N-Acetylcysteine Supplementation and Oxidative Damage and Inflammatory Response After Eccentric Exercise

Luciano A. Silva, Paulo C.L. Silveira, Cléber A. Pinho, Talita Tuon, Felipe Dal Pizzol, and Ricardo A. Pinho

The objective of the study was to verify the effect of N-acetylcysteine (NAC) supplementation on parameters of oxidative damage and inflammatory response after high-intensity eccentric exercise (EE). 29 participants with a mean age of 21.3 ± 4 yr, weight of 74.5 ± 7.7 kg, and height of 177.2 ± 6.9 cm were selected and divided randomly into 3 groups: placebo (21 days; n = 8), NAC (21 days; n = 9), and NAC plus placebo (14 days; n = 8). Four participants withdrew from the study for personal reasons. 14 days after starting supplementation, the participants performed EE: 3 sets until exhaustion (elbow flexion and extension on the Scott bench, 80% 1RM). Blood samples were collected before and on the 2nd, 4th, and 7th day after EE. Muscle soreness (MS), lipoperoxidation, protein carbonylation, tumor-necrosis factor-α (TNF-α), and interleukin 10 (IL-10) were determined. Results showed a significant increase in MS in all the groups on the 2nd day after EE and a decrease in the following days. A significant increase was observed in malondialdehyde and carbonyl levels on the 4th and 7th days after EE in all groups. TNF-α increased significantly on the 2nd day after eccentric exercise and decreased in the following days irrespective of NAC supplementation; concentration of IL-10 increased significantly on the 4th day in all groups. Only the supplemented groups maintained high levels of IL-10 on the 7th day after EE. The results 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.

Keywords: free radical, physical exercise, antioxidant, muscle

High-intensity eccentric exercise increases the production of reactive oxygen species (ROS), altering the antioxidant defense system (Childs, Jacobs, Kaminski, Halliwell, & Leeuwenburgh, 2001; Goldfarb, Bloomer, & McKenzie, 2005). This can lead to oxidative stress and contribute to a decrease in performance, fatigue, muscle damage, and muscle soreness (Avery et al., 2003; Ji, 1996; McBride, Kraemer, Triplett, & Sebastianelli, 1998).
During the performance of eccentric physical exercise, neutrophils and macrophages migrate to and infiltrate the lesioned muscle tissue, activating proinflammatory cytokines and producing additional ROS (Childs et al., 2001; Goldfarb et al., 2005). ROS is also produced in other ways, however, such as activation of xanthine oxidase, production of NADPH oxidase, ischemia reperfusion, increase of phagocyte activity, protein breakdown, and excessive accumulation of calcium (Goldfarb et al., 2005).

Several studies have suggested that antioxidant supplementation decreases ROS production during physical exercise, as well as improving the body’s defense systems against the attack of free radicals (Avery et al., 2003; Bloomer, Goldfarb, McKenzie, You, & Nguyen, 2004; Ji, 1996). Therefore, although many studies have pointed to antioxidant supplementation as essential in fighting oxidative damage induced by eccentric physical exercise, it remains unclear which biological mechanisms are involved in this process and which supplementation model can improve the response of oxidative defense (Beaton, Allan, Tarnopolsky, Tiidus, & Phillips, 2002).

N-acetylcysteine (NAC) is a donor of antioxidant thiols used in clinical practice to facilitate glutathione biosynthesis, improving the enzymatic defense system, as well as decreasing the harmful effects of ROS (Pinho et al., 2005). NAC acts as a scavenger of ROS including hypochlorous acid, hydroxyl radical, and hydrogen peroxide (Aruoma, Halliwell, Hoey, & Butler 1989; Medved et al., 2004). In high doses, in case of homeostasis breakdown, or after reacting with transition metals, however, it can cause a cytotoxic effect and produce additional ROS such as superoxide, hydrogen peroxide, and hydroxyl radical (Halliwell & Gutteridge, 2007; Neal, Cooper, Gurer, & Ercal, 1998).

