

Full Length Research Paper

The protective role of oral consumption of N-acetyl cysteine during a single session of exhaustive exercise in untrained subjects

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The aim of the current study was to verify the effect of N-acetyl cysteine (NAC) supplementation on markers of oxidative stress and inflammatory response during a single session of exhaustive exercise. In a randomized placebo-controlled double-blind clinical trial, thirty healthy, untrained young males and females with a mean age of 21.33 ± 2.39 years, weight of 59.63 ± 9.24 kg and height of 166.20 ± 10.15 cm were selected and divided into 2 groups. Before starting the supplementation, blood samples were collected from all the participants. The next blood samples were collected just before the exercise started, immediately after the exhaustive exercise and after one hour of rest. Malondialdehyde (MDA), total antioxidative capacity (TAC), CRP, BMI and Vo_2 max were determined. A significant increase was observed in MDA and CRP levels during the experiment in the placebo group. But in the treated group, the concentrations remained the same throughout the experiment. The TAC levels were significantly raised in the samples collected after NAC supplementation as compared to the placebo group. No changes were observed in the time to fatigue. Results of the current study suggest that oral consumption of n-acetyl cysteine for 24 h before a single bout of an exhaustive physical exercise could significantly reduce the harmful effects of oxidative stress.

Key words: N-acetyl cysteine, oxidative stress, exhaustive exercise, reactive oxygen species, time to fatigue.

INTRODUCTION

There have been several investigations to show that, exhaustive exercise especially in untrained individuals can cause oxidative stress and lead to increase in the

production of reactive oxygen species in different tissues (Valko et al., 2007). Overproduction of reactive oxygen/nitrogen species known as pro-oxidants can result from

different stressors including vigorous physical exercise (Poljsak et al., 2011).

As the consumption of oxygen increases during exhaustive physical exercise, it leads to acute state of oxidative stress (Poljsak et al., 2011). It has been shown that the initial stage of inflammatory processes involve an increase in the production of highly reactive oxygen species (Xinyuan et al., 2013). Also it is well known that highly reactive oxygen species can easily react with macromolecules including lipids, proteins and DNA within the cells (Urso and Clarkson, 2003). Polyunsaturated fatty acids in the cell membranes are the most frequent targets; they undergo a chain of oxidative reaction called lipid peroxidation which leads to decrease in the membrane fluidity and making it more difficult for the proteins and nutrients to pass through (Bloomer et al., 2007). Several factors such as duration, intensity, fitness, breed; health, athletic ability and environmental conditions are likely to have an impact on the severity of oxidative stress and damage (Fisher-Wellman et al., 2009; Volvaard et al., 2005).

Following the cessation of a session of exhaustive exercise, especially in untrained subjects, excessive generation of pro-oxidants occurs in the muscles and tissues Sen et al. (2000). Strenuous exercise in an unconditioned individual or someone unaccustomed to exercise will induce oxidative damage and result in oxidative stress and then tissue injury Evans (2000).

Hence, supplementation with varieties of anti-oxidants to reduce free radical production and subsequent oxidative damage during and following an exhaustive physical exercise has been a priority of much research activity (Bloomer et al., 2007; Fisher-Wellman et al., 2009). N-acetyl cysteine (NAC) is a by-product of glutathione which is a tri-peptide in the cytoplasm of the cells and acts as a reducing agent (Childs et al., 2001). Both glutathione and NAC are antioxidants and they have the ability to minimize oxidative stress and its negative effects on different tissues (Kretschmar et al., 1991). Always intense and unaccustomed exercise could lead to oxidative stress and its detrimental effects (Alessio et al., 2000). N-acetyl cysteine has been used in clinical practice to facilitate glutathione (the major non-enzymatic endogenous antioxidant) biosynthesis, thereby improving the intracellular enzymatic antioxidant defense system and possibly decreasing the damaging effects of reactive oxygen species (Zhang et al., 2011). The role of glutathione as a reducing agent in ameliorating the complications of exercise induced oxidative stress has been studied by many investigators (Fisher-Wellman et al., 2009). Furthermore, it has been shown that NAC decreases free radical production and oxidative stress both at rest and during pro-

longed exercise (Jammes et al., 2005). N-acetyl cysteine significantly attenuated the rise in plasma $[K^+]$ followed by fatigue during a prolonged endurance exercise. This result confirms that the antioxidant NAC attenuates muscle fatigue, in part via improved K^+ regulation, and points to a role for reactive oxygen species in muscle fatigue (McKenna et al., 2006).

