CHAPTER 5

DISCUSSION

To the author's knowledge, this study was the first to investigate the effects of N-acetylcysteine on oxidative stress and physical performance after short heavy exercise in sedentary men at a dose of 1,200 mg daily for a short period (7 days). Results showed the direct effect of short heavy exercise on a treadmill in a control and an NAC supplement group at baseline (day 0) by decreasing TAC and GSH, and increasing nitrite and MDA. A previous report showed that heavy physical exercise is associated with increased oxygen uptake in skeletal muscle (61). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are believed to be a series of biochemical and physiological changes during exercise, via xanthine oxidase catalyzed reaction and neutrophil activation in the ischemia-reperfusion injury (62). Moreover, mitochondrial electron transport, catacholamines, and protanoids were also higher during exercise; and post exercise, prostanoids, proteolysis, inflammation and calcium homeostasis were involved if muscle fiber was injured (63).

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Total antioxidant capacity (TAC) and Glutathione (GSH)

Total antioxidant capacity (TAC) is preferred to overall antioxidant status in the system, and most studies have shown that TAC decreases quickly after strong exercise (64-66). GSH is also a main antioxidant within the cells of the body, including red blood cells, and it completely decreases and returns to basal levels in

approximately 30 min (10, 67). In both parameters (Figure 4 and 5) , the results in this study showed depletion after short heavy exercise at day 0 and day 8 in the control group and at day 0 in the NAC supplement group (Table 2). Glutathione (GSH) is an endogenous antioxidant that controls free radical formation. The general mechanism of GSH is able to scavenge free and non-radicals directly by glutathione peroxidase (GPX). Moreover, GSH reduces organic hydroperoxide (ROOH) to H_2O as well as nitrite to GSNO (68, 69). Previous work has noted that rats swimming until exhaustion had increased lipid peroxidation and decreased GSH/GSSG ratio (70).

Nitrite

Nitrite is generated continuously by skeletal muscle; a production that is increased by contractions (71). Skeletal muscle normally expresses the neuronal (type I or nNOS) and endothelial (type III or eNOS) isoforms of NO synthase (NOS). The nNOS is strongly expressed in fast-twitch muscle fibers and localized to the muscle sarcolemma, where it is associated with the dystrophin-glycoprotein complex. It appears to be the prime source of the nitrite released from skeletal muscle (72). The high level of nitrite in cardiac muscle was a reversible regulation of respiration in cardiac tissue in situations of hypoxia and reoxygenation (73). The results of this study showed the nNOS responds from short heavy exercise by increasing nitrite levels at either day 0 or day 8 in the control group and at day 0 in the NAC supplement group (Figure 6). It is still argued whether or not increasing nitrite and ROS in muscle is related to forcing the muscle. Nitrite involves the cyclic GMP at the subsarcolemma (74) via dependent and independent mechanisms (27). Although Nitrite is a free radical, bifunction is proposed with antioxidant at a low, but not high

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concentration. Nitrite derivatives can inhibit the activity of glyceraldehdye-3-phosphate dehydrogenase (75, 76) and creatine kinase (77), which could limit ATP production, but there is no evidence to support this.

Malondialdehyde (MDA)

MDA is an important target measurement in oxidative stress that has three carbon chain aldehydes and can be detected by TBAS. Increasing MDA has been proved in both maximal and submaximal exercises in humans (78, 79). From this study we found the aggrasive affect on the lipid peroxidatin after short heavy exercise either in control group at day 0 and day 8 (Figure 7) or at day 0 in supplement group.

Interleukin-2 (IL-2)

Short heavy exercise depressed the IL-2 in both the control group (3.2 ± 0.65 to 2.36 ± 0.37 pg/ml) and day 8 (3.11 ± 0.38 to 2.31± 0.52 pg/ml) and NAC supplement group (3.09 ± 0.71 to 2.25 ± 0.36 pg/ml) at day 0 (Figure 8). Previous study showed the effect of strenuous exercise, which decreased the percentage of type 1 of T cells in the circulation (80), and this phenomenon is still found in oxidative stress from endurance exercise (81). Thus, short heavy exercise increases the risk of infection or depletes the immune system. IL-2 is classified as a type 1 cytokine and plays an important role in both intra- and extra-cellular infection (82) by regulating growth and function of cells involved in cell-mediated and humoral immune systems (48, 83). Increase in IL-2 level was also reported to relate to the increase in NK-cell count, when prolonged interval exercise at moderate intensity is performed (84).

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Thus, inducing the IL-2 expression may be best when exercising for a longer duration (45-60 minutes) and prolonged period (85).