Thus, it is possible that antioxidant supplementation can reduce the oxidative responses and muscle soreness induced by eccentric exercise. The objective of the study was to verify the effect of NAC supplementation on the development of muscle soreness, parameters of oxidative damage, and inflammatory response after high-intensity eccentric exercise.

**Materials and Methods**

**Participants**

Twenty-nine healthy male volunteers and students of UNESC (Universidade do Extremo Sul Catarinense, Criciuma, Santa Catarina state, Brazil) with a mean age of 21.3 ± 4 years, weight of 74.5 ± 7.7 kg, and height of 177.2 ± 6.9 cm were selected. They were nonsmokers, did not take NAC or related supplements, had not participated in resistance training or any other form of structured exercise for at least 6 months, did not have a history of muscle lesion, and were not carriers of any disease that might compromise the results or be aggravated by physical exercise. All the participants were informed about the purpose of the study and the associated risks, and all of them gave written informed consent. Approval for this study was obtained from the ethics committee of UNESC.
Supplementation

Participants were randomly selected in a single-blind manner to receive either a placebo (capsule containing starch) or NAC supplementation (capsule containing 10 mg/kg body mass), without previous control of the diet. Volunteers received one capsule per day for 14 days before the eccentric-exercise (EE) protocol and for 7 days postexercise and were randomly allocated to three groups: placebo (21 days; \( n = 8 \)), NAC (21 days; \( n = 9 \)), and NAC plus placebo (14 days of NAC + 7 days of placebo; \( n = 8 \)). During the study, 4 participants withdrew from the groups for personal reasons. Participants were instructed to maintain their normal diet throughout the duration of the study and to take one capsule daily with their largest meal. Fourteen days after starting supplementation, the participants performed EE.

EE Protocol

EE of short duration and high intensity was performed 12 hr after the supplementation, with elbow flexion and extension on a Scott bench at an intensity of 80% of one maximum repetition—1RM (Bompa, 2001). The concentric phase of the exercise was performed with manual assistance from the instructor. The eccentric phase was performed for a duration of 6–8 s. Three sets of the exercises were performed, with 2-min rest intervals, until exhaustion. The 1RM test was performed 24 hr before the supplementation.

Blood Collection

Blood samples were collected before the exercise and on the second, fourth, and seventh days after the exercise. Blood (10 ml) was obtained from the cubital vein of the right arm and collected in Vacutainer tubes without additives. It was then processed and the serum separated, aliquotted, and immediately stored in a freezer at –80 °C for later analysis.

Measurements

Muscle-soreness levels were rated on a visual analog scale. Participants marked their subjective rating of muscle soreness from 0 (without pain) to 10 (extreme pain; Revill, Robinson, Rosen, & Hogg, 1976). As an indicator of lipid peroxidation, the formation of substances that react to the heating of thiobarbituric acid (malondialdehyde [MDA]) were measured spectrophotometrically (532 nm) and expressed as MDA equivalents (Draper & Hadley, 1990). Oxidative damage in proteins was measured by determining the carbonyl grouping based on the reaction with 2,4-dinitrophenylhydrazine. Carbonyl content was determined spectrophotometrically (370 nm) using a coefficient of 22,000 M (Levine et al., 1990). Tumor-necrosis factor-α (TNF-α) and interleukin 10 (IL-10) were determined by ELISA with commercially available kits (R&D Systems, Minneapolis, MN). The quantity of proteins in thiobarbituric acid–reactive species and carbonyl assays was measured using the technique of Lowry, Rosebough, Farr, and Randall (1951).
Statistical Treatment

Data were expressed as mean and standard error of the mean (SEM) and statistically analyzed using two-way analysis of variance (ANOVA), followed by the SNK post hoc test. The level of significance established for the test was $p < .05$. SAEG (General Statistical Analysis System) version 9.0 was used.

Results

The results showed a significant increase in muscle soreness in all the groups on the second day after EE and a significant decrease in those values in all the groups from the fourth day after EE (Table 1).

Oxidative damage to membrane lipids was evaluated by the formation of MDA, a byproduct of lipoperoxidation. The results show (Figure 1) a significant increase in MDA levels on the fourth and seventh days after the eccentric exercise, in all groups.