To the best of our knowledge there has been no study to show that oral consumption of pure NAC can ameliorate the possible damages caused by the generation of reactive oxygen species during a single session of exhaustive exercise in untrained subjects. The results of an investigation suggest that treatment with NAC represents an important factor in the defense against muscle soreness and has different effects on oxidative damage and pro- and anti-inflammatory cytokines following a session of exhaustive exercise (Silva et al., 2008). The aim of the present study was to evaluate the protective role of oral N-acetyl cysteine during a single bout of exhaustive exercise in untrained young individuals.

MATERIALS AND METHODS

Study design and participants

In a randomized placebo-controlled double-blind clinical trial, thirty healthy untrained young males and females (Age = 21.33 ± 2.39 years) from Azad University of Bushehr, Iran, were divided into two groups; the placebo and treated groups of 15 each. For randomization, the eligible participants received the treatment tablets and the placebo in random order. The order of the treatments was randomly generated by computer and sealed in sequentially numbered opaque envelopes and assigned to subjects in the order of their first study visit. Both the clinical team and participants were blinded from the time of randomization until analysis was complete. A questionnaire containing the information about their foods consumed and the physical activity they had for the past 6 months, was filled by each individual. The main inclusion criteria were participants aged between 21 and 25, either sex, having Vo_2 max between 35 and 40 ml/kg/min and living in the same dormitory and all having the same type of food. The subjects who were consuming energetic drugs, smokers and those having any type of regular sport activity were excluded from the study. None of the participants were on antioxidant supplementation. All the experimental procedures were performed in accordance with the Helsinki Declaration and the policy statement of the American College of Sports Medicine on research with human subjects. The ethic Committee of Bushehr University of Medical Sciences approved all the experimental protocols. Also, prior to participating in this investigation, informed written consent was obtained from all subjects.

Participants in the intervention group received 600 mg doses (four times with an interval of 6 h) of effervescent tablets of N-acetyl cysteine (Zambon, Srizzera SA, Swissmedics Company), starting from 24 hours before the exhaustive exercise. The last dose was given just one hour before the exercise. The control group consumed dextrose 5% dissolved in water (it has no side effect

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Abbreviations: BMI, body mass index; CRP, C-reactive protein; MDA, malondialdehyde; CK, creatine kinase; TAC, total antioxidative capacity; NAC, N-acetyl cysteine; Vo_2 max, maximal oxygen uptake; TBARs, thio-barbituric acid reactive substance; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione.

Table 1. The general characteristics and anthropometric parameters of the treated and the control group.

Parameter	Placebo	Treated	P-values
Age (years)	21.33 ± 2.39	21.33 ± 2.33	0.823
Height (centimeter)	166.83±11.18	166.2±10.15	0.0923
Weight (kg)	66.2± 12.67	59.63± 9.24	0.543
BMI (kg/ m ²)	23.93±4.45	21.55±2.47	0.323
f.m (kg)	16.89±8.58	14.06±6.12	0.091
f.f.m (kg)	49.37±10.57	45.61±10.46	0.561
f.m (%)	25.20±10.04	23.84±9.98	0.087

Data is presented as mean ±SD, BMI = body mass index, f.f.m = fat free mass, f.m = fat mass and P <0. 05 are considered as significant.

when consumed orally). The clinical trial was registered as Irct ID: IRCT201208238129N2.

Procedures

Anthropometric measurements and the general characteristics of the participants including age, sex, height, weight, fat free mass (f.f.m), fat mass (f.m), Vo₂ max and body mass index (BMI), were recorded. Height was measured to the nearest 0.5 cm, and the weight, to the nearest 0.1 kg. Body fat composition was measured by using X-Scan plus II, JAWON Medical, Korea, which provides segmental obesity and segmental edema. Vo₂ max was determined according to Cooper Standard Aerobic Test (distance in meters minus 504.9 divided by 74.44). Body mass index was calculated as weight in kilograms divided by the square of height in meters (Grundy et al., 2004). The first blood samples were taken 24 h before starting the experiment (time 1). The second sample was collected at time 2 (just before the experiment started) and immediately after the exhaustive exercise utilizing Bruce examination protocols in the form of flat treadmill running (time 3). The last sample was collected after one hour of rest (time 4). The serum samples were immediately separated and stored at -80°C for further analysis. Malondialdehyde, the byproduct of lipid peroxidation was measured by using an improved thiobarbituric acid reactive substance (TBARS) based method (Manciet and Copeland, 1992). The determination of total antioxidant capacity was performed using commercial Kits from Biovision-Co, USA which uses Trolox equivalents to standardize the antioxidants. C-reactive protein was measured by ELISA technique based on the principle of a solid phase enzyme-linked immunosorbent assay. Inter assay coefficient of variation (CV) for the method was <20%.

