037), but no effect was found in other groups. In conclusion, different exercise intensities induce a different modulation pattern of proteins which might be responsible for cardiac adaptation, especially angiogenesis.

Key words: angiogenesis, exercise, CD34⁺, HIF-1 α , myocardium, PGC-1 α , VEGF

Introduction

Cardiovascular disorders, such as coronary heart disease are one of the world's leading causes of death. Regular aerobic exercise or training has been recognized to improve cardiac fitness and delay myocardial impairment (Allen 2004, Piepoli 2017). Aerobic exercise is a regular physical exercise for a certain period and intensity, which depends primarily on the aerobic energy-generating process (Allen 2004, Piepoli 2017). Cardiac responses to exercise depend on the type, intensity, and duration of the training; and this adaptation is typically referred to the athlete's heart (Weeks and McMullen 2011, Golbidi and Laher 2012, Wilson et al. 2016).

Aerobic endurance training such as running or swimming enhances the demand for oxygen and nutrients leading to stimulation for forming new capillaries in the myocardium, called cardiac angiogenesis (Thijssen et al. 2009, Lansford et al. 2016). The upregulation of cardiac angiogenesis will increase cardiac perfusion; furthermore, it may maintain cardiac function and reduce cardiovascular diseases (Volaklis et al. 2013, Lansford et al. 2016). Training increases not only the number of blood vessels (angiogenesis) but also the diameter (arteriogenesis) of the arterial blood vessel in the myocardium, transformation in the blood flow, myocardium contraction, and oxygen-related level with a hemodynamic mechanical instance in heart function (Volaklis et al. 2013).

Vascular endothelial growth factor (VEGF), the primary regulator for angiogenesis, increases the migration of endothelial progenitor cells (EPCs) such as CD34⁺ from bone marrow to the damaged area (Koutroumpi 2012, Recchioni et al. 2016, Kutikhin et al. 2018). This growth factor has become the initiator for inducing vessel wall remodeling and activating growth factors that induce the proliferation, migration, and tube formation of endothelial cells in cardiac and skeletal muscle (Koutroumpi 2012, Recchioni et al. 2016, Kutikhin et al. 2018). At the molecular level, aerobic training enhances an angiogenesis mechanism in the myocardium due to the hypoxia myocardium (Laufs et al. 2004).

Exercise activates VEGF to promote angiogenesis through hypoxia-inducible factor (HIF) upregulation. This mechanism known as HIF-dependent angiogenesis regulation (Shibuya 2011, Ohno et al. 2012, Serocki et al. 2018). HIF-1 α expression may depend on the level of aerobic training intensity (Hoppeler and Vogt 2001, Lindholm et al. 2014, Lindholm and Rundqvist 2016, Grespan et al. 2019). On the other hand, physical exercise produces mechanical stress that will trigger the production of reactive oxygen species (ROS) (Mason et al. 2016, Broxterman et al. 2017, Di Meo

et al. 2019). The production of ROS levels may differ on the level of intensity of physical exercise. Thus, it is likely to have different downstream of reduction – oxidation reactions (Mrakic-Sposta et al. 2001, Larsen et al. 2016, Roh et al. 2017). ROS will increase AMP/AMPK signal activation and thus may be able to activate the peroxisome proliferator-activated receptor- γ (PPAR) coactivator 1 α (PGC-1 α) gene and may cause an increase in VEGF through the estrogen-related receptor alpha (ERR- α) pathway, referred to as HIF-independent angiogenesis regulation in several studies (Arany et al. 2008, Chinsomboon et al. 2009, Kang et al. 2009, Rowe et al. 2011, Radak et al. 2013). PGC-1 α will be rapidly induced in a condition that requires high energy to increase mitochondrial biogenesis (Scarpulla et al. 2012, Wenz 2013).

Interestingly, information regarding the role of training intensity in altering the molecular pathway of angiogenesis is limited and the effect of intense aerobic exercise on the molecular pathway of angiogenesis is also remains unclear. Thus, In this present study, we explored the important role of different intensities of aerobic training in inducing different molecular pathways in cardiac angiogenesis.