N-acetylcysteine (NAC)

NAC contains the thiol group of cysteine that has been proposed as a precursor of GSH, which enters the cell by co-translation with Na^+ via the amino acid transport system (41). Increasing NAC supplement, NAC and cysteine concentrations peak in plasma within 60 – 120 min after ingestion (86). Although the bioavailability of orally administered NAC is low (87), Matuszczak et al. (42) observed that oral administration of 150 mg/kg NAC solution increased plasma NAC concentration by 20- to 30-fold, and plasma cysteine concentration by 10- to 15-fold. A similar dose administered via intravenous infusion yielded higher plasma NAC concentrations in order of magnitude (54, 88). Both routes of administration inhibited oxidation of circulating glutathione during exercise to a similar degree. This "ceiling effect" of NAC may be a consequence of glutathione regulation of glutathione synthesis through a negative feedback mechanism (89, 90). In this study, the result of GSH level at pre-exercise on day 8 was higher than that on day 0 (59.58 ± 1.81 and 57.18 ± 2.55 μmol/g Hb, respectively) and as same as in TAC level (2.45 ± 0.11 mmol Trolox/L) on day 0 (Figure 5). Preserved GSH helped to control the lipid peroxidation within the muscle (91), when GSH from exercise was consumed in skeletal muscle to inhibit lipid peroxidation. Therefore, the level of GSH after post-exercise did not deplete quickly and MDA did not increase, which was in contrast to the non- NAC supplement group (Figure 5 and 7). The results were also similar to a previous study (53), in which NAC improved the net peroxyl radical scavenging capacity (PSC) at

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resting after NAC supplementation at 800 mg per day for 2 days, and the levels of MDA were maintained from the maximal bicycle ergometer exercise test (92). For the nitrite levels, from this study didn't show the benefit effects of NAC supplementation (Figure 6), in control and supplement groups, by increasing of nitrite levels after short heavy exercise. Although, in supplement group, the increasing level looked lower than another levels after exercise, but no statistical difference ($p > 0.05$).

In Figure 8 shows the improvement of IL-2 level after exercise at day 8 in only supplement group significantly. Increasing in IL-2 level can be explained with active function of T-cells. IL-2 is released from T-cell for activation the T-helper cells or relates to the T-cell periliferation, thus, IL-2 level was directed relation to the number of T-cells within the blood circulation (83, 84). Thus, NAC may be a source and activation synthesis of intracellular GSH within the T-cells and finally, this help to protection the inflammatory or infection condition in the body.

Physical performance and Running time

For physical performance, the running time until exhaustion stage, with 85% of MHR and RPE at 15, was evaluated. From the result, showed non significant difference either in control and supplement group, although, the running time in supplement group was slightly increase (15.94 ± 1.55 min) but didn't significant compared with before supplementation (15.45 ± 1.65 min). Whereas in control group, there was not improvement (day 0 = 14.13 ± 1.65 and day 8 = 13.94 ± 12.02 min). Because of difference in physical strength between groups; although the target heart rate in both groups didn't difference but the RPE in supplement group for stopping

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exercise (day 0 = 13.62 ± 0.80 and day 8 = 13.34 ± 0.92) was lower than in a control group (day 0 = 14.26 ± 0.70 and day 8 = 14.67 ± 0.86). But the results of this study didn't show the effect of NAC on running time (Table 3). This confirmed that in the previous study, GSH and NAC decreased exercise-induced GSH oxidation, but did not improve the endurance to exhaustion in a rat model (92). In similar results of a previous study by Medved and co-workers (93), the effects of NAC infusion on muscle fatigue, at 125 mg/kg/h for 15 min following 25 mg/kg/hr, showed that NAC did not alter the time to fatigue in 8 untrained men, when compared to saline infusion. Running time in this study was recorded when the heart rate was up to 85% of MHR per individual, because heart rate is a significant correlation with maximal consumption (94). Then, the time of reflex to the aerobic capacity in the body, or ATP production, was increased for a little longer. However, the mechanism still needs to be confirmed and studied deeply. Side-effects of NAC can limit experimental use. These include anaphylactoid reactions, hypotension, light-headedness, nausea, diarrhea, sleepiness, and dysphoria, among others (42, 95, 96). At much lower doses, patients with chronic obstructive pulmonary disease reported no side effects from ingesting 1,800 mg/day of NAC (capsules) for 4 days (97). In this study, less than 20% of the NAC supplement group experienced sleepiness or light-headedness, while on the other hand, stronger force and freshness were shown.

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Conclusion

NAC is an interesting target drug that is not only used in COPD patients, but also applied as a possible anti-oxidant from short heavy exercise in sedentary subjects. NAC is the acetylated derivative of cysteine. The sulfhydryl residue of this amino acid confers with antioxidant property, which can react directly with a variety of biological oxidants, including ROS and RNS. It supports glutathione biosynthesis and improves the total antioxidant capacity in the body system, including improvement of IL-2 release in the case of anti-inflammation. However, this study had a small group of sedentary men on short time NAC supplement, and a larger group taking supplement for a longer period of time should be studied to establish the long term benefit of NAC in humans.

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