Statistical analysis

Normal distribution of the data was controlled with the Kolmogorov-Smirnov test. Probability values <5% were considered statistically significant. The significance of the difference in the results between the two groups was determined with Chi-square analysis using 2 x 2 contingency tables. Independent unpaired t tests was used for continuous normally distributed variables. The Mann-Whitney U Test was used to compare differences between the two independent groups when the dependent variable was continuous but not normally distributed. The Wilcoxon signed-rank test was used as an alternative to the paired Student's t-test for dependent samples when the variables cannot be assumed to be normally distributed. Kruskal-Wallis test was used to compare independent variables across several groups. Spearman correlation coefficient was used to test relationship between different variables. All statistical analy-

ses were performed using the PASW Statistics 16 (SPSS Inc., Chicago, IL).

RESULTS

The general characteristics and anthropometric parameters of the treated and the control group are given in Table 1. There was no difference in age, sex, weight and BMI between the two groups (Table 1). The concentration of CRP in the control group increased during the experimental procedure but, it was significant only at one hour after the exhaustive exercise (Figure 1). However, in the treated group the concentration remained the same during the study (Figure 1). The concentration of malondialdehyde in the serum samples of treated subjects remained the same during the study (Figure 2). However, in the control group the concentrations raised significantly at times 3 and 4 during the experiment (Figure 2). The blood levels of total antioxidant capacity at time 1 (before the consumption of N-acetyl cysteine) was the same in both groups (Figure 3), but at times 2, 3 and 4 the concentrations decreased significantly in the control group (Figure 3). In the treated group, the total antioxidant capacity levels were significantly raised at times 2 and 4 but at time 3 no change was observed (Figure 3). Moreover, oral consumption of n-acetyl cysteine starting from 24 h before the exhaustive exercise had no significant effects on the values of Vo₂ max and then, the time to fatigue (Table 2).

DISCUSSION

The novelty of this study was the oral consumption of NAC (600 mg, without any other antioxidant ingredients) by the participants who were doing exhaustive exercise for the first time. The results of the present study showed that the serum concentration of MDA and CRP known as the markers of lipid peroxidation and cell damage respectively, keep on increasing in the subjects undergoing an intense and unaccustomed exercise. This is in accordance with the results from other investigators showing that plasma TBARs and CK levels increased after a 45

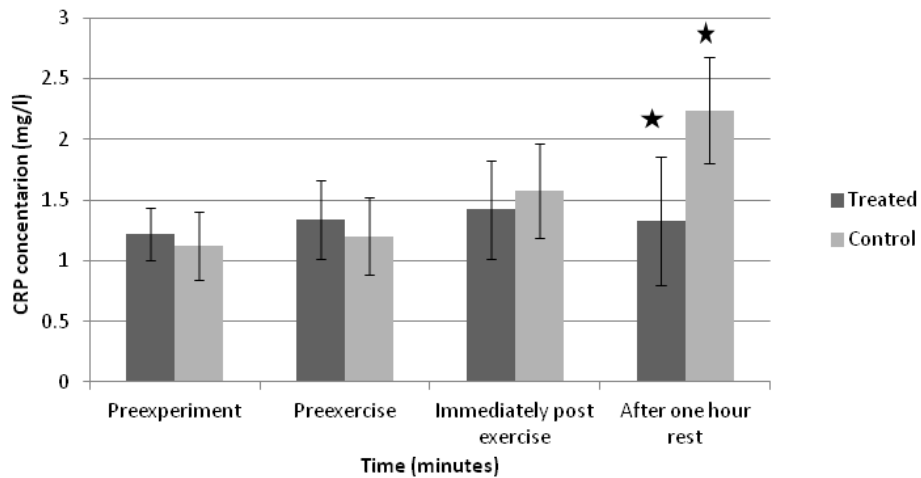


Figure 1. The differences in the concentration of CRP between the two groups (treated and the control). The stars show a significant difference.