Table 1  Muscle Soreness in Young University Students After Muscle Lesion Induced by Eccentric Exercise

<table>
<thead>
<tr>
<th>Group</th>
<th>Muscle Soreness</th>
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<tbody>
<tr>
<td></td>
<td>Pre–eccentric exercise</td>
</tr>
<tr>
<td>Placebo</td>
<td>0</td>
</tr>
<tr>
<td>14 days of N-acetylcysteine</td>
<td>0</td>
</tr>
<tr>
<td>21 days of N-acetylcysteine</td>
<td>0</td>
</tr>
</tbody>
</table>

Note. Participants marked their subjective rating of muscle soreness on a scale from 0 (without pain) to 10 (extreme pain). The values are presented as $M ± SD$, and the difference used in relation to pre–eccentric exercise ($^*$) and in relation to Day 2 ($^+$) was significant at $p < .05$.

Figure 1 — Lipoperoxidation levels in the serum of young university students after muscle lesion induced by eccentric exercise. The values are presented as $M ± SEM$ and the results expressed in nmol of malondialdehyde (MDA)/mg of proteins. The difference used in relation to pre–eccentric exercise (EE; $*$) and in relation to placebo ($†$) was significant at $p < .05$. NAC = N-acetylcysteine.
To verify the oxidative damage in proteins, we evaluated the carbonyl groups based on the reaction with dinitrophenylhydrazine. Similar to lipoperoxidation, the results (Figure 2) also show a significant increase in PC on the fourth and seventh days after eccentric exercise in all groups, and NAC supplementation did not alter these results.

To quantify the inflammatory response, we measured tumor-necrosis factor-α (TNF-α) and interleukin 10 (IL-10). All the groups significantly increased TNF-α on the second day after eccentric exercise. The supplemented groups maintained high levels of TNF-α until the fourth day after EE, decreasing on the seventh day (Figure 3). Serum concentration of IL-10 increased significantly on the fourth day in all the groups. Only the supplemented groups maintained high levels of IL-10 on the seventh day after EE (Figure 4).

**Figure 2** — Carbonylation levels in the serum of young university students after muscle lesion induced by eccentric exercise. The values are presented as $M \pm SEM$ and the results expressed in nmol/mg of proteins. The difference used in relation to pre–eccentric exercise (EE; *) and in relation to placebo (†) was significant at $p < .05$. NAC = N-acetylcysteine.

**Figure 3** — Tumor-necrosis factor-α (TNF-α) in the serum of young university students after muscle lesion induced by eccentric exercise. The values are presented as $M \pm SEM$ and the results expressed in pg/ml of serum. The difference used in relation to pre–eccentric exercise (EE; *) and in relation to placebo (†) was significant at $p < .05$. NAC = N-acetylcysteine.
The purpose of the study was to verify the effect of NAC supplementation on the development of muscle soreness, parameters of oxidative damage, and inflammatory response after high-intensity eccentric exercise.

Initially, we verified the markers of muscle lesion. Several indicators have been used to determine the level of muscle lesion induced by EE, such as creatine kinase, myoglobin, and muscle soreness (Avery et al., 2003; Beaton et al., 2002; Lee et al., 2002). We used muscle soreness as the marker of cell damage. Exercise can induce microlesions in the active muscles, especially when it is relatively intense, of long duration, and includes eccentric contractions (Beaton et al., 2002). Clinically, this presents as muscle discomfort and pain in the stressed muscles, reaching a peak 24–48 hr after exercise (Armstrong, Warren, & Warren, 1991; Paschalis et al., 2006). Our results show a significant increase in muscle soreness on the second day after the EE in all the groups, and these values decreased in the following days irrespective of NAC supplementation (Table 1). Our results are in accordance with those of other studies that did not find a protective effect of antioxidants against muscle soreness (Beaton et al., 2002; Childs et al., 2001; Jamurtas et al., 2005; Shafat, Butter, Jensen, & Donnelly, 2004). It is possible that ROS has an important role in the etiology of skeletal-muscle damage and muscle soreness via oxidation of ion-transport systems, leading to disruption of Ca^{2+}-ion homeostasis, impaired mitochondrial respiratory control, distortions in signal-transduction pathways, and, ultimately, cell dysfunction (Kourie, 1998). Therefore, we believed that quenching of ROS by NAC could protect against muscle soreness caused by eccentric exercise.

