CHAPTER 2

LITERATURE REVIEW

The classification of exercise (13)

Levels of exercise can be divided into 3 types by classification from heart rate as follows**:**

1. ***Mild exercise*** immediately increases heart rate to approximately 55-65% of MHR, but it soon decreases slightly and remains at a constant rate throughout the duration of exercise. When exercise stops, the heart rate gradually returns to normal in only 1-2 minutes.

2. ***Moderate exercise*** increases heart rate rapidly to approximately 65-80% of MHR, depending on the gravity of the exercise or whether the increased heart rate is relatively constant throughout the duration of exercise. When exercise is stopped, the heart rate gradually returns to baseline level, but this takes longer than after mild intensity exercise.

3. ***Heavy exercise*** increases heart rate to more than 85% of MHR throughout the duration of exercise, which can only be carried out for a short period of time.

Oxidative stress

Oxidative stress was defined as imbalance between free radicals and antioxidants level in the cell and mitochondria (1, 3). Oxidative stress can occur in the cell and relates to various pathogenesis of disease (2). However under conditions of oxidative stress in overall cellular bimolecular are potential targets for reactive oxygen species (ROS) and reactive nitrogen species (RNS) (2, 3).

Reactive oxygen species

Reactive oxygen species (ROS) is free radical molecule that increases under oxidative stress conditions. The oxygen molecular is not very reactive as such as, it has two unpaired electrons with parallel divides on last electron sheath (3). Free radicals derived from oxygen indicate more important of free radical species generated in cell systems such as superoxide ($O_2^{\cdot-}$), hydroxyl radical (OH°), hydrogen peroxide (H_2O_2), and perhydroxyl radical (HO_2°) (3).

Reactive nitrogen species

Reactive nitrogen species (RNS) are also classifed in a free radical molecules derived. Dominant type of RNS is nitric oxide NO) that produced in above the organisms by one of oxidation of one of the determinant guanido-nitrogen atoms of L-arginine (3). This procedure is catalyzed by the enzyme nitric oxide syntheses (NOS) (2, 3). Depending on the microenvironment, nitric oxide (NO) can be converted to many other RNS, peroxynitrite ($ONOO^-$) (1, 3).

Free radicals (19)

A free radical is an unpaired electron and unstable molecule, and highly sensitive to reaction with other molecules in biological compartments such as protien, lipid, and DNA. Free radicals are superoxide ($O_2^{-\circ}$), hydroxyl (OH°), peroxyl (RO°) and perhydroxyl (HO_2°). Most free radicals, the hydroxyl radical (OH°) and hydrogen peroxide (H_2O_2), are highly dangerous in biological compartments.

Lipid peroxidation from a chain reaction is a main process of oxidation on unsaturated fatty acid and phospholipids (20, 21). Lipid hydroperoxide (LOOH) on structure can be converted to hydrocarbons, ketone or aldehyde (malondialdehyde) (12). Protein is an important molecule that can be oxidized by free radicals (22). Various proteins in the cell have their specific functions, such as enzymes, immune protein or structure compartment, so their unstable structure after oxidation causes cellular dysfunction. Oxidized mitochondrial of DNA (mtDNA) is a result of organ dysfunction with a mutation, and many of them enhance disease progressions such as asthma, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS) and lung cancer (23). Moreover, oxidative stress is related to the immune system in the body and can cause immune-related diseases (autoimmune diseases), such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (24). In the cardiovascular system, over oxidative stress induces various diseases, e.g. cardiovascular disease (CVD) or myocardial infarction (25). It is proposed that muscle weakness is related to over oxidative stress (19), and many proteins within the muscle cell act as signal transcriptors (regulatory protein) that control the contraction and

extension of muscle fiber. Nitric oxide (26) is a substance from the activity of nitric oxide syntheses (NOS) at the blood vessel wall and in the muscle, and is divided into 2 types; Type I (Neuronal-type NO syntheses) and Type 2 (endothelial isoform of NOS). NO, in particular, controls the growth of blood vessels and blood flow. However, when over releasing NO, an unclear mechanism shows negative effects on muscle contraction (18). Some evidence suggested that NO inhibited calcium-dependent ATPase, actin-myosin cross-bridge cycling, mitochondrial oxygen utilization, and finally, accelerated muscle fatigue process (27).