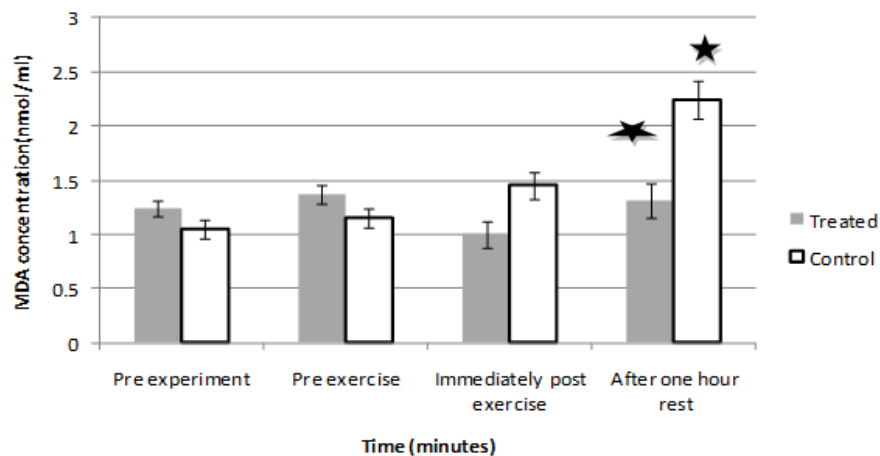


Figure 2. The differences in the concentration of MDA between the two groups (treated and the control). The stars show a significant difference.

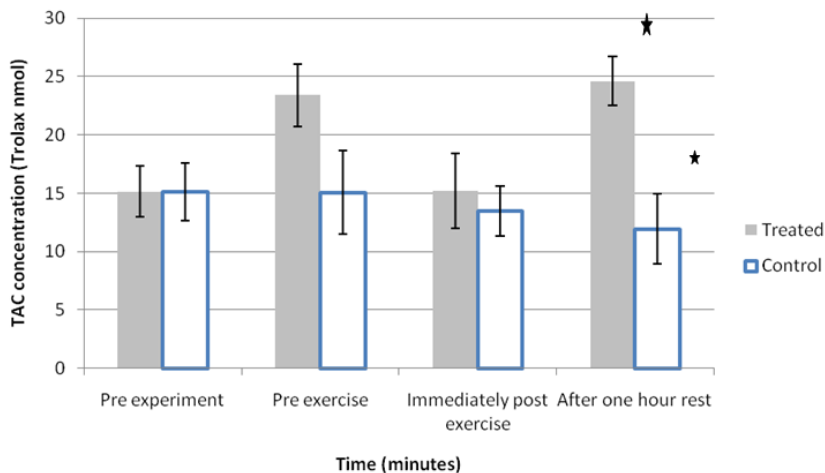


Figure 3. The differences in the concentration of TAC between the two groups (treated and the control). The stars show a significant difference.

Table 2. The comparison of Vo_2 max between the case and the control group, before and after the exercise.

Treated group		Placebo group	
Before the exercise	After the exercise	Before the exercise	After the exercise
36.07±9.30	20.44±9.35	32.38±6.75	21.95±15.12

Data is presented as mean ±SD.

min session of flat treadmill running (Maughan et al., 1989; Kanter et al., 1988). Different studies by using various forms of exercise have reported significant increase in plasma levels of malondialdehyde (Bryant et al., 2003; Ramel et al., 2004; Rodriguez et al., 2003). Also, generation of reactive oxygen species has been found to be increased after exhaustive aerobic and isometric exercise (Alessio et al., 2000). However, changes in MDA due to the intense exercise are not reported consistently (Kelley et al., 2006). There are several studies suggesting that regular aerobic exercise has the potential to lower the concentrations of inflammatory biomarkers including CRP in individuals with conditions associated with elevated inflammation (Andersson et al., 2010; Lakka et al., 2005; King et al., 2003). Our study has shown elevated CRP level after an intense exhaustive exercise in young untrained individuals with no sign of inflammation. This shows that compared with mild and regular aerobic exercise, intense exhaustive physical training especially in unaccustomed subjects can result in the elevation of inflammatory markers. Several studies show that the more intense the exercise the more production of reactive oxygen species and the oxidative stress (Chevion et al., 2003).