Materials and Methods

Study design

Twenty male Wistar rats aged 8 weeks and weighing 200 \pm 20 g were purchased from PT. Biofarma, Bandung, Indonesia. The rats were housed in a standardized cage. They were given a pellet rodent diet (Prospects Rat Standard Chow Diet, Indonesia) and were free to access water daily (Guid. Care Use Lab. Anim. 2011). The rats were kept in the Animal Laboratory Faculty of Medicine Universitas Padjadjaran. The environment was maintained at 12 h dark and light cycle with a temperature of around 22°C-24°C and a stable humidity of 60%. The rats adapted to the set environment for 2 weeks, and food and water were provided ad libitum. Animal procedures and treatment were conducted according to the laboratory animals guide and were approved by the Research Ethics Committee of Universitas Padjadjaran, approval number 466/UN6.KEP/EC/2019.

Animal and exercise training protocol

Rats were randomly allocated to four groups (n=5 for each group) upon arrival and then acclimatized and habituated to the environment for 2 weeks, followed by treadmill adaptation for another 2 weeks, and then further treadmill training for 12 weeks, with a frequen-

cy of 5 days per week and duration of 30 min per day. Four groups were in Group I as the control group, and three others were assigned as exercise groups (Group II = low-intensity with treadmill speed 10 m/min; Group III = moderate-intensity with treadmill speed 20 m/min, and Group IV = high-intensity with treadmill speed 30 m/min). The treadmill training intensities were defined based on lactate accumulation levels and followed a previous study (Guid. Care Use Lab. Anim. 2011, Lesmana et al. 2016, Gunadi et al. 2020). The rats were sacrificed immediately after the last training day, under inhaled isoflurane concentration of 5% or greater, continued until 1 min after breathing stopped (Jones 2007). Isoflurane was chosen as an anesthetic drug in compliance with the ethical approval issued (Flecknell 2009). Heart samples were collected, weighed, and dissected to separate the left ventricle myocardium. Cardiomyocyte samples were snap-frozen in liquid nitrogen and stored at -80°C until use.

Western blotting

The dissected cardiomyocyte samples were weighed and homogenized in lysis buffer containing 10 mM Tris – HCl (pH 7.8), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, and protease inhibitors. Protein samples were denatured at 96°C for 5 min after centrifugation (BioRad 2016). Protein samples (10 μg) were loaded equally to each lane and separated using SDS-PAGE electrophoresis and then transferred to a nitrocellulose membrane (GE Healthcare) for 1 h at room temperature. The membrane was blocked overnight at 4°C in 2% blocking reagent (GE Healthcare) in Tris-buffered saline buffer with 0.1% Tween 20. Immunoblotting of the primary antibody was conducted at 4°C overnight using HIF-1 anti rabbit alpha polyclonal antibody (Thermo Scientific # PA1-16601, USA) at a dilution of 1:300 (Huang, 2015); PGC-1 alpha anti rabbit polyclonal antibody (Thermo scientific # PA5-38022, USA), at a dilution of 1:300; VEGF polyclonal anti rabbit antibody (Thermo scientific # MA5-12184), at a dilution of 1:300 (Zhang, 2014); CD34⁺ monoclonal antibody (Thermo scientific # MA1-10202); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Thermo scientific # AM4300), at a dilution of 1:1000 (Fernandez-Miranda, 2019). The next, membrane were incubated in secondary antibody; anti Rabbit HRP (Thermo Scientific) and anti Mouse HRP (Thermo Scientific, with 1:5000 dilution. The membrane were visualized using Amersham ECL Western Blotting Detection Reagents (GE Healthcare, GERPN2109) and image were taken using LI-COR C-DiGit Blot Scanner (Licor Inc, Nebraska, USA). The band density were determined using ImageJ software. Blots were stripped using Restore Western Blot Stripping Buffer (Thermo Scien-

tific) according to the manufacturer's protocols and reprobed using an anti-GAPDH as an internal control to monitor protein level (Jensen 2012).

