**N-acetylcysteine enhances muscle cysteine and glutathione availability and attenuates fatigue during prolonged exercise in endurance-trained individuals**

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Medved, I., M. J. Brown, A. R. Bjorksten, K. T. Murphy, A. C. Petersen, S. Sostaric, X. Gong, and M. J. McKenna. N-acetylcysteine enhances muscle cysteine and glutathione availability and attenuates fatigue during prolonged exercise in endurance-trained individuals. J Appl Physiol 97: 1477–1485, 2004. First published June 11, 2004; 10.1152/japplphysiol.00371.2004.—The production of reactive oxygen species in skeletal muscle is linked with muscle fatigue. This study investigated the effects of the antioxidant compound N-acetylcysteine (NAC) on muscle cysteine, cysteine, and glutathione and on time to fatigue during prolonged, submaximal exercise in endurance athletes. Eight men completed a double-blind, crossover study, receiving NAC or placebo before and during cycling for 45 min at 71% peak oxygen consumption (V˙O2peak) and then to fatigue at 92% V˙O2peak. NAC was intravenously infused at 125 mg·kg−1·h−1 for 15 min and then at 25 mg·kg−1·h−1 for 20 min before and throughout exercise. Arterialized venous blood was analyzed for NAC, glutathione status, and cysteine concentration. A vastus lateralis biopsy was taken preinfusion, at 45 min of exercise, and at fatigue and was analyzed for NAC, total glutathione (TGSH), reduced glutathione (GSH), cysteine, and cysteine. Time to fatigue at 92% V˙O2peak was reproducible in preliminary trials (coefficient of variation 5.6 ± 0.6%) and with NAC was enhanced by 26.3 ± 9.1% (NAC 6.4 ± 0.6 min vs. Con 5.3 ± 0.7 min; P < 0.05). NAC increased muscle total and reduced NAC at both 45 min and fatigue (P < 0.005). Muscle cysteine and cysteine were unchanged during Con, but were elevated above preinfusion levels with NAC (P < 0.001). Muscle TGSH (P < 0.05) declined and muscle GSH tended to decline (P = 0.06) during exercise. Both were greater with NAC (P < 0.05). Neither exercise nor NAC affected whole blood TGSH. Whereas blood GSH was decreased and calculated oxidized glutathione increased with exercise (P < 0.05), both were unaffected by NAC. In conclusion, NAC improved performance in well-trained individuals, with increased muscle cysteine and GSH availability a likely mechanism.

reactive oxygen species; antioxidants; muscle fatigue; cysteine; cystine

**IN SKELETAL MUSCLE, AN INTEGRATED SYSTEM OF ENDOGENOUS ANTIOXIDANTS AND PROTEIN AND NONPROTEIN THIOL COMPOUNDS, INCLUDING SUPEROXIDE DISMUTASE, GLUTATHIONE PEROXIDASE, CATALASE, AND GLUTATHIONE, MINIMIZES THE ACCUMULATION OF REACTIVE OXYGEN SPECIES (ROS; REF. 37). HOWEVER, THIS ANTIOXIDANT CAPACITY IS SMALL AND IS OVERWHELMED DURING EXERCISE, RESULTING IN INCREASED ROS (3, 10, 17). INCREASED ROS PRODUCTION ACCELERATES MUSCLE FATIGUE IN RAT (33) AND CANINE DIAPHRAGM (29, 43) AND MOUSE LIMB SKELETAL MUSCLE (5). THEREFORE, ENHANCING THE SKELETAL MUSCLE ANTIOXIDANT CAPACITY MAY BE BENEFICIAL FOR MUSCLE PERFORMANCE.**

N-acetylcysteine (NAC), a thiol-containing compound, attenuates fatigue in rabbit and rat diaphragm (12, 42, 44). Furthermore, NAC infusion attenuated fatigue of human muscle, during both low-frequency electrical stimulation of the tibialis anterior muscle (34) and inspiratory resistive loading of the diaphragm (46). Our laboratory developed an NAC infusion model for use during voluntary whole body exercise in humans, finding no effect on intense, intermittent exercise performance (26). However, during prolonged, submaximal exercise, time to fatigue was greater in three well-trained individuals during NAC infusion, with performance change induced by NAC correlated to peak oxygen consumption (V˙O2peak) (27). This suggested a performance-enhancing effect of NAC, but the limited sample size precluded definitive conclusions. Therefore, the first hypothesis tested in this study was that NAC infusion would increase time to fatigue during prolonged, submaximal exercise performance in a homogenous group of well-trained individuals.

