Effects of Moderate Intensity Exercise on Glutathione Peroxidase Activity and VO$_2$ max in Elderly Women

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Introduction

Aging is a multifactorial process that results in age-related bodily dysfunction resulting in a decrease in life span, quality of life, and independence [1]. Aging is associated with a reduced ability to repair oxidative damage due to increased free radicals in the mitochondria and nuclei in the cell [2]. Aging progressively associated with high levels of oxidative biomolecular reactions with free radicals cause increased damage to proteins, fats, and DNA. Under normal conditions, the formation of mild reactive oxygen species (ROS) in mitochondria due to light intensity exercise will be corrected quickly by the antioxidants in the mitochondria, but continuous increase in ROS due to age and sedentary behavior causes imbalance (fewer antioxidants are formed while more ROS is released). This causes damage to mtDNA progressively which will cause degenerative diseases [3].

Regular moderate intensity aerobic exercise will lead to adaptation the higher antioxidants in mitochondria through activation nuclear factor erythroid 2 (NRF2)-related factor due to induced of ROS [4]. It will increase glutathione peroxidase (GPx) [5]. GPx catalyzes H$_2$O$_2$ become H$_2$O and O$_2$ and modulating redox as a cell response to regulate the mitochondrial function [6]. Moderate intensity aerobic exercise is highly recommended for the elderly because this kind of exercise cannot damage the structure and function of cells [7]. Regular moderate and light intensity aerobic exercise involves many large muscle groups with a long enough period of time so that the fuel source can be converted into adenosine triphosphate using the citric acid cycle and electron transport chain as the main metabolic pathway [8]. This type of exercise is an effective strategy to counteract some effects due to aging of the arteries in particular large increase in elastic arterial stiffness and vascular endothelial dysfunction.

Aging accompanied by a sedentary lifestyle decreases of VO$_2$ max by ±1% per year starting from middle age, whereas an active lifestyle can inhibit a reduction to half [9], [10]. Decreasing of VO$_2$ max over 4–5 mLO$_2$/kgbb/min/decade is largely due to decrease in maximal cardiac output and artery venous oxygen level (a-v) O$_2$ increased peripheral blood resistance, decreased muscle capillary density, endothelial dysfunction, changes in skeletal muscle mitochondrial structure, and decreased oxidative capacity of muscles [9].
VO₂max calculation can be done using conventional training and using prediction calculations. The 6-min walking test (6 MWT) is one-way to measure the VO₂max and a recommended method for the elderly because the measurement is non-invasive, safe, and simple for determine maximal aerobic strength in the elderly [11], [12]. VO₂max is significantly associated with 6 MWT [13].

This study was to investigate the effects of moderate intensity aerobic exercise on GPx activity and VO₂max in elderly women.

Materials and Methods

Subjects
The research was quasi-experimental (control group [CG] pre-test post-test design) study and 73 subjects sedentary elderly women with the age of 65.86 ± 5.21 years were participated, divided in two group; 37 subjects experimental group (EG) and 36 subjects CG. Each person was selected based on the inclusion criteria by interview and activity daily living (ADL) Barthel score >12, pedometer >5000 step per day, in good balance and cooperative cognitive status. The subjects had physical examination such as body weight, height, blood pressure, and heart rate 60–100 beats/min. Drop out criteria were if the subjects did not discipline in the research program.

Materials and equipment
It consists of sphygmomanometer, stethoscope, weight scale, heart rate monitor, spirometer, stop watch, pulse oximeter, and pedometer.

Protocol experiment
Before starting the exercise, subject had 5 min warm up and stretch and after exercise had 5 min cooling down. Design moderate intensity aerobic exercise was achieved if 50–85% of maximal heart rate. 3 time in a week for 12 week, and 30 min/session, the type of exercise was aerobic walking and monitored during period of training with pulse rate monitor every 10 min [13]. The VO₂max was measured by 6 MWT.

