International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 1(G); January 2018; Page No. 9184-9191
DOI: http://dx.doi.org/10.24327/ijcar.2018.9191.1508

Research Article

MANUAL ACUPUNCTURE EFFECT ON SUPEROXIDE DISMUTASE ENZYME IMMEDIATELY AFTER ACUTE PHYSICAL EXERCISE IN UNTRAINED MAN

Rachma Novita Indrarini[1]., Hasan Mihardja[1]., Adiningsih Srilestari[1]., Nora Sutarina[2] and Shinta Dewi Sukandar[1]

[1] Departement of Medical Acupuncture, Faculty of Medicine University of Indonesia
[2]Departement of Sport Medicine, Faculty of Medicine University of Indonesia

A R T I C L E I N F O

Article History:
Received 11th October, 2017
Received in revised form 10th November, 2017
Accepted 26th December, 2017
Published online 28th January, 2018

Key words:
Physical exercise, ROS, SOD, Acupuncture

A B S T R A C T

Physical exercise is important for health but can also increase oxidative stress that induce Reactive Oxygen Species (ROS). Superoxide dismutase (SOD) is endogenous antioxidants found in the body, an enzyme that catalyzes the dismutation of radical superoxide ions (O2) into hydrogen peroxide (H2O2) and oxygen molecules (O2) against oxidative stress Acupuncture is one of the therapeutic modalities that is expected to reduce oxidative stress that occurs due to physical exercise. The study was conducted on thirty untrained men who were randomly divided into two groups, the manual acupuncture group (n = 15) performed acupuncture therapy at bilateral ST36 and SP6 acupuncture points, and the placebo group (n = 15) performed the needle stitching on the plaster without penetrating the skin. Acupuncture therapy is performed once for 30 minutes immediately after the subjects have finished acute physical exercise. Assessment of the blood SOD level was assessed before physical exercise and one hour after physical exercise. The results of this study showed a statistically significant difference in the difference between the level of SOD before and after physical exercise between the manual acupuncture group and placebo group p = 0.001.

INTRODUCTION

Regular physical exercise or exercise and physical activity are important to support one's physical and mental health. Physical exercise that is done regularly with a long period of time can be beneficial for long-term health. In addition, regular physical exercise can also reduce the risk of developing or developing a disease, increase metabolism, improve mood, reduce stress, strengthen bone and joint muscles, increase bone density, and boost the immune system (www.bimbie.com, 2016) (Brown *et al*, 2015).

When doing physical exercise there is an increased need for oxygen, it occurs due to the addition of workload. In physical exercise there is also a change in body physique, can be temporary that we are familiar with the response or are sedentary we are familiar with the adaptation (Sugama *et al*, 2015). Different effects can be found on various intensity exercises performed. Physical exercise with moderate and sustained intensity provides health and body resilience, whereas high intensity physical exercise with long duration actually gives bad effect for the body (Shockett *et al*,2016). There are also different effects on acute exercise and chronic exercise. Acute exercise may cause endothelial damage due to reduced oxygen supply and energy resulting from vascular ischemia. However, regular exercise (chronic) can provide the benefits of body adaptation to cardiorespiratory, musculoskeletal, and other systems (Luk *et al* 2016). Physical exercise has been shown to increase the activity of antioxidant enzymes, but at the same time there is an increased need for oxygen which causes increased Reactive Oxygen Species (ROS) (Kanda *et al*, 2013). When compared to at rest, oxygen uptake increases 10-20 times during exercise. ROS can provide a good effect on the body, but it can also have a negative effect on the body if there is an imbalance between the formation and removal of free radicals. This causes a pathological condition called oxidative stress. Oxidative stress causes damage to DNA molecules, lipids, proteins and carbohydrates that can lead to cell dysfunction or death (Liu *et al*, 2012) (Liu *et al*, 2016). Superoxide dismutase (SOD) is an enzyme that catalyzes the dismutation of radical superoxide (O_2^-) ions into hydrogen peroxide (H_2O_2) and oxygen molecules (O_2), is the first-line defense mechanism against oxidative stress and endogenous antioxidants found in various tissues (Liu *et al*, 2016) (Nunes *et al*, 2014).

Corresponding author:* **Rachma Novita Indrarini
Departement of Medical Acupuncture, Faculty of Medicine University of Indonesia

Acupuncture is a traditional Chinese medicine that has existed since 2,500 years ago but since the last 30 years acupuncture has been widely used in western medicine. Acupuncture is an act of stimulating certain points on the body for promotive, preventive, rehabilitative andcurative purposes (Iraldo *et al*,2009). In Asia acupuncture has been widely used to improve physical conditions, particularly in sports, especially athletes (Mendhan *et al*, 2011).

Various acupuncture studies have been widely practiced in the field of sports, providing excellent results especially in overcoming post-sport trauma, as well as effective in reducing muscle tension, increasing blood flow, increasing pain threshold and modulating the autonomic nervous system (Waite, 2016). Matchmaking techniques are important in acupuncture acts. Manual acupuncture is the most commonly used technique because it is simple, cheap but gives a good effect. Liu *et al* in 2013 conducted a study of 30 subjects divided into three groups, namely control group, intensive training group and electroacupuncture group (EA). Subjects were asked to do 15 days of physical exercise with frequency once a day for 15 minutes, then performed acupuncture acts. The acupuncture group was given electroacupuncture at Xuehai point (SP10), Zusanli (ST36), Taichong (LR3), and Sanyinjao (SP6) bilaterally for 30 minutes, once daily for 15 days, then examined the serum levels of malondialdehyde (MDA) and SOD levels before exercise and one day after physical exercise and 15 days after EA intervention. The result of MDA levels in the EA group was significantly lower than in the exercise group, whereas the serum SOD group EA was significantly higher than in the physical exercise group (Hubscher *et al*, 2010). The existing research shows that acupuncture can have an effect on free radicals and can increase SOD. This study was conducted to show the effect of manual acupuncture on SOD in untrained men after physical exercise.