Short Acute exercise and oxidative stress: effect of gender

This study used male subjects, who were previously reported to show how the sex hormone, estrogen, affected antioxidant properties in female humans (28) or rats (29). Moreover, in short, female rats had lower levels of oxidative stress when compared to resting males (30).

Chung and co-workers (31) investigated the role of estrogen in reduced exercise-induced oxidative stress and found minimal difference in oxidative stress levels in women during both the luteal and follicular phases of their menstrual cycle. That, and several other studies, reported no difference in exercise-induced oxidative stress response between men and women following submaximal aerobics, long duration aerobics, and isometric exercise (31). Although no difference in oxidative stress response occurred between women and men from short exercise, higher antioxidant was seen more in females than in men during the resting period (32-34).

The inflammatory processes and exercise

Oxidative stress affected the enzymes of inflammatory processes; MAPK, NF-kB and COX-2, which are more active during oxidative stress, as well as TNF-alpha and IL-8 that are released during inflammation. Additionally, the high level of lipid peroxides and 4-HNE stimulated the MAPK (35) and IL-2 excretion in blood circulation (36, 37). Interleukin 2 (IL-2) is a glycogen with an equivalent weight to 15,000 dalton, and it affects activity and growth of T-cells by producing $CD4^+$ and $CD8^+$ T cells. Heavy exercise is harmful and activates the inflammation processes in the body from muscle tissue injury at the Sarcolemma. Thus, T-cell interleukin 1 and 2 proteins are produced to inhibit inflammation (6).

Antioxidant substances within the body are in 3 groups as follows (38-40):

1. Group of antioxidant enzymes: Superoxide dismutase (SOD), Catalase, Glutathione peroxidase (GSH-Px), Glutathione reductase (GRx), Glutathione-S-Transferase (GST), Ascorbate peroxidase and Cytochrome C peroxidase.

2. Group of non-enzymes.

    2.1. Protein metal; ceruloplasmin.

    2.2. Protein sulfide; albumin.

3. Group of small molecular substances.

    3.1. Types of water soluble substances; vitamin C, sugar, uric acids and glutathione.

    3.2. Types of fat soluble substances; vitamin E and carotenenoid.

N-acetylcysteine (**NAC**) is a commercial drug with a cysteine amino acid. Cysteine has a thiol group and can be used to synthesize GSH within the cell (35, 41), then its anti-free radical activity can prevent muscle fatigue (27).

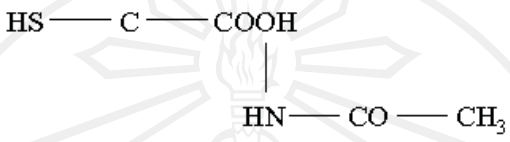

Figure 1. The structure of N-acetylcysteine (NAC).

Side effects of N-acetylcysteine

In the first minute after supplementation, dry mouth, sweating, insomnia and nausea can occur. Nausea, diarrhea, and abdominal pain may occur after 3 minutes, after which all symptoms disappear (42, 43).

Clinical application in NAC

Van Schooten and co-workers (44) studied the effects of N-Acetyl-L-cysteine by oral administration for six months in 41 healthy smokers (aged 42 $\pm$ 2.3 yrs), and showed that NAC supplementation at 1,200 mg daily can modulate certain smoking-associated biomarkers (urine mutagenicity).

Zembron-Lacny and co-workers (45) studied the effects of NAC supplementation at 1,200 mg for 8 days in sedentary men (20.3 $\pm$ 2.3 yrs, n=15). The results showed that NAC improved antioxidant status and immunological response.