Statistical analysis

SPSS 20.0 software was used for statistical analysis; results were presented as mean \pm standard error of the mean. One way analysis of variance (ANOVA)/Kruskal-Wallis tests were used to examine mean differences between groups continued with the Tukey post hoc test (for data with normal distribution) or the Mann-Whitney test (for data without normal distribution), with a 95% confidence interval ($p < 0.05$) (Kremelberg 2014). The Pearson test or Spearman test were used to analyze the correlation between HIF-1 α and VEGF protein expression and the correlation between PGC-1 α and VEGF protein expression among groups. A simple linear regression analysis test was then used to quantify the impact of protein HIF-1 α or PGC-1 α on protein VEGF in each training group (Kremelberg 2014).

The output of regression analysis was denoted by r squared (r^2) or by the coefficient determination that indicated the percentage of the variance in the dependent variable (expression of protein VEGF) and independent variable (expression of protein HIF-1 α or PGC-1 α), with the equation $KD = r^2 \times 100\%$ (Frost 2013).

Results

Effect of exercise intensity on angiogenesis parameters

To study the effect of exercise intensity in the modulation of angiogenesis, we measured HIF-1 α , PGC-1 α , VEGF and CD34⁺ protein expression in cardiomyocyte (Fig. 1A).

HIF-1 α protein levels significantly increased in the exercise groups compared to control (0.93 ± 0.30 vs 1.61 ± 0.64 vs 1.65 ± 0.41 vs 1.72 ± 0.42 AU; $p = 0.049$). The highest HIF-1 α protein expression was observed in the high-intensity exercise group followed by the moderate-intensity exercise group and then the low-intensity exercise group (Fig. 1B)

PGC-1 α protein levels significantly increased in the training group compared to control (0.64 ± 0.10 vs 1.21 ± 0.49 vs 1.20 ± 0.37 vs 0.77 ± 0.65 ; $p = 0.003$), and descriptively, PGC-1 α protein levels in the low and moderate-intensity exercise groups had the highest level protein expression compared to the control and high-intensity exercise groups (Fig. 1C)

VEGF protein levels significantly increased in all exercise groups compared to control (1.23 ± 0.093