Theoretical Review

Physical training

Physical activity is any activity that exerts physical strength and voluntary movements that burn calories. Examples of daily activities such as learning, walking, and others. Physical exercise is a planned, structured, and repetitive physical movementdoes not involve competition. Exercise involves physical activity and physical exercise that have rules, or goals to be an the body there is a physiological response and increased body metabolism that occurs due to increased intensity of one's physical exercise. Increased levels of lactate, respiratory rate and pulmonary ventilation and increased stress hormones indicate a body stress response due to increased exercise intensity (Lin *et al*, 2011). There is research showing moderate intensity performed in a short period of time may lead to an increase in ROS (Ahmedov *et al*, 2010).

Oxidative Stress

In general, the body produces reactive oxygen species (ROS) and reactive nitrogen species (RNS) continuously at rest. But at the time of exercise and certain diseases increased ROS. Excessive formation of ROS causes tissue damage, known as oxidative stress, whereas ROS at low or moderate concentrations plays a role in cellular function regulation. Oxidative stress can occur when natural ROS production can not be balanced by the capacity of tissue antioxidants (Matsubara *et al*, 2010) (Amulic *et al*, 2012) (Kolaczkowska *et al*,2013). Excessive formation of ROS damages almost all types of biomolecules, such as lipids, proteins, and DNA (Bhattacharya, 2015). In the 1970s and 1980s, the term "oxidative stress" was used to refer to processes of destruction by ROS. Then, German biochemist Helmut SIE defines oxidative stress as an imbalance between oxidants and antioxidants in overcoming oxidants, potentially causing damage. Oxidative stress is believed to significantly contribute to the development of a number of diseases, especially age-related diseases. In 1968, a major breakthrough in the field of free radical biology by Irvin Fridovich invented superoxide dismutase (SOD), a specific enzyme that catalyzes the transition from O2 to H2O2. A few years later, Chance and colleagues reported that mitochondria are key generators of O2- in cells. Both findings are important because they show that free radicals, on the one hand, are produced in biological systems, and on the other hand, there is an enzymatic mechanism regulating their concentration. It clearly shows that free radicals are formed on biological systems and may have certain functions. Since then, much research has been done to understand the biological functions of free radicals.

Evolutionarily, the induction of oxidative stress develops into an important part of the innate immune system as a defense mechanism against bacteria. However, ROS produced by the immune system can also damage host cells (Held, 2015).

Skeletal muscle is a very specialized tissue with excellent plasticity in response to external stimuli such as physical exercise and training. Repeated muscle contractions during resistance training cause a variety of physiological responses. These responses include activation of mitochondrial biogenesis, muscle fiber transformation, and angiogenesis. High muscle activity also causes a sharp increase in ROS. Since more than three decades ago it has been said that muscle activity leads to increased production of ROS and free radical concentration. Highly reactive molecules have many damaging effects, such as decreased muscle strength and increased muscle atrophy.

Mice and human studies have shown that aerobic or anaerobic exercise as well as chronic exercise lead to ROS formation and it is assumed that ROS potentially harms muscle function and causes muscle fatigue and muscle atrophy. Some potential ROS manufacturers have been identified in muscle cells that are likely to be activated by different stimuli. Among these are mitochondria, nicotinamide adenine dinucleotide phosphate (NADPH), nitrogen oxidase (NOX), phospholipase A2 (PLA2), xanthine oxidase (XO) and lipoxygenases. In addition to intracellular sources, ROS has been shown to be produced from non-muscle sources. Severe exercise can cause muscle injury, which then leads to activation of neutrophils and macrophages through interferon-γ (IFN-γ), interleukin-1 (IL-1) and tumor necrosis factor (TNF). These immune cells overload produce ROS (oxidative burst), which is a major component of the neutrophil defense mechanism. In addition, increased catecholamines (adrenaline, noradrenaline, dopamine) also play a role in ROS generation, as well as ROS derived from endothelium in muscles that do not experience fatigue, intracellular ROS seems important to increase the production of strength. Increased ROS due to intense exercise leads to various adaptations of muscle cells. Depending on the concentration of ROS, the duration of ROS exposure and individual training status, ROS can have beneficial and adverse effects. The exhausting single exercise has been

shown to cause oxidative damage in untrained people while in the untrained person, no such effect was observed due to increased resistance of those individuals to oxidative stress. A sharp rise in ROS after weight training, aging and / or illness (eg, chronic heart failure, COPD, cancer) can cause contractile dysfunction and muscle atrophy, both of which cause muscle weakness and fatigue (Nunes *et al*,2014)

The best physiological defense against O_2^- is the superoxide dismutase enzyme, which converts two superoxide anions into one molecule of hydrogen peroxide and one oxygen. The SOD subunit shows the structure of two domains: one domain containing α-helix and the second consisting of both α-helix and β-sheet. Mammals have 3 SOD isoforms produced by different genes: Cu / Zn-SOD, a homodimer of 32 kDa, localized in the cytosol or mitochondrial membrane space; Mn-SOD, homotetramer of 88 kDa, localized in mitochondria (matrix and inner membrane), and extracellular EC-SOD form, an extracellular tetrameric glycoprotein of 135 kDa.