Study ethics
If subjects have complaint (such as leg pain and head ache) during exercise, will be got a treatment until the complain loss. Subject explained more detail the procedures and the aim of the study before joining the program. Informed consent was confirmed. The ethical clearance approved by Ethics Committee of the Faculty of Medicine, University of Indonesia.

Biochemical analysis
Blood samples were drawn at 5 min before exercise and considered as baseline and 24 h after exercise through vena puncture. The blood samples were centrifuged 3000 rpm for 30 min and 1.5 mL plasma was stored frozen at −20°C until assay. All samples were measured the GPx activity using ELISA according GPx Assay Kit method.

Statistical analysis
Data were analyzed using SPSS package program version 20.0. Unpaired t-test and Pearson test were used to compare the mean of GPx activity level and VO₂max between CG and EG at 0 and 12 weeks after exercise, p < 0.05 was significantly different.

Results
The subject characteristics such as age, weight, height, body mass index (BMI), pedometer, and ADL between CG and EG were homogen and normally distributed (p > 0.05).

| Test/unit     | CG (n=36) mean ± SD | EG (n=37) mean ± SD | p    |
|--------------|----------------------|----------------------|------|
| Age (years)  | 65.29 ± 4.84         | 66.71 ± 4.99         | 0.181|
| Body weight (kg) | 55.11 ± 12.15       | 56.67 ± 10.17       | 0.739|
| Height (cm)  | 147.19 ± 4.78        | 150.09 ± 4.28       | 0.098|
| BMI (kg. m⁻²) | 25.48 ± 4.76         | 25.14 ± 4.36        | 0.811|
| Pedometer (step) | 2800.95 ± 1383.43   | 3448.87 ± 1231.78   | 0.139|
| ADL           | 19.24 ± 0.76         | 19.47 ± 0.68        | 0.300|

*test unpaired, **Mann–Whitney U-test. *Value p<0.05=significance differences two groups (homogen).

ADL Barthel: Activity daily living, SD: Standard deviation, EG: Experimental group, CG: Control group, BMI: Body mass index.

Changes of plasma GPx activity and VO₂max

Significant difference was found in ∆ GPx activity and ∆ VO₂max as shown in Table 2.

We found the difference of VO₂max between the CG and the treatment group (p < 0.05) in the third exercise time (Table 2). The VO₂max change of the EG group was significantly higher compared to CG (p = 0.026) at 0–6 weeks exercise and became more increases at the 0–12 weeks of exercise (p = 0.001) (Figure 1).
Table 2: Changes of plasma GPx activity and VO\(_2\)max

| Variable                  | CG (n=36) mean ± SD | EG (n=37) mean ± SD | p   |
|---------------------------|---------------------|---------------------|-----|
| VO\(_2\)max (ml/kg/min)   | 7.403 ± 36.801      | 40.160 ± 30.253     | 0.001* |
| % GPx                     | 0.183 ± 3.929       | 2.091 ± 3.237       | 0.026* |
| % ∆1                      | 0.617 ± 3.179       | 4.104 ± 2.429       | 0.001* |
*Uji t-test unpaired p<0.05, value ∆=The difference absolute value, value ∆2=The difference data between 0 and 6 weeks exercises, value ∆1=The difference absolute value between 0 and 12 weeks exercises. SD: Standard deviation, EG: Experimental group, CG: Control group, GPx: Glutathione peroxidase.

The results showed, there was a significant increase of plasma GPx activity by 40.16% (73.27 U/mg) in EG at week 12 from baseline and reversely, we found tend of decrease in plasma GPx activity by 7.40% (~54.46 U/mg) at 12 weeks from baseline in CG (Table 3 and Figure 2).