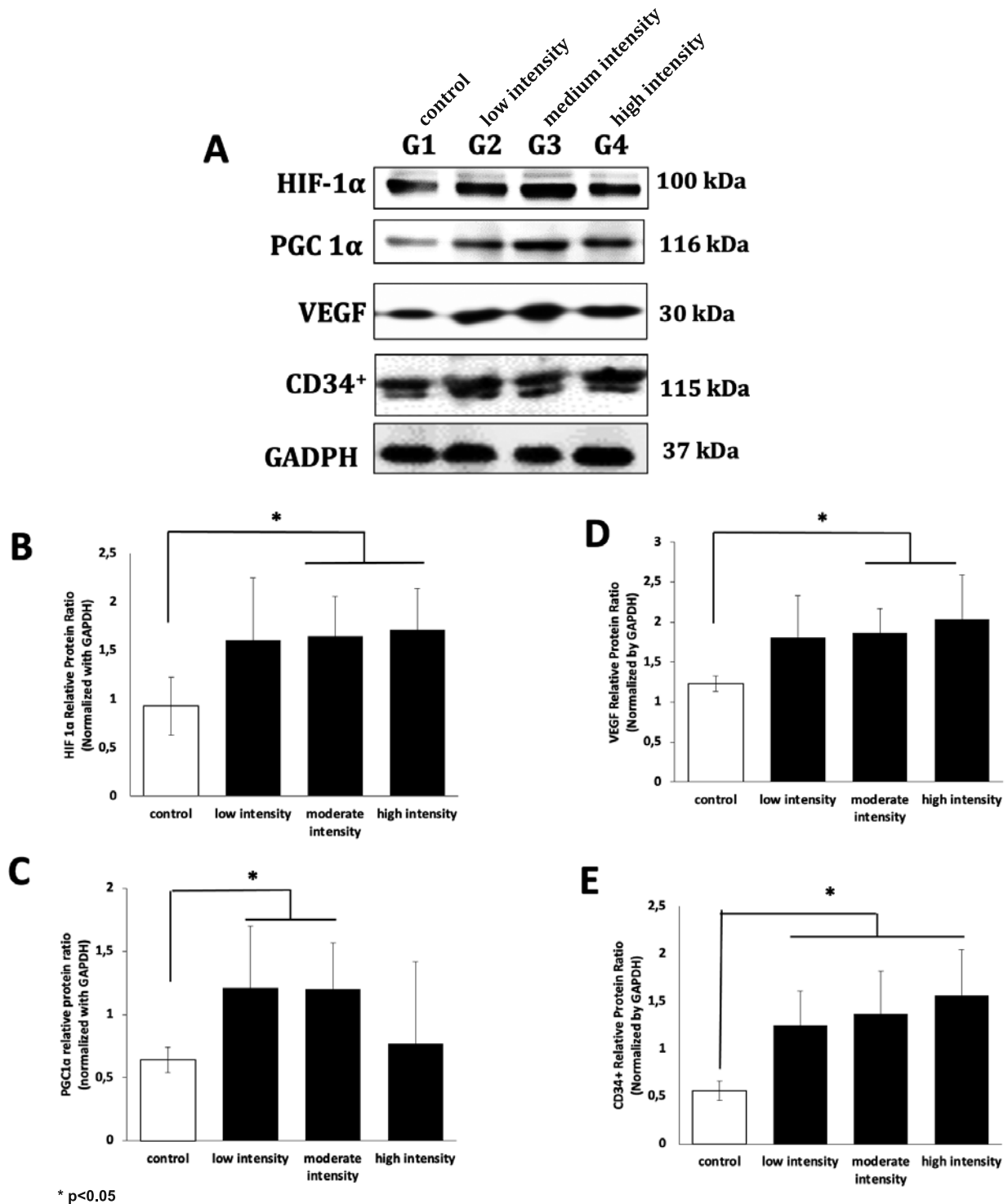


Fig. 1. Effect of different intensities of rat training to HIF-1 α , PGC-1 α , VEGF, CD34⁺ protein expression among groups.

Representative figure of Western blot membrane (A); Quantification of HIF-1 α (B); PGC-1 α (C); VEGF (D); and CD34⁺ (E). Protein expression level among different intensity groups. Data is presented as mean and bar graph represents Standard Deviation. Asterisk * indicates p<0.05.

vs 1.81 ± 0.523 vs 1.87 ± 0.396 vs 2.040 ± 0.55 ; $p=0.024$), and VEGF protein levels reached the highest level in the high-intensity exercise group, followed by the moderate-intensity and low-intensity groups sequentially (Fig. 1D).

CD34⁺ protein levels significantly increased

in the exercise groups compared to control (0.56 ± 0.10 vs 1.25 ± 0.36 vs 1.37 ± 0.45 vs 1.56 ± 0.48 ; $p=0.007$). CD34⁺ protein levels showed the highest response in the high-intensity exercise group followed by the moderate-intensity exercise group then the low-intensity exercise group compared to control (Fig. 1E).

Table 1. Correlation between HIF-1 α and VEGF, PGC-1 α and VEGF.

Group	parameters	VEGF r	VEGF p
Group II	HIF-1 α	0.813	0.047*
(low intensity)	PGC-1 α	0.400	0.505
Group III	HIF-1 α	0.578	0.154
(medium intensity)	PGC-1 α	0.900	0.037*
Group IV	HIF-1 α	0.943	0.008**
(high intensity)	PGC-1 α	-0.600	0.285

Correlation analysis between HIF-1 α or PGC-1 α on VEGF

We examined the correlation between HIF-1 α and PGC-1 α on VEGF in each training group (Groups II, III, and IV) and analysed the impact of protein expression HIF-1 α or PGC-1 α on VEGF (Table 2). Pearson analysis showed that there was a significant positive correlation between HIF-1 α and VEGF in Group II (low-intensity exercise) and Group IV (high-intensity exercise), but no correlation in Group III (moderate-intensity exercise). On the other hand, a significant correlation between PGC-1 α and VEGF was only observed in Group III. We analysed the impact of the HIF-1 α and PGC-1 α on VEGF with a simple linear regression study.