SOD's main role is to neutralize O_2^- produced in the cytosol, mitochondria, and cell endoplasmic reticulum. However, SOD can also have a pro-oxidant effect because O_2^-dissociation produces hydrogen peroxide (H_2O_2), which is a toxin for cells. To overcome the harmful H2O2 is needed another antioxidant system, such as CAT and GPx, which will turn H2O2 into H2O. SOD ratio imbalances to CAT and GPx can cause several diseases.

Oxidative stress and inflammatory processes are closely related. Inflammation is a manifestation of oxidative stress, and pathways that produce inflammatory mediators, such as adhesion molecules and interleukins, are all caused by oxidative stress. Thus, the reduction of the inflammatory process is closely related to the antioxidant effect of SOD (Sharman *et al*,2012).

The primary mechanism in cellular defense against oxidative stress activates the nuclear-factor-E2-related factor (Nrf2) antioxidant signal response element, which controls the expression of protein-coding genes involved in the detoxification and elimination of reactive oxidants through conjugative reactions and by increasing cellular antioxidant capacity (Capersen *et al*, 1985) (Norton *et al*,2010). Induction of antioxidant enzymes can be regulated at the level of gene transcription through specific enhancer, antioxidant response element (ARE), in the gene promoter of antioxidant enzymes (Sharman *et al*,2012). In addition to its involvement in induced gene expression induction, ARE is also responsible for low-level constitutive expression (or basal) of some genes in non-stress conditions. Because ROS and other endogenous reactive molecules continue to be generated from normal aerobic metabolism, ARE's involvement in controlling constitutive gene expression implies an important role of enhancers in the maintenance of cellular redox homeostasis under stress and non-stress conditions (Norton *et al*,2010).

Acupuncture

Acupuncture comes from the Latin *acus* which means needle and *punctura* which means piercing, which is done at a certain point on the body. Along with the progress of knowledge about acupuncture, there are many researches that show the effectiveness of acupuncture is good for health In various studies have shown that stabbing acupuncture points can increase the specific signal molecules that can affect the function of the cell so that it can achieve the circumstances that homeostasis (Plowman *et al*, 2011). Stimulation at a particular acupuncture point can achieve favorable regional or systemic effects. Preventive acupuncture significantly inhibits systemic inflammatory responses and increases survival rates in mice with fatal endotoxemia (Pai *et al*,2015).

Scientific evidence suggests that acupuncture stimulation can reduce oxidative damage to pathological processes of organisms, but the exact mechanism of action remains unclear. Literary studies have found an association between acupuncture and redox modulation in various diseases, such as vascular dementia, Parkinson's disease, and hypertension, ranging from redox systems, antioxidant systems, anti-inflammatory systems to Brain Derived Neurotrophic Factor (BDNF) and the nervous system for signaling pathways. Experimental studies have reported that electroacupuncture effectively minimizes lipid peroxidation and malondialdehyde (MDA) levels by increasing the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in the CA1 hippocampus in mice with vascular dementia. Siu *et al*. says that electroacupuncture in ST36 Zusanli can increase Trx expression in ischemic reperfusion of brain tissue, which then increases antioxidant activity, shifts intracellular oxidative status toward redox balance, and then suppresses ROS production (Kim *et al*,2010). Acupuncture can improve cognitive abilities and reduce oxidative stress by suppressing activity AChE in the hippocampus (Kavoussi *et al*, 2007). In addition, Acupuncture with bee venom at ST36 may increase immune regulation in symptomatic hSOD1G93A transgenic mice by decreasing levels of Iba-1, COX2, and TNF-α (Takahashi *et al*,2009).

Acupuncture has been shown to play an important role in regulating the redox balance, selectively neutralizing excess ROS and activating the intracellular anti-oxidant system. Acupuncture can increase the nuclear translocation of Nrf2 in neurons, increase the expression of Nrf2 and the target gene Nrf2.30 The Nrf2 / ARE signal pathway is essential for increasing gene expressionantioxidants in response to various stimuli, and also protects cells against oxidative stress and inflammation (Pai *et al*, 2015).

Acupuncture in ST36 is known as a modulator of immune function and is often used in clinical disorders of the immune system. The research of Yu JB *et al*. in the Acute Lung Injury rabbit showed that acupuncture in ST36 increased the activity of SOD, GPx and CAT with an increase in Nrf2 and HO-1 expression. The study for the first time confirms that acupuncture provides a strong protective effect against lung injury through activation of the Nrf2 / ARE pathway and subsequent antioxidant enzyme induction. Consistent with the results of Western Blot and real-time PCR analysis, immunoflorescence staining showed an increase in Nrf2 protein accumulation in the cell nucleus after acupuncture stimulation (Pai *et al*,2015). In addition, Acupuncture with bee venom in ST36 may increase immune regulation in symptomatic hSOD1G93A transgenic mice by decreasing Iba levels -1, COX2, and TNF-α (Takahashi *et al*, 2009). Acupuncture in ST36 and SP6 effectively reduced the intensity of Iba-1 staining and the microglia activation cluster in the A53T model rat. Increased proinflammatory cytokines, such as TNF-α and IL-1β, in the midbrain and striatum may be blocked by acupuncture stimulation. Acupuncture also derived

monocyte chemotactic protein-1, IL-6, and interferon-γ (Wong *et al*, 2010).