To assess the oxidative damage we measured MDA levels (Figure 2) and protein carbonylation (Figure 3). Contrary to what we expected, the results showed a significant increase in the level of lipoperoxidation and protein carbonylation on the fourth and seventh days after EE in all supplemented groups. It is possible that those results are related to the presence of iron in the serum. The EE increased
inflammation and the level of free iron (Childs et al., 2001). According to Pinho et al. (2005), the use of NAC alone might have limitations and present pro-oxidant effects, because of the facility with which it interacts with iron. This mechanism can lead to the formation of additional ROS and an increase in oxidative damage.

We also observed that NAC supplementation did not decrease protein carbonylation after EE. These results can be related to phagocytic cells migrating to the tissue after muscle injury (Goldfarb et al., 2005; Stupka et al., 2000).

Previous studies have shown the role of antioxidants in EE-induced cytokine production (Petersen et al., 2001; Toft et al., 2002). Those studies showed that proinflammatory cytokines increase progressively after EE. The cytokine response to exercise represented a reaction to exercise-induced muscle injury and inflammation (Vassilakopoulos et al., 2003).

TNF-α consists of the early-response, proinflammatory cytokines that are most likely synthesized by resident macrophages and local postcapillary vascular endothelium, with synthesis occurring rapidly after the onset of injury or infection (Cavaillon, 1994). Changes in serum TNF-α have been reported after a bout of strenuous exercise (Ostrowski, Rohde, Schjerling, & Pedersen, 1999; Paschalis et al., 2006). Here, we demonstrate that TNF-α serum concentrations (Figure 3) increased significantly on the second day after EE, decreasing in the following days irrespective of NAC supplementation.

The increase in TNF-α on the second and fourth days after EE in supplemented groups could be related to muscle damage. TNF-α might accumulate in the cell or be released, suggesting a dissociation between intracellular TNF-α production and release (Smith et al., 2000). It is possible that the decreases on the fourth and seventh days after EE were caused by rapid clearance or an undetectable accumulation at the site of injury (Cavaillon, 1994).

We also observed that serum concentration of IL-10 increased significantly on the fourth day after EE in all groups and on the seventh day only in the groups supplemented with NAC (Figure 4).

IL-10 is a primary anti-inflammatory cytokine that acts by inhibiting proinflammatory cytokine production by activated monocytes and macrophages (Ostrowski et al., 1999). Studies have shown a significant increase immediately after intense exercise (MacIntyre, Sorichter, Mair, Berg, & McKenzie, 2001; Ostrowski et al.; Smith et al., 2000), although it is not clear why elevations were observed at different times. In the current study, however, this time period would fit well with the period of resolution of an acute inflammatory response (Smith et al., 2000) and might, in part, be responsible for the significant reduction in proinflammatory TNF-α during those periods.

It is possible that the mechanism by which NAC stimulates the production of anti-inflammatory cytokines on the fourth and seventh days after EE can be related to the inhibition of ROS production with a concomitant decrease in NF-kB activation and expression of cytokine-induced neutrophil chemoattractant (Blackwell, Blackwell, Holden, Christman, & Christman, 1996). In models of preexisting inflammation, it has been shown that NAC can also modulate phagocytic activity by suppressing oxidative burst and by potentiating host defense (Stolarek, Bialasiewicz, & Nowak, 2002; Villagrasa et al., 1997).
In conclusion, the results of this study suggest that treatment with NAC does not alter oxidative damage caused by EE (lipoperoxidation and protein carbonylation). It seems that NAC has some anti-inflammatory effects and acts on the down-regulation of proinflammatory cytokines. We suggest further studies to clarify the effects of NAC supplementation on pro- and anti-inflammatory cytokines.

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References