Based on simple linear regression and the coefficient determination equation explained above, it is shown that on the lowest impact of HIF-1 α to the variation in protein expression, VEGF happened in Group III (33.4%). In Group II, 66.1% of the various protein expressions VEGF can be explained by HIF-1 α . Meanwhile, Group IV was the highest, in which HIF-1 α could be attributed to 88.9% of the various protein expressions VEGF. Table 5 shows that protein PGC-1 α has a significant impact only on Group III, that is, 95.6% of the variation in protein expression VEGF can be explained by PGC-1 α activity (p<0.05).

Discussion

Angiogenesis is the process of forming new blood vessels from existing vascular tissue by growing new capillaries (Chen et al. 2010, Davies 2016). Angiogenesis plays a role in the process of renewal and growth of vascular tissue. During the process, mature endothelial cells will combine and form new capillaries (Chen et al. 2010, Korivi et al. 2010, Davies 2016). Angiogenesis is a natural process that can be activated by various caused, including physical stress such as exercise, metabolic stress in the form of hypoxia, decreased pH, hypocalcemia, inflammation, activation of oncogenes, wound healing, and tissue repair (Chen et al. 2010, Korivi et al. 2010, Davies 2016). Although,

several researcher report on biomolecular mechanisms regarding exercise inducing angiogenesis, this information is still very limited (Korivi et al. 2010).

In physiological conditions, angiogenesis will be regulated by endogenous pro-angiogenic activators such as VEGF and anti-angiogenic inhibitors under strict control, fibroblast growth factor (β -FGF), and insulin-like growth factor-1 (IGF-1) (Chen et al. 2010). VEGF signals are required for the implementation of vasculogenesis and angiogenesis processes (Shibuya 2011). However, the biomolecular activity of endogenous activators and angiogenic inhibitors has receive little research concern . Under normal conditions, new blood vessel formation requires endothelial cell activation, degradation of the basement membrane, migration, and proliferation; these steps are regulated by cell interactions, growth factors, and protein matrices. Growth factors such as VEGF, β -FGF, and IGF-1 promote endothelial cell proliferation and migration. Matrix proteins such as fibronectin, laminin, and collagen type-1 provide the stages of angiogenesis (Chen et al. 2010). Results of previous studies indicate that the growth and maturation of new blood vessels is a very complex and coordinated process, requiring a sequential activation of a series of receptors by multiple ligands (Chen et al. 2010). However, VEGF signaling is often an essential step in physiological angiogenesis (Shibuya 2011).

Our study showed that VEGF activity is significantly different between the training and control groups, and descriptively, VEGF activity reached the highest level in the high-intensity exercise group, followed by the moderate- and low-intensity groups sequentially (Table 1 and Fig. 1D). On previous study, Sprague-Dawley rats that performed physical activity treadmill running for 60 min, 5 days per week for 6 weeks, in light-, moderate-, and high-intensity groups. There was an increase in myocardial VEGF protein expression in the moderate- and high-intensity groups compared with that in the control group, but there was no significant difference in the VEGF protein expression in the moderate- and high-intensity groups (Tang et al. 2011).

Table 2. Impact of HIF-1 α to VEGF on low, moderate and high intensity exercise groups.

Group	VEGF			
	n=5	B	Std. Error	p
Group II	Constant	0.750	0.468	0.207
	HIF-1 α	0.660	0.273	0.094
Group III	Constant	0.947	0.773	0.308
	HIF-1 α	0.558	0.455	0.308
Group IV	Constant	-0.212	0.455	0.680
	HIF-1 α	1.295	0.264	0.016**

Group II: low intensity; Group III: moderate intensity; Group IV: high intensity

Table 3. Coefficient determination test HIF-1 α to VEGF on low, moderate and high intensity exercise groups.

Groups	Model	r	r ²	Adjusted r ²	Std. Error
Group II	1	0.813 ^a	0.661	0.548	0.351649560
Group III	1	0.578 ^a	0.334	0.112	0.373921454
Group IV	1	0.943 ^a	0.889	0.852	0.219758160

Group II: low intensity; Group III: moderate intensity; Group IV: high intensity

Table 4. Impact of PGC-1 α to VEGF on low, moderate and high intensity exercise groups.