RESEARCH METHODOLOGY

This study was conducted after the approval of the Medical Research Ethics Committee FKUI-RSCM. The selected study design in this study was a single blinded randomized clinical trial with placebo control. The research was conducted at the facility of Indonesia Sports Medicine Center, Gelora Bung Karno Area, Jl. Jend. Sudirman, Central Jakarta. The research subjects of 30 people with affordable population are employees of PT Kamlu (head office: Jl Bungur Besar Raya No. 56 Central Jakarta) that meets the inclusion and exclusion criteriaThe inclusion criteria in this study were men aged 20-35 years, eligible for physical exercise assessed from history, vital sign checks, and general physical examination, and willing to sign informed consent and follow the study to completion. Exclusion criteria in this study was a history of cardiovascular, pulmonary, metabolic, or malignant diseases; history of musculoskeletal injuries in the last six months; consumption of nonsteroidal anti-inflammatory drugs, glucocorticoids, vitamin supplements and antioxidants in the past week; exercise or physical exerciseany form within 72 hours prior to the implementation of the intervention; doing regular physical exercise at least once per week in the last three months; and there is a wound on the acupuncture point to be treated.

The subjects were randomly allocated using a computer-based random number table, divided into two groups, the manual acupuncture group and the placebo acupuncture group. Physical exercise in this study is run for 30 minutes using a treadmill. Preparations that need to be considered before starting physical exercise are sufficient sleep subjects on the previous night, breakfast of bread and drinking water at least 200 - 400 mL in 1 hour before starting physical exercise, as well as wearing sportswear (short sleeve shirts, pants, sports shoes) which is comfortable to run. Before the physical exercise begins, the subjects warm up, consisting of a slow walk around the exercise area for 3-5 minutes and seven stretching movements involving the torso and large muscles (hamstring, quadriceps, calf). During the physical exercise, the researchers will monitor the subjects' pulse rate at moderate intensity (64-76% HRmax). In the first five minutes, the treadmill speed gradually rises slowly from 2 km / h to 5 km / h, with an increase of 1 km / h every minute. After five minutes, the treadmill speed is set to 6 km / h and maintained until the 30th minute. For the study subjects who did not achieve 64% HRmax at 10 minutes, the treadmill speed will be increased to 7 km / h, and so on, and maintained until the 30th minute. Physical exercises were discontinued in intensity exceeding 76% HRmax or the study subjects were exhausted and unable to continue the protocol. Physical exercise ends with a stretch as it does when heating.

For manual acupuncture group treatment, the subject is in a recumbent position. Before stimulation of acupuncture points, performed aseptic and antiseptic action and plasterin plaster plastering that has been perforated at the location of ST36 Zusanli and SP6 Sanyinjiao. After that, stabbing acupuncture needles until the sensation of matchmaking (feeling sore, sharp, feeling like shocked) and left for 30 minutes, then the needle removed and thrown into medical bins. For the placebo group treatment, the subjects in a position lying on their backs. Prior to stabbing, aseptic and antiseptic action and plasterine plastering were performed at ST36 Zusanli and SP6 Sanyinjiao locations. After that, stabbing of acupuncture needles on plaster with no conditionabout the skin of the study subjects and left for 30 minutes, then the needle removed and thrown into the medical waste bin.

The results of the study were blood SOD levels. SOD blood checks were performed twice. First, after the subjects were divided into groups of manual and placebo acupuncture and before doing physical exercise, the second was 1 hour after physical exercise ended and had acupuncture manual and placebo acts. Blood collection is done by experienced analysts. Blood specimens were prepared according to requirements and taken to the University of Indonesia's Univ. Integrated Laboratory for SOD blood glucose and yield readings.

The acupuncture point used in this study was ST36 Zusanli, located on the anterior side of the lower limb, on the line connecting ST35 and ST41, 3 B-cun below ST35, and Sanyinjiao SP6, located in the lower limb, posterior to the medial boundary of the tibia , 3 B-cun above the prominence of malleolus media (WHO,2008).

Data collected in the research processed using SPSS 20 statistical program. Against all numerical data is done data normality test with saphiro-wilk. Numerical data with normal distribution will be presented in the mean and standard deviation values, whereas if the data distribution is abnormal it is presented in the median and range values. Statistical analysis of blood SOD levels before and after intervention in each group was performed by paired t test when the normal data distribution or Wilcoxon test when the data distribution was not normal. Statistical analysis for the comparison of blood SOD levels between the two groups was performed by unpaired t test when the normal data distribution or Mann-Whitney test when the data distribution was not normal. When the comparative hypothesis test obtained p value of $<\alpha$ ($p < 0.05$) then it can be said there are statistically significant differences between variables compared.