In NAC supplementation taken orally at 600 mg, the highest level of NAC in the blood can be detected after 60 minutes and remains at 8.1 μM, as well as cysteine, which stays at up to 21.2 μM (46). In a clinical study, Sadowska et al., (16) demonstrated the short term effects of NAC supplementation. One hundred and ninety ARDS patients in an intensive care unit (ICU) received NAC at 900 mg per day for 8 days by intravenous route or 1,240 mg in 60 kg weight by vein route. The results showed that the process of phagocytosis significantly decreased after the subjects had received NAC for at least 5 days. Moreover, long term NAC supplementation had been studied in chronic COPD patients, who were administered 1,200 mg per day for 2 months; resulting in a significant reduction in the level of hydrogen peroxide. Especially, the level of blood glutathione improved after 5 days of NAC supplementation taken at 600 mg per day. Thus, an NAC dose of at least 600 mg per day, for a short period of within 5 days, could be applied in this study in order to control oxidative stress, but the physical performance has yet to be evaluated.

Several studies found that moderate and light exercise intensity activated IL-2, while at heavy intensity IL2 release was inhibited. The basic theory of the "J-Shape Pattern" describes the effect of exercise on the body's immunity (47). The J-Shape curve of the relationship between exercise intensity and infection of the upper respiratory system is shown, and also inhibition of NK-cell activity. In contrasting results of exercise intensity and IL-2, Smith (48) found that the level of IL-2 suddenly decreased after exercise, whereas the study of Feng et al., (49) found that IL-2 increased after exercise with different exercise intensities. Feng's study (49) found that the IL-2 level increased after 120 minutes of exercise. Heavy exercise (intensity

levels greater than 75% VO_2 max) reduced activation of the body's immune system by reducing the number of lymphocytes and decreasing IL-2, while moderate exercise (intensity level of 50% VO_{2max}) did not affect the IL-2 level, as in Esperson's study (50).

Nutrient or multivitamin supplementation has been encouraged and promoted for controlling oxidative stress in all athletes. Antoni et al., (51) studied the response of antioxidant to oxidative stress after heavy exercise in 8 male spin bike athletes and it showed that NAC improved antioxidant enzyme and enhanced the glutathione level.

N-acetylcysteine (NAC) has a high potential for reducing oxidative stress in clinical treatment for chronic obstructive pulmonary disease (COPD) (52). Previous reports showed that NAC supplementation at 600 mg per day did not affect lung function within 7 days, but the GSH level significantly increased after NAC supplementation at 1,200 mg per day for 5 days (17). In healthy subjects (n = 9), only two days of NAC supplementation at 800 mg per day did not change the level of GSH, MDA, or PSC (53), but a higher dose (125 mg per 1 kg body weight or 25 mg per 1 kg body weight) significantly increased the NAC in muscles for 20 min after supplementation (54). Moreover, a minimal dose of NAC can improve phagocytosis, scavenge superoxide radical, and reduce glutathione peroxidase significantly at 600 mg per day for 14 days (55). For the effect of NAC on muscle strength, the study of Matuszczak showed that the strength of hand grip improved significantly by 15% after NAC supplementation at 150 mg per 1 kg body weight or 4,500 mg in 60 kg body weight of sedentary subjects (n = 18) (42).

According to the above review, the effectiveness of NAC on exercise performance related to oxidative stress was studied and found. NAC is used in clinical treatment, especially for COPD patients. It is composed of cysteine with a thiol group. However, there is little evidence of studies on NAC supplementation and physical performance in healthy humans. Therefore, the aim of this study was to ascertain whether short periods of 7 days NAC supplementation, with a high concentration of 1,200 mg per day, can control oxidative stress, and enhance physical performance and interleukin-2 from short heavy exercise in healthy subjects.