The mechanism(s) underlying this potential ergogenic effect during prolonged exercise was not studied (27) but also deserves investigation. The first problem is that it is not known whether NAC crosses the sarcolemma and thereby directly affects muscle ROS or redox status during exercise. Our laboratory found that NAC infusion increased red blood cell (RBC) NAC concentration ([NAC]) during exercise (26, 27), suggesting that NAC might also enter myocytes, but there are no reports of muscle NAC. Thus the second hypothesis tested was that NAC infusion would increase NAC content in both skeletal muscle and RBCs during prolonged exercise.

NAC potentially reduces the deleterious effects of ROS by direct scavenging of ROS (2) and/or supplying cysteine (Cys) for enhanced glutathione synthesis (9). Our laboratory has demonstrated that NAC maintained blood redox status during high-intensity, intermittent exercise, indicated by an attenuated decline in reduced glutathione (GSH) and rise in oxidized glutathione (GSSG; Ref. 26). It is, therefore, possible that...
muscle ROS and glutathione status might also be protected by NAC during prolonged exercise, but these effects of NAC in human muscle are unknown.

A complicating factor is that the reported effects of exercise on human skeletal muscle glutathione are scarce and their findings conflicting, with reports of GSH being unchanged (8) or decreased (45) and of GSSG being increased (8) or unchanged (35). A critical role is suggested for endogenous GSH in alleviating exercise-induced oxidative stress and affecting exercise performance (38). In rats, exogenous glutathione administration enhanced glutathione synthesis and increased swim performance by up to 141% (7, 30), whereas glutathione deficiency reduced endurance time by ~50% (38).

Cys is a precursor to glutathione synthesis, and increased intracellular Cys availability enhanced intracellular glutathione (9, 16, 41). Supplementation with a Cys donor increased performance during sprint cycling (22), suggesting that enhanced glutathione synthesis can augment performance in humans. No studies have comprehensively investigated the effects of prolonged exercise or NAC infusion on muscle total glutathione (TGS), GSH, Cys, and cystine. We therefore also tested the hypotheses that fatiguing exercise would decrease muscle GSH and increase GSSG and that each of TGS, GSH, and Cys would be augmented by NAC infusion.

METHODS

Subjects

Eight healthy men (age, 27.1 ± 5.6 yr; body mass, 76.7 ± 10.9 kg; height, 180.3 ± 5.4 cm; means ± SD) volunteered for the study after being informed of all risks and giving written, informed consent. The subjects were endurance trained, completing either running or cycling activity, four to five times per week for 1–2 h, for a minimum of 2 yr. Subjects refrained from vigorous activity and avoided ingesting caffeine, alcohol, or other drugs and also consumed standard food packages for 24 h before their two experimental trials. Ethical approval was obtained from the Victoria University of Technology Human Research Ethics Committee.

Exercise Trials

Overview. Subjects attended the laboratory on six separate occasions, separated by a 7-day period. All exercise trials were completed on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands). Subjects first completed an incremental exercise test to determine their VO2 peak, with all equipment, calibration, and procedures as previously detailed (24, 26).

Prolonged, submaximal exercise. An identical exercise protocol was used as previously described (27), with subjects cycling at 70% VO2 peak for 45 min and then to volitional fatigue at 90% VO2 peak (27).

Experimental trials. The two experimental prolonged, submaximal exercise trials were conducted in a double-blind, randomized, crossover design, to determine the effects of NAC (Parvolex, Faulding Pharmaceuticals) or saline [control (Con)] infusion on exercise performance and on muscle and blood thiols (26, 27). For ethical reasons, the attending medical practitioner was nonblinded. To prevent possible unblinding of experimenters due to the NAC odor, all ampules and syringes containing NAC and saline were handled and sealed in a room separate to the laboratory. The medical practitioner also removed the cannulas after the experiment.

NAC Infusion

The NAC intravenous infusion protocol comprised an initial loading dose of 125 mg·kg−1·h−1 for 15 min to increase plasma [NAC], followed by a constant infusion of 25 mg·kg−1·h−1 to achieve a plateau in [NAC], with exercise commencing after 20 min of constant infusion (26, 27). NAC infusion was continued throughout exercise until fatigue, and any adverse reactions were assessed according to the scale previously detailed (27).