RESULTS

Characteristics of Research Subject

Table 1 Characteristics of research subject

Characteristic	Manual Acupuncture	Placebo	P
Age	23,13± 3,48	23,53± 2,722	0,556*
Smoking habit			
Yes	12 (80%)	13(86,7%)	1,000**
No	3 (20%)	2 (13,3%)	
Body mass index	20,26± 3,161	20,42±2,409	0,561*
Systolic blood pressure	128,47±12,129	128,60±10,225	0,974***
Dyastolic blood pressure	75,00±13,180	73,20±10,150	0,678***
Pulse	83,33±11,108	81,53±13,223	0,690***
Early SOD blood levels	1,66±0,416	1,82±0,290	0,255*

*Mann-Whitney **Fisher's Exact ***Independent sample t-test

The Fisher Exact test showed no difference (p = 1,000) for smoking habits. Mann-Whitney test showed no difference between the two groups for the age variable (p = 0,556) and body mass index (p = 0,561). The unpaired t-test showed no difference between the two groups for the systolic blood pressure variables (p = 0.974), diastolic blood pressure (p = 0.678), and pulse rate (p = 0.690). The Mann-Whitney test

showed no difference between the two groups for baseline SOD levels (p = 0.255). From the above data, overall there is no difference (p> 0.05) for the basic categories of research subjects between the two groups, so that the appropriate groups to compare.

Comparison of Mean Blood SOD Levels Before and After Physical Exercise on Manual Acupuncture Group

Table 2 Comparison of mean blood SOD levels before and after physical exercise in the manual acupuncture group

Variable	Beforeexercise	After exercise	Difference	P
SOD blood level (u/gHb)	1,66± 0,416	2,09 ±0,66	0,43 ± 0,487	0,005*

*Wilcoxon

Comparison of Mean Blood SOD Levels Before and After Physical Exercise in Placebo Groups

Table 3 Comparison of mean blood SOD levels before and after physical exercise in the placebo group

Variable	Beforeexcercise	After exercise	Difference	P
SOD blood level (u/gHb)	1,82± 0,290	1,78 ±0,176	-0,04 ± 0200	0,483*

*Paired-t-test

Average Comparison of Changes in Blood SOD Levels Before and After Physical Exercise between Manual Acupuncture Group and Plasebo

Table 4 Average comparison of changes in neutrophil count before and after exercise between the manual acupuncture group and placebo

Variable	Manual acupuncture	Placebo	P
SOD blood level (u/gHb)	0,43± 0,487	-0,04 ± 0,200	0,001*

*Mann-Whitney

The mean changes of blood SOD levels before and one hour after physical exercise in the manual acupuncture group were 0.43 ± 0.487 u / gHb, whereas the mean changes in blood SOD levels before and after physical exercise in the placebo group were -0.04 ± 0.200 u / gHb. Mann-Whitney test results showed that there were statistically significant differences for changes in SOD levels before and after physical exercise between manual acupuncture group and placebo group (p = 0.001).

DISCUSSION

This acupuncture study was conducted to assess blood SOD levels in subjects who did physical exercise. This study is the first study conducted in Indonesia, using a single blinded randomized clinical trial technique conducted on 30 subjects meeting the inclusion and exclusion criteria. Using computers to randomly divide into two groups, manual acupuncture and placebo acupuncture. In this study there are no participants who dropped (drop out).

In the basic characteristics of age, body mass index, smoking habit, pulse rate, blood pressure and baseline SOD levels in the manual and placebo acupuncture group there was no significant difference so that the study subjects were considered equivalent and feasible to be compared. The subject of research is a janitor with the same education that is SMU working on PT Kamlu. Before doing research subjects are required to sleep enough, eat bread for breakfast and drink 200-400 ml of water before doing physical exercise.

This study was conducted in Indonesia Sport Medicine Center (Bung Karno Gelora) which has a treadmill instrument with pulse indicator, therapy room and an appropriate walking area for this research. So that the execution of this research can be done at one place starting from blood taking, blood pressure and pulse test, warm up, physical exercise, stretching continued therapy and blood taking after therapy. The activity was performed approximately 1 hour 50 minutes on each subject. This study aims to assess the immediate effects of acupuncture on changes in blood SOD levels that occur immediately after one physical exercise.

In this study, the physical exercise used was running on a treadmill at a moderate intensity for 30 minutes. Running is chosen because it can be done by everyone with techniques that are not difficult so it is considered safe from the risk of injury. Previously had to start with a warm up and stretch the muscles after running. The reason for the selection of moderate intensity is due to the research subjects taken are people who are not trained and not accustomed to doing physical exercises regularly. The reference used to see increased intensity in physical exercise is the pulse rate, in the range 64% to 76% maximum pulse rate (calculated from 220 minus the age of the subject) is considered moderate intensity.

Muscle contraction during physical exercise leads to an increase in ROS levels in the skeletal muscle.Much literature supports the important role of ROS in intracellular signaling. Superoxide produced by mitochondria (O_2^-) is often associated with regulation of inflammatory processes, such as in the generation of inflammatory reactions, cytokine synthesis, and innate immune mechanisms. ROS has been shown to play a role in the transcription of the NF-κB-dependent gene and a number of other cascade signals. However, the highly reactive molecule also has damaging effects, such as decreasing strength and causing muscle atrophy. During physical exercise, free radicals increase due to electron leakage of the mitochondrial respiration chain, due to the failure of complex coupling processes I and III. The release of free radicals causes oxidation reactions and destroys various molecules (proteins, lipids, DNA), as well as this is reflected in plasma and blood cells. In addition, oxidative stress is also associated with muscle metabolism and muscle damage, and is often associated with aging and age-related diseases, such as malignancy, cardiovascular disease, Alzheimer's, and so on (Radom *et al*,2010).

In endurance training, an increase in oxygen uptake is accompanied by a maximal increase in mitochondrial oxidative capacity. Several studies have reported on the impact of duration and intensity of physical exercise performed throughout life against systemic oxidative stress. This raises the debate about oxidative stress caused by certain physical exercises and their relation to the aging process (Radom *et al*,2010).