Group	VEGF			
	n=5	B	Std. Error	p
Group II	Constant	0.858	0.522	0.199
	PGC-1 α	0.748	0.407	0.146
Group III	Constant	0.637	0.159	0.028
	PGC-1 α	1.026	0.127	0.004**
Group IV	Constant	5.5235	3.383	0.219
	PGC-1 α	-4.202	4.406	0.411

Table 5. Coefficient determination test PGC-1 α to VEGF on low, moderate and high intensity exercise groups.

Groups	Model	r	r ²	Adjusted r ²	Std. Error
Group II	1	0.748 ^a	0.559	0.412	0.400816339
Group III	1	0.978 ^a	0.956	0.941	0.096090978
Group IV	1	0.482 ^a	0.233	-0.023	0.577404286

The enhancement of VEGF activity leads to the differentiation and migration of EPCs to the endothelium to conduct the angiogenesis process. One of the mature EPCs is CD34⁺ (Lian et al. 2014, Li et al. 2017). Both acute and chronic exercises have been reported to induce EPC mobilization (Lian et al. 2014, Lansford et al. 2016, Li et al. 2017). This study showed that CD34⁺ activity significantly increased in the exercise groups compared with that in the control group. Although the post hoc test result was not significant, descriptively, CD34⁺ activity was highest in the high-intensity exercise group, followed by moderate-intensity exercise group and then the low-intensity exercise

group (Table 1 and Fig.1E), equally with research on healthy sedentary men running for 30 min with an anaerobic threshold of light, moderate, and severe intensities. The data showed a significant increase in EPC in men who did moderate- and heavy-intensity exercises. Another study on mice divided into groups that did voluntary running for 7, 14, and 28 days showed a significant increase in EPC in these mice, respectively, 267%, 289%, and 280% compared with those in the control group (Laufs et al. 2004).

Exercise increases the oxygen consumption in muscle cells by 50 times compared with when we are rest. Blood flow increases 25 times to compensate for the