Blood Processing and Analyses

A 20-gauge catheter was inserted into a dorsal hand vein for arterialized venous blood sampling and a 22-gauge catheter was inserted into a superficial median forearm vein for infusion of either NAC or saline. Arterialized venous blood was sampled from a dorsal hand vein at 13) at 0, 1, 2, 5, 10, 15, 25, and 35 min during the preexercise infusion period. Further samples were taken at 15, 30, and 45 min and at fatigue during exercise and during recovery at 1, 2, 5, 10, and 30 min. A 5-ml sample was used for measurement of reduced and total thiols in blood and plasma, with all processing and analyses as previously detailed (26, 27). Blood and plasma thiol concentrations, including NAC, total glutathione (TGS), GSH, and Cys, were analyzed by high-pressure liquid chromatography (HPLC; Waters Associates, Milford, MA), with fluorescence detection (Hitachi, Tokyo, Japan). Because of laboratory freezer failure, for three subjects whole blood total and reduced cysteine could not be measured and consequently cystine and RBC concentrations could not be calculated.

Muscle Biopsy Sampling and Analyses

Muscle biopsy sampling. After injection of a local anesthetic (1% Xylocaine) into the skin and fascia, three small incisions were made in the midportion of the vastus lateralis muscle. Muscle samples were taken preinfusion, after 45 min of exercise, and at fatigue and analyzed for muscle thiols, including NAC, glutathione, and Cys. The subject’s contralateral leg was biopsied on their second experimental trial. The time taken for the subject to stop pedaling at 45 min, undergo a muscle biopsy and recommence cycling did not differ between trials (Con 55 ± 7 s vs. NAC 53 ± 7 s).

Muscle thiol analyses. The muscle sample was immediately blotted on filter paper, frozen in liquid nitrogen, and stored at −80°C for later analysis of muscle thiols. Approximately 20 mg of frozen muscle were homogenized for 20 s in 200 ml of 20 mM monobromobimane by using a handheld homogenizer (Omni 1000, Omni International, Gainesville, VA). For analysis of total thiols, 100 ml of homogenate were added to 200 ml of 4 mM diithiothreitol in an Eppendorf tube. The sample was immediately vortexed and left at room temperature for 10 min to reduce oxidized thiols. Free thiols were derivatized with 50 ml of 20 mM monobromobimane, vortexed, and incubated at room temperature in darkness for 10 min. Proteins were precipitated with 25 ml of sulfosalicylic acid (50% wt/vol) and immediately vortexed and centrifuged at 1,000 g for 5 min. The supernatant (50 ml) was injected into the HPLC for analysis. For reduced thiols, 100 ml of muscle homogenate was mixed with 250 ml of H2O and 25 ml of sulfosalicylic acid (50% wt/vol) in an Eppendorf tube, immediately vortexed, and centrifuged at 1,000 g for 5 min, before injection of 50 ml of the supernatant into the HPLC for analysis. The HPLC method and conditions were identical to those used for plasma and blood thiols analyses (26).

The HPLC mobile phase was methanol (18.82 vol/vol) and 20 mM KH2PO4 at pH 2.9 and 5 mM octanesulfonic acid running through a 150 by 3.9-mm NovaPak C18 column (Waters Associates) at 1 ml/min, with fluorescence detection at 400-nm excitation and 475-nm emission. This gives baseline separation of thiol compounds from each other and the reagent peaks, with a quantitation limit of ~100 nM and coefficient of determination of <5% for each. Total concentrations of the thiols were determined in a similar manner except that the oxidized thiols were reduced with diithiothreitol before a second derivitization with monobromobimane was performed. The supernatant was then extracted and injected for HPLC analysis.
Thiol concentrations in RBCs were calculated as previously described (26, 27). Hct could not be measured in one subject because of technical difficulties, and consequently RBC NAC data are reported for seven subjects. Individual coefficients of variation (CV) for time to fatigue were calculated for all subjects within the exercise protocol and averaged to obtain an overall CV (18). This allowed clearer delineation of NAC effects from typical test variation.

Statistical Analyses

All data are presented as means ± SE, except anthropometric data. Single comparisons (e.g., time to fatigue) were analyzed by using a paired Student’s t-test. A one-way ANOVA with repeated measures was used for blood and plasma [NAC]. All other blood and muscle analyses were analyzed by using a two-way (treatment, time) ANOVA with repeated measures on both factors. Post hoc analyses were conducted by using the Student-Newman-Kuels test. Significance was accepted at P < 0.05.

RESULTS

Exercise Performance Variability and Effects of NAC

The subjects’ $\dot{V}O_2$ peak was 65.6 ± 2.2 ml·kg$^{-1}$·min$^{-1}$, and their submaximal work rates were 239 ± 20 and 336 ± 25 W, corresponding to 71 ± 1.3 and 92 ± 1.9% $\dot{V}O_2$ peak, respectively. Time to fatigue at 92% $\dot{V}O_2$ peak was reproducible during the two variability trials (CV 5.6 ± 0.6%, Table 1), and no trial order effect was observed (data not shown). NAC increased, by 26.3 ± 9.1% (P < 0.05), time to fatigue at 92% $\dot{V}O_2$ peak (Con 5.3 ± 0.7% vs. NAC 6.4 ± 0.6 min) and thus also work done (Con 104.9 ± 15.3 KJ vs. NAC 126.5 ± 11.6 KJ).