Stronger ROS enhancement through intense physical exercise leads to various processes of muscle cell adaptation. In untrained people, a single, exhausting physical exercise can cause oxidative stress, while in a trained person, the effect is not found because there has been an increase in body resistance to stressful conditions. In addition to its effect on contractile kinetics, the ROS produced during physical exercise is also capable of modulating various signaling pathways, such as calcium, tyrosine kinase and phosphatase

proteins, serine / threonine kinase, and phospholipase. It then causes changes in gene expression, cell function, metabolism or cell damage. Chronic oxidative stress affects protein loss and muscle atrophy (Liu *et al*, 2015).

Miostatin, a factor of inhibition of muscle differentiation, can provide ROS production signals via canon smad3, NF-κB, and TNF-α in muscle cells. When there is no Smad3, miostatin induces ROS production through activation of p38 and ERK pathways mitogen-activated protein kinase (MAPK) mediated TNF-α, IL-6, NOX (NADPH oxidase), and XO (xanthine oxidase). Phospholipase A2 (PLA2) enzyme also contributes to increased intra- and extracellular ROS during muscle contraction. PLA2 causes the breakdown of arachidonic acid from phospholipids in the plasma membrane, sarcoplasmic reticulum, or mitochondrial membrane. Arachidonic acid is an important lipid signaling molecule and is the substrate for lipoxygenase for ROS production (Liu *et al*,2015).

Oxidative stress is defined as a balance disorder between ROS production and antioxidant defense. The complex network of metabolites and antioxidant enzymes work together to prevent oxidative damage. The best physiological defense against O_2^- is the superoxide dismutase (SOD) enzyme, which converts two superoxide anions into one molecule of hydrogen peroxide and one oxygen. Antioxidant enzymes such as SOD play a key role in reducing oxidative stress. Thus, the function of the antioxidant system is not to completely remove the oxidant, but to keep it at an optimum level, since ROS also has cellular functions useful in redox signaling (Capersen *et al*,1985).

The induction of various cytoprotective enzymes in response to reactive chemical stress is regulated primarily at the level of transcription. The transcription response is mediated by a cis-acting element called the ARE (antioxidant response element), initially found in gene promoters encoding two major detoxifying enzymes, GSTA2 (glutathione S-transferase A2) and NQO1 (NADPH quinone oxidoreductase 1). ARE has structural and biological features that are uniquely responsive to oxidative stress (Norton *et al*,2010).

The nuclear-factor-E2-related factor (Nrf2) transcription factor is known to be a central protein that interacts with ARE to modulate gene transcription, including antioxidant enzymes and inflammatory proteins. Nrf2 transcription factors bind to small Maf proteins with ARE in the regulatory region of target genes, and Keap1 (Kelch ECH associating protein 1)is a protein that binds Nrf2 and causes its degradation via the ubiquitin proteasom pathway (Capersen *et al*,1985).

Keap1's involvement in stimulating degradation Nrf2 raises the understanding that the interaction between the two proteins is a dynamic process that must be regulated through a particular pathway that allows Nrf2 to control its gene expression in basal and induced conditions. Nrf2's ability to control its basal gene expression suggests that Nrf2 is a functional active transcription factor, which can be found in the nucleus at homeostasis (Norton *et al*,2010). Acupuncture came from China more than 5,000 years ago. Currently acupuncture practice has expanded to Western countries and is used to treat various diseases, even as surgical anesthesia. Science has determined that meridians and acupuncture points identified by traditional Chinese medicine actually correlate with anatomical and neurophysiological systems (Natural Standard, 2013). Various studies have shown that some areas of the upper brain stem and hypothalamus with acupuncture stimuli can release opioids and serotonin. Acupuncture can achieve such stimulation by placing needles at various acupuncture points (Lin and Chen, 2008).

In this study, the manual acupuncture group was treated with filiform needle stabbing at the acupuncture points of ST36 and SP6 until a matchmaking sensation was achieved, while the placebo group of filiform needles was inserted only on the plaster regardless of the subject's skin. Against all subjects, plaster patching was done on the acupuncture points to disguise the difference in treatment between the two groups. In the manual acupuncture group, the plaster has been perforated in the middle to allow the filiform needle to penetrate the skin. The excitation technique chosen in this study is manual acupuncture. The reason for using the technique is simple, economical, easy, safe, and effective. Acupuncture interventions allow to be applied in first-rate health care facilities.

Acupuncture has a protective effect as anti-oxidative and anti-inflammatory which involves an activation mechanism of Nrf2. In the study of Wang XR *et al*, acupuncture has been shown to increase Nrf2 nuclear translocation in neurons and Nrf2 gene expression and its target genes, suggesting that one of the effects of acupuncture therapy is through the Nrf2 function. Acupuncture not only regulates the expression of Nrf2, but also causes translocation of Nrf2 fromcytoplasm to the nucleus and regulate the expression of NQO1 and HO-1. Nrf2 regulates gene expression of a number of enzymes that serve to detoxify pro-oxidative stressors. The antioxidant effects of Nrf2 are regulated through the association of Keap1 and translocation from the cytoplasm to the nucleus, where it regulates the cytoprotective gene and encodes antioxidants, including SOD. Several other studies have also shown similar results in various animal models of inflammation (da Silva *et al*,2011).