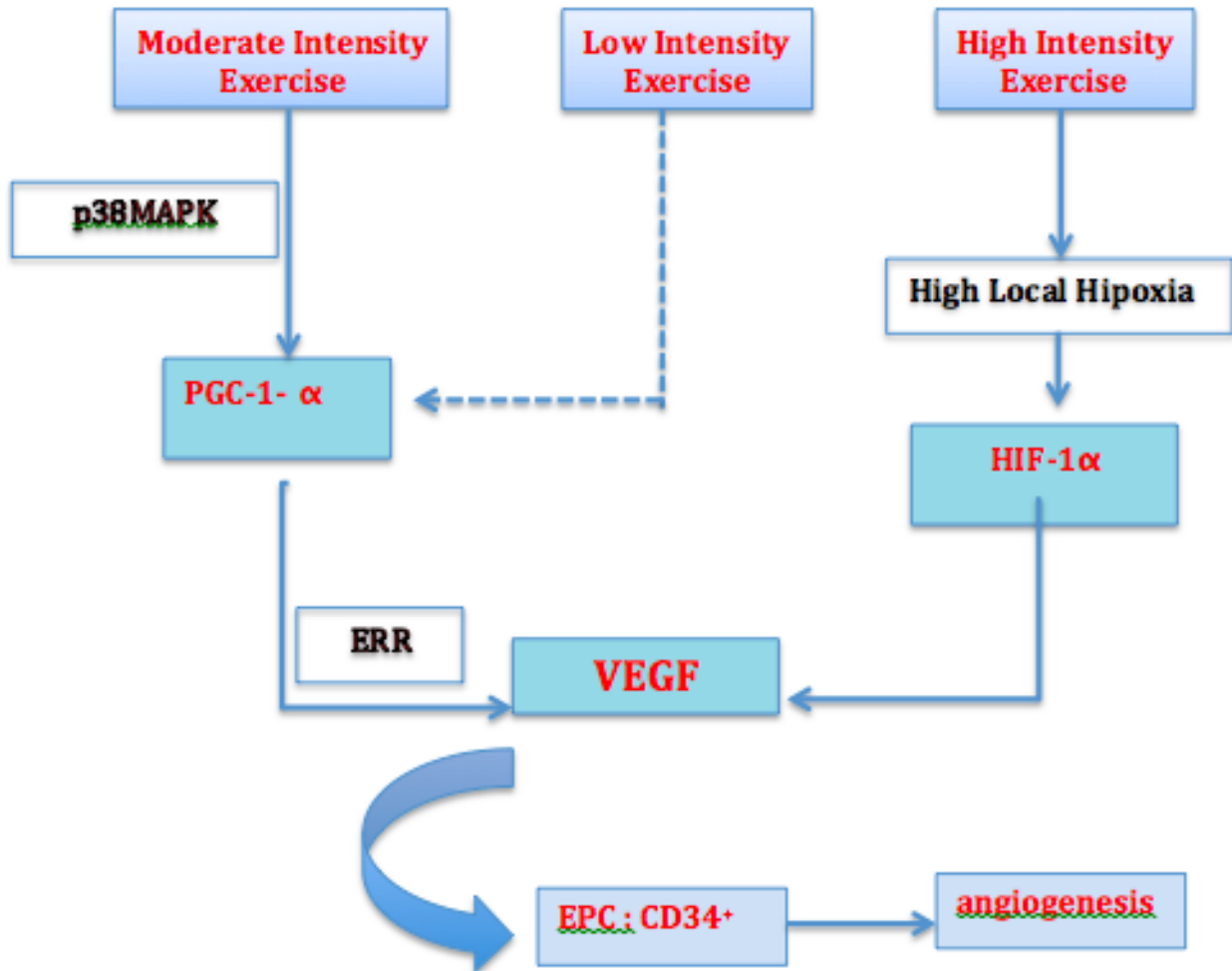


Fig. 2. Proposed mechanism of myocardium adaptation in different intensity exercise.

In the high hypoxia condition, such as during high-intensity exercise with high HIF-1 α activity, HIF-1 α decreases mitochondrially the encoded component of the oxidative phosphorylation pathway in skeletal muscle via communication with myc-1, HIF-1 also actively suppresses oxidative capacity through restriction functions of PGC-1 α . These circumstances also occurred in our study, specifically PGC-1 α activity in the high-intensity exercise group was significantly lower than in the moderate-intensity exercise group.

increased oxygen demand. As we know, an imbalance between the high demand for oxygen and limited oxygen supply because of muscle contraction during exercise or training will cause hypoxic conditions (Mac Gabhann et al. 2007). Hypoxia in tissues inhibits the activity of the prolyl hydroxylase domain (PHD) and factor-inhibiting HIF-1 (FIH-1) because these two enzymes require oxygen as a co-substrate (Niecknig et al. 2012). The inhibition of PHD and FIH-1 results in stable HIF-1 α and can bind to the CBP/p300 coactivator to complete transcription activity. Hypoxic conditions result in the accumulation of HIF-1 α in the cytoplasm occurring rapidly (Semenza 2011). During exercise, HIF-1 α activation may occur through a regulatory pathway that depends on oxygen levels (Lindholm and Rundqvist 2011). This study showed that HIF-1 α protein expression significantly increased in the exercise groups compared with that in the controlled group. Although the HIF-1 α protein expression was not

significantly different among the exercise groups, descriptively, the level of HIF-1 α protein expression was highest in the high-intensity exercise group, following by the moderate- and low-intensity exercise groups (Table 1 and Fig.1B). This result was consistent with that in a previous study in the rat Wistar strain group that performed physical activity running on the treadmill at light intensity for 60 min, 4 days a week for 8 weeks, showing a significant increase in myocardial HIF-1 α mRNA expression compared with that in the control group (9,015 vs 1.49; $p=0.001$) (Zokaei and Javid 2017). This was in contrast to our previous study on male BALBc mice that performed moderate-intensity swimming exercise for 4 weeks, 30 min/day for 5 days, showing that HIF-1 gene expression significantly decreased 0.4 times lower than that in the control group (0.35 vs 0.52; CI=95%, 0.27-0.31; $p=0.025$) (Sylviana et al. 2018).