Table 3: The percentage changing of GPx activity and VO\(_2\)max between CG and EG

| Variable                  | CG (n=36) mean ± SD | EG (n=37) mean ± SD | p   |
|---------------------------|---------------------|---------------------|-----|
| % GPx                     | 0.183 ± 3.929       | 2.091 ± 3.237       | 0.026* |
| % ∆1                      | 0.617 ± 3.179       | 4.104 ± 2.429       | 0.001* |
*Uji t-test unpaired p<0.05, value ∆=The difference absolute value between 0 and 6 weeks exercises, value ∆2=The difference absolute value between 0 and 12 weeks exercises. SD: Standard deviation, EG: Experimental group, CG: Control group, GPx: Glutathione peroxidase.

Discussion

Chronic and continuous intensity aerobic exercise will be adapted by repairing the oxidative damage due to ROS through increasing availability of oxygen concentration [10]. Routine exercise in the elderly can control the production of ROS mitochondrial associated with antioxidant repair resulting decrease of ROS production during basal conditions [14], [15].

![Figure 1: Comparison of VO\(_2\)max between the control group and the experiment group](https://www.id-press.eu/mjms/index)

In this study, there was a significantly increasing of antioxidants (GPx activity) by 40.16%. It is found in the mitochondrial matrix to against ROS due to oxidative damage in the mitochondria [6]. The GPx activity was positively correlated with VO\(_2\)max (r = 0.223). It showed that moderate-intensity exercise can improved the function of cardiorespiratory system by increasing of GPx activity.

GPx activity may improve the mitochondrial function, thus increasing O\(_2\) uptake to elevate of VO\(_2\)max. According to Robergs et al., oxygen cellular outages in mitochondria are important that can increase VO\(_2\)max. In this research found improvement of oxygen uptake was a marker of increased mitochondrial respiration [10].

![Figure 2: Comparison of glutathione peroxidase activity between control groups and experiment groups](https://www.id-press.eu/mjms/index)

Increased of VO\(_2\)max due to moderate intensity aerobic exercise in elderly women supported by Murias et al. [16], which stated that the adaptation of there was increasing of VO\(_2\)max 0.16 l/min every 3 weeks and totally there was an increase of 31 ± 10% for 12 weeks exercise. Although the increase in cardiorespiratory function due to exercise has not been optimally seen, this change has been able to show improvement in the elderly's cardiorespiratory function.

This study found a significant increase of plasma GPx activity as an antioxidant by 40.16% (p = 0.001) in the treatment group, while the CG actually decreased by 18.51% (p = 0.285) at the 12th weeks. GPx activity was also found to be positively correlated with VO\(_2\)max (p = 0.041, r = 0.223). The low GPx activity in sedentary elderly found in this study is thought to be caused by the absence of stimulation of transcription factor activation (NRF2) which serves to increase antioxidant expression. The aging process decreases NRF2 expression and dysregulates the NRF2 activation [16]. It causes lower antioxidant levels in older adults.

Acute exercise is an immediate response that helps the body as an intermediary in the adaptation response to improve the cell function and the cardiorespiratory system due to exercise performance [10]. For the elderly, it is recommended to do 12 weeks of aerobic exercise with a frequency 3 times a week regularly to see the exercise adaptation process is carried out, because 1 h after exercise (recovery period), the activity of the antioxidant enzyme returns to the basal level, but 24 h later the enzyme increases again due to an increase in the genetic regulation of the enzyme [17]. Exercises that carried out regularly and continuously will accumulate the antioxidant production thereby improving the mitochondrial function.

In this study, based on the correlation between age and variables research, the physical functions would decrease the aging process. The decreasing of physical function could be repaired by performing moderate-intensity aerobic exercise regularly so that the function of cell and cardiorespiratory system
becomes improved. This improvement derived from the change and correlation of the parameters to the exercise. Furthermore, this study found a positive correlation between $\text{VO}_{2}\text{max}$ and GPx activity.

**Conclusion**

There was a positive correlation between $\text{VO}_{2}\text{max}$ and GPx activity. Moderate intensity exercise regularly for 12 weeks had revealed the improvement of cell function based on GPx activity. It was followed by improving cardiorespiratory system function.

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