Acupuncture on ST36 Zusanli and SP6 Sanyinjiao is known to modulate immune function and is often used in clinical disorders of the immune system. Acupuncture action increases the activity of SOD, GPx, and CAT by increasing the Nrf2 and HO-1 expression that follow it. The research of Yu JB *et al*. showed that electroacupuncture in ST36, SP6, and BL13 bilateral produce strong protection against lung injury through activation of Nrf2 / ARE pathway and antioxidant enzyme induction. Consistent with Western blot and real-time PCR analysis, immunofluorescence staining showed an increased accumulation of Nrf2 protein in the cell nucleus after electroacupuncture stimulation (Liu *et al*,2015).

The results of this study showed a significant difference in blood SOD levels before and after physical exercise between the manual acupuncture group compared to the placebo group ($p = 0.001$). In the in-group analysis, subjects who received manual acupuncture treatment had significant changes in blood SOD levels before and one hour after physical exercise (0.43 ± 0.487 u / gHb, $p = 0.005$), suggesting that acupuncture was effective in overcoming inflammatory responses and oxidative stress triggered by physical exercise, and increased levels of SOD as an antioxidant. This was not found in the placebo group, as seen in the results of the analysis in the group showing no significant difference between blood SOD levels before and one hour after physical exercise (-0.04 ± 0.200 u / gHb, $p = 0.483$).

The results of this study are expected to be applicable to other specific populations, such as elite athletes. Elite athletes are often confronted with a high-intensity physical exercise and competition schedule, with high intensity and long duration. Such conditions can lead to more potent oxidative stress even in the athlete's body there has been a physiological adaptation process. Oxidative stress and its accompanying complaints, such as pain and decreased muscle strength or the incidence of acute respiratory infections, may affect the performance of athletes during competition. Therefore, acupuncture acts can be an option to improve athlete's performance.Limitations of this study were no longer performed blood levels of SOD blood levels, ie 2, 3, 4, and 24 hours after physical exercise, due to limited funding and research time.

CONCLUSION

The results suggest that manual acupuncture has a better effect on the amount of SOD in untrained men who perform acute physical exercise. The mean SOD blood levels in untrained men after performing acute physical exercise in the manual acupuncture group were significantly better than the placebo acupuncture group ($p = 0.001$).

Suggestion

Manual acupuncture acts can be one of the supporting therapies in overcoming oxidative stress triggered by physical exercise. Further studies that evaluate subjective complaints, such as muscle pain, fatigue, and follow-up of accompanying complaints, compare the effectiveness of various modalities of acupuncture therapy in physical exercise, as well as subjects with other populations, such as trained people or athletes, to assess effectiveness and determine the dose and technique of acupuncture stimulation that suits the needs of each individual, as well as the effect of acupuncture on SOD with a longer time and done serially to know the graph of increasing and decreasing SOD levels so it can be known how long the decrease in SOD levels can survive.

References

1. Akimoto T, Nakahori C, Aizawa K, Kimura F, Fukubayashi T, Kono I. Acupuncture and Responses of Immunologic and Endocrine Markers during Competition. *Medicine & Science in Sports & Exercise*. 2003; 35(8):1296-1302.
2. Ahmedov S. Ergogenic effect of acupuncture in sport and exercise: A brief review. *The Journal of Strength and Conditioning Research.* 2010; 24(5): 1421-7.
3. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil Function: From Mechanisms to Disease. *Annual Review of Immunology*. 2012; 30:459-89.
4. Bimbingan Belajar untuk Pembelajar. Masalah Olahraga yang Paling Sering Muncul. Disitasi dari http://www.bimbie.com/masalah-olahraga.htm pada 2 September 2016.
5. Brown WM, Davison GW, McClean CM, Murphy MH. A Systematic Review of the Acute Effects of Exercise on Immune and Inflammatory Indices in Untrained Adults. *Sports Medicine* -Open. 2015;1(35):1-10.
6. Bhattacharya S. Reactive Oxygen Species and Cellular Defense System. Dalam: Rani V and Yadav UCS. Free Radicals in Human Health and Disease. 2015;XVI:17-29.
7. Capersen CJ, Powell KE, Christenson GM. Physical Activity, Exercise, and Physical Fitness: Definitions and Distinctions for Health-Related Research. *Public Health Reports.* 1985;100(2):126-31.
8. da Silva MD, Guginski G, Werner MF, Baggio CH, Marcon R, Santos AR. Involvement of Interleukin-10 in the Anti-Inflammatory Effect of Sanyinjiao (SP6) Acupuncture in a Mouse Model of Peritonitis. Evidence-Based Complementary and Alternative Medicine. 2011;2011:1-9.
9. Giraldo E, Garcia J, J, Hinchado M, D, Ortega E, Exercise Intensity-Dependent Changes in the Inflammatory Response in Sedentary Women: Role of Neuroendocrine Parameters in the Neutrophil Phagocytic Process and the Pro-/Anti-Inflammatory Cytokine Balance. *Neuroimmunomodulation*. 2009;16:237-44.
10. Hübscher M, Vogt L, Ziebart T, Banzer W. Immediate effects of acupuncture on strength performance: a randomized, controlled crossover trial. *European Journal of Applied Physiology*. 2010;110:353–8.
11. Held P. An Introduction to Reactive Oxygen Species: Measurement of ROS in Cells. BioTek Instruments, Inc. *White Paper Review* 2015;1:1-21.
12. Kim SK, Bae H. Acupuncture and immune modulation. Autonomic Neuroscience: *Basic and Clinical*. 2010;157:38–41.
13. 13.Kanda K, Sugama K, Hayashida H, Sakuma J, Kawakami Y, Miura S, *et al*. Eccentric exercise-induced delayed-onset muscle soreness and changes in markers of muscle damage and inflammation. *Exercise Immunology Review.* 2013;19:72-85.
14. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nature Reviews Immunology*. 2013;13:159-75.
15. Kavoussi B, Ross BE. The Neuroimmune Basis of Anti-inflammatory Acupuncture. *Integrative Cancer Therapies.* 2007; 6(3):251-7.
16. Luk HY, McKenzie AL, Duplanty AA, Budnar RG, Levitt D, Fernandez A, *et al*. Leukocyte Subset Changes in Response to a 164-km Road Cycle Ride in a Hot Environment. *International Journal of Exercise Science.* 2016;9(1):34-46.
17. Liu M, Timmons BW. The Effect of Acute Exercise on the Production of Reactive Oxygen Species and Inflammatory Markers in Healthy Prepubertal and Adult Males. A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements for the Degree Master of Science. 2012:1-107.
18. Liu M, Timmons BW. The Effect of Acute Exercise on Neutrophil ROS Production and Inflammatory Markers in Healthy Pre-Pubertal and Adult Males. *Pediatric Exercise Science.* 2016; 28(1):55-63.
19. Lin ZP, Chen YH, Fan C, Wu HJ, Lan LW, Lin JG. Effects of Auricular Acupuncture on Heart Rate, Oxygen Consumption and Blood Lactic Acid for Elite Basketball Athletes. *The American Journal of Chinese Medicine.* 2011; 39(6):1131-8.
20. Liu CX, Guo LH, Tian GH, Ren LN, Zhang R, Yin Y, *et al*. Effect of electroacupuncture intervention on