The downregulation of HIF-1 expression associated

with regular exercise occurs because of the appearance of negative regulators such as PHD2, FIH-1, and Sirtuin, as shown by Lindholm et al. published research (Lindholm and Rundqvist 2011, Lindholm et al. 2014, Lindholm and Rundqvist 2016). In that research, they observed activation of the HIF-1 negative regulation after 6 weeks of regular exercise. It can be speculated that a subsequent inhibition of HIF-1 activity is part of the shift from angiogenic response to metabolic adaptation (Lindholm et al. 2014). However, in our study, Group IV that performed high-intensity exercise still resulted in the highest HIF-1 protein expression. This might be because switching of the angiogenic response to metabolic adaptation had not occurred. This condition may indicate a strong positive correlation between HIF-1 and VEGF, a growth factor that initiates the angiogenic response shown in Group IV, a group of Wistar rats that performed high-intensity exercises (Table 3). In this study, 88.9% of the increased protein expression VEGF in that group might be attributed to HIF-1 α activity (Table 4). The low-intensity group (Group II) also has a significant positive correlation between HIF-1 α and VEGF activity, although only 66.1% of the various protein expression VEGF were explained by HIF-1 α .

In the moderate-intensity exercise group, the VEGF protein expression was still higher than in the control group, although our research showed that there was no significant correlation between HIF-1 α activity and VEGF; in this group, HIF-1 α activity had a weak impact on VEGF protein expression (33.4%). Some researchers considered that other regulations can induce VEGF, where it was not dependent on HIF-1 α activity (HIF-1 α -independent regulation) that involves another coactivator, for example, PGC-1 α through nuclear receptor ERR- α (Arany et al. 2008). On moderate-intensity, chronic exercise conditions could have occurred, switching to metabolic adaptation (Lindholm and Rundqvist 2016, Arany et al. 2008). Therefore, PGC-1 α activity in low- and moderate-intensity exercise groups has the highest level of protein expression compared with that in the control and high-intensity exercise groups (Table 1 and Fig. 1C). There was a strong positive correlation between PGC-1 α and VEGF activities in the moderate-intensity exercise group. Thus, 95.6% of the variation in protein expression VEGF on this group was explained by PGC-1 α (Tables 4 and 5). In addition, in the low-intensity exercise group, PGC-1 α protein expression also had a strong tendency to have a strong relationship with VEGF protein expression, although the impact of PGC-1 α activity on 55.9% VEGF protein expression was not statistically significant ($r=0.748$).

ROS produced during exercise will increase the

activation of AMP/AMPK signals that induce PGC-1 α expression as an adaptive metabolic response to exercise. PGC-1 α will be induced rapidly in conditions that require high energy, to increase mitochondrial biogenesis (Lindholm and Rundqvist 2016). In the high hypoxia condition, such as during high-intensity exercise with high HIF-1 α activity, HIF-1 α decreases mitochondrial-ly the encoded component of the oxidative phosphorylation pathway in skeletal muscle via communication with myc-1 (Lindholm and Rundqvist 2016). HIF-1 also actively suppresses oxidative capacity through restriction functions of PGC-1 α (Lindholm and Rundqvist 2016). These circumstances also occurred in our study, specifically with PGC-1 α activity in the high-intensity exercise groups significantly lower than in the moderate-intensity exercise group (Fig. 2).

Conclusion

Different intensities of aerobic exercise induce different molecular pathways in cardiac angiogenesis. In moderate-intensity exercise, the increase in myocardial VEGF protein expression is strongly influenced by independent HIF-1 α regulation. In our study, we investigated the strong impact of PGC-1 α on increasing VEGF protein expression. Conversely, in low- and high-intensity exercises, cardiac angiogenesis occurs strongly because of the effect of HIF-1 α . We recommend on the future research may examine factors that inhibit hypoxia such as FIH-1 and PHD to confirm that intensity of exercise might lead to cardiac adaptation during regular exercise. In general, future research should identify the novel exercise-induced molecules impacted by intensity. These molecules may play a role in the process of adaptation and enhancement of heart function to overcome cardiovascular diseases.

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