The result of some studies confirmed that a correlation exists between Vo_2 and oxidative stress (Chevion et al., 2003). However, other findings show that intense aerobic exercise does not increase the markers of oxidative stress (Chevion et al., 2003). These contradictory results could be explained by antioxidant nutritional status. It was also found that trained subjects can exhibit oxidative stress as well as unaccustomed subjects (Pincemail et al., 2000; Palazzetti et al., 2003). Potential mechanisms of increased generation of reactive oxygen species include the leakage of electron from electron transport chain during mitochondrial respiration and subsequent production of superoxide anions, activity of xanthine oxidase in the catabolism of nucleoproteins pathway, autooxidation of catecholamines or the activity of NAD(P)H oxidase (Sen et al., 2000).

The results of our study showed that, the serum MDA and CRP levels were not changed in the group taking the antioxidant N-acetyl cysteine, as compared to the control group. This is in line with the result from an investigation which shows that administration of N-Acetyl cysteine (1200 mg), for 8 days and in two doses has reduced malondialdehyde significantly (Zembron-Lacny et al., 2009). The results of previous study indicated that NAC

at 1,200 mg daily can reduce oxidative stress from short exhaustive exercise. However, this still needs more proof because low levels of free radicals that produced in mitochondria are very important for normal muscle function, and they increase more with stronger force (Wilmore et al., 2008). The *in vivo* and *in vitro* studies have shown that the synergic consumption of N-acetyl-cysteine and antioxidants such as vitamin C and vitamin E might act as pro-oxidant by inhibiting the defense mechanism or adaptive response from exercise induced free radicals (Ristow et al., 2009). Also, some studies concluded that production of low concentration of free-radicals during a moderate bout of exercise, in fact induces the expression of intra cellular antioxidant enzymes including super oxide dismutase, catalase and glutathione peroxidase as a defense mechanism (Carmen et al., 2008). However, in many other investigations N-acetyl-cysteine has been used as an effective antioxidant (Wilmore et al., 2008; Waris and Ahsan, 2006). In our study, the blood total antioxidant capacity of the subjects in the control group decreased significantly. However, it was elevated in the treated group during the experiment. This is in agreement with the results from some studies but not all (Peake et al., 2007). The result of an investigation has suggested that even with short bouts of high-intensity exercise, NAC is effective at promoting a positive redox balance within the cell (Peake et al., 2007). This supports our results regarding the benefits of using NAC orally during short sessions of high-intensity exercise. Recently, it was reported that supplementation with vitamin C and NAC together increases oxidative stress in humans after an acute muscle injury induced by eccentric exercise (Childs et al., 2001). In our study, we used pure NAC (without vitamin C) to examine the impact of NAC alone on oxidative stress. It was found that the total antioxidant capacity decreased immediately after the exhaustive time in the treated group; this may be because of the antioxidant components used to quench the over production of harmful radicals at this particular time. This was supported by the investigation which shows that the antioxidant capacity may be temporarily reduced during and immediately post exercise (Teixeira, 2009; Kerksick and Willoughby, 2005; Steinberg et al., 2006). In our study, there was no change observed in the time to fatigue regarding the subjects treated with NAC as compared to the control. This is in agreement with the result reported that N-acetyl cysteine infusion alters blood redox status but not time to fatigue during intense exercise in humans

(Kerksick and Willoughby, 2005). However, the result from an investigation concluded that NAC may be helpful at delaying fatigue as well as the building of oxidative stress; they suggested that more systemic, translational research on humans needs to be conducted (Teixeira et al., 2009). The result from an investigation suggested that even with short sessions of high-intensity exercise, NAC is effective at promoting a positive redox balance within the cell (Massimo et al., 2004; Bloomer and Goldfarb, 2004). However, the result from a recent clinical trial has shown that, thiol-based antioxidant supplementation enhances GSH availability in skeletal muscle, but at the same time it could disrupt the skeletal muscle inflammatory response and repair capability, potentially because of a blunted activation of redox-sensitive signalling pathway (Michalilidis, 20013). Finally, we conclude that oral administration of N-acetyl cysteine starting from one day before a single session of exhaustive exercise program can significantly reduce the harmful effects of oxidative stress, but not time to fatigue. We suggest that total level of thiols which are highly altered by NAC and also the activity of antioxidant enzymes such as GPx, GR which are directly related to GSH be determined in future studies.

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