serum IL-17 and IL-23 contents in rheumatoid arthritis rats. *Zhen Ci Yan Jiu*. 2015;40(2):141-5.
21. Mendham AE, Donges CE, Liberts EA, Duffield R. Effects of mode and intensity on the acute exercise-induced IL-6 and CRP responses in a sedentary, overweight population. *European Journal of Applied Physiology*. 2011; 111:1035-45.
22. Matsubara Y, Shimizu K, Miyamoto T, Kono I. Effects of Acupuncture Stimulation on the Response of Leukocyte and Lymphocyte by High-Intensity Exercise. *The Journal of the Japanese Society of Balneology, Climatology and Physical Medicine*. 2010; 73(2):92-100.
23. Nunes-Silva A, Bernardes PTT, Rezende BM, Lopes F, Gomes EC, Marques PE, *et al*. Treadmill Exercise Induces Neutrophil Recruitment into Muscle Tissue in a Reactive Oxygen Species-Dependent Manner. An Intravital Microscopy Study. Public Library of Science. 2014; 9(5):1-11.
24. Norton K, Norton L, Sadgrove D. Position statement on physical activity and exercise intensity terminology. *Journal of Science and Medicine in Sport*. 2010;13: 496–502.
25. Plowman SA, Smith DL. Exercise physiology for health, fitness, and performance. 2011; 3:219-664. Lippincott Williams & Wilkins, Philadelphia, USA.
26. Pai HJ, Azevedo RS, Braga AL, Martins LC, Saraiva-Romanholo BM, Martins MA, *et al*. A randomized, controlled, crossover study in patients with mild and moderate asthma undergoing treatment with traditional Chinese acupuncture. *Clinics*. 2015; 70(10):663-9.
27. Radom-Aizik S, Zaldivar F, Oliver S, Galassetti P, Cooper DM. Evidence for microRNA involvement in exercise-associated neutrophil gene expression changes. *Journal of Applied Physiology*. 2010; 109:252-61.
28. Sugama K, Suzuki K, Yoshitani K, Shiraishi K, Miura S, Yoshioka H, *et al*. Changes of thioredoxin, oxidative stress markers, inflammation and muscle/renal damage following intensive endurance exercise. *Exercise Immunology Review*. 2015; 21:130-42.
29. Shockett PE, Khanal J, Sitaula A, Oglesby C, Meachum WA, Castracane VD, *et al*. Plasma cell-free mitochondrial DNA declines in response to prolonged moderate aerobic exercise. *Physiological Reports*. 2016, 4(1):1-9.
30. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *Journal of Botany*. 2012; 2012:1-26.
31. Takahashi T, Sumino H, Kanda T, Yamaguchi N. Acupuncture Modifies Immune Cells. *Journal of Experimental & Clinical Medicine*. 2009;1(1):17-22. 78910. 11. 12.
32. Waite L. The Benefits of Acupuncture Therapy for Athletes. Disitasi dari http://www.Active.com pada 6 April 2016.
33. Wong MC, Shen HJ. Science-based Mechanisms to Explain the Action of Acupuncture. *Journal of the Association of Traditional Chinese Medicine.* 2010; 17(2):5-10.
34. World Health Organization. WHO standard acupuncture point locations in the Western Pacific region. 2008.

How to cite this article:

Rachma Novita Indrarini *et al* (2018) 'Manual Acupuncture Effect on Superoxide Dismutase Enzyme Immediately After Acute Physical Exercise in Untrained Man', *International Journal of Current Advanced Research,* 07(1), pp. 9184-9191. DOI: http://dx.doi.org/10.24327/ijcar.2018.9191.1508

****