NAC and Adverse Reactions

Reactions. No moderate or severe adverse reactions to NAC were observed during the preinfusion, exercise, or recovery periods (Table 2).

Plasma, blood, and RBC NAC. The [NAC] measured in whole blood and plasma, and calculated for RBC is shown in Fig. 1. During the 15-min loading infusion phase, total plasma [NAC] increased progressively until a peak of 305.2 ± 38.6 mg/l at 15 min (P < 0.005), decreased during the maintenance infusion phase (P < 0.005) to 214.7 ± 17.5 mg/l immediately before exercise, and then plateaued, with no further changes during exercise. In recovery, total plasma [NAC] decreased rapidly from fatigue levels but remained higher than preinfusion at 30 min postinfusion (P < 0.05). A similar pattern of change was found for reduced plasma [NAC] (Fig. 1). An identical pattern of change was found for both total and reduced forms of NAC in whole blood during the loading,

Table 1. Individual time to fatigue during preexperimental prolonged, submaximal exercise trials

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Variability trial 1</th>
<th>Variability trial 2</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.32</td>
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<td>2</td>
<td>4.94</td>
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<td>3</td>
<td>6.06</td>
<td>5.62</td>
<td>5.3</td>
</tr>
<tr>
<td>4</td>
<td>5.05</td>
<td>4.67</td>
<td>5.5</td>
</tr>
<tr>
<td>5</td>
<td>3.25</td>
<td>3.56</td>
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<td>6</td>
<td>4.15</td>
<td>4.58</td>
<td>7.0</td>
</tr>
<tr>
<td>7</td>
<td>5.06</td>
<td>5.21</td>
<td>2.1</td>
</tr>
<tr>
<td>8</td>
<td>4.79</td>
<td>5.13</td>
<td>4.9</td>
</tr>
</tbody>
</table>
Mean ± SE    | 4.8 ± 0.3           | 5.0 ± 0.3           | 5.6 ± 0.6 |

Each exercise trial comprised 45 min at 71% peak oxygen consumption and then 92% peak oxygen consumption continued to fatigue. Time to fatigue at 92% peak oxygen consumption was used as an index of performance. CV, coefficient of variation.

Table 2. Lack of severe or moderate adverse reactions with N-acetylcysteine or saline infusion before and during prolonged, submaximal exercise

<table>
<thead>
<tr>
<th>Reaction Frequency and Severity</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction</td>
<td>NAC</td>
<td>Con</td>
<td>NAC</td>
<td>Con</td>
</tr>
<tr>
<td>Erythema</td>
<td>6</td>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Swelling</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Flushing</td>
<td>6</td>
<td>7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Coughing</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sweating</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Ichy skin</td>
<td>7</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Results are from 8 subjects. NAC, N-acetylcysteine; Con, saline (control).
maintenance, exercise, and recovery periods. RBC NAC also followed a similar pattern during exercise and recovery periods being 38.9 and 23.8 mg/l during exercise for total and reduced forms, respectively. (Fig. 1).

Muscle NAC. NAC was not detected in muscle at preinfusion or during Con trials. During NAC infusion, muscle total and reduced NAC were elevated at 45 min and at fatigue, with 72.8 ± 7.3 and 68.7 ± 6.3% present in the reduced form, respectively (Fig. 2).

Muscle Thiols

Cysteine and cystine. Muscle total and reduced Cys as well as cystine were unchanged from preinfusion levels during Con and were not different between NAC and Con before infusion (Fig. 3). However, NAC markedly increased both total and reduced Cys and cystine in muscle compared with preinfusion levels (P < 0.001). Muscle total and reduced Cys and cystine were greater at 45 min and fatigue with NAC than in Con (P < 0.005; Fig. 3).

Glutathione. Muscle TGSH was decreased by 37.7 ± 17.9% at 45 min of exercise and remained depressed at fatigue (Fig. 4; P < 0.05). Exercise tended to decrease muscle GSH (P = 0.06), whereas muscle calculated GSSG (cGSSG) was unaltered with exercise (Fig. 4). Both muscle TGSH and GSH were higher during NAC compared with Con (P < 0.05; Fig. 4). No change in muscle GSH-to-TGSH, GSSG-to-TGSH, or GSSG-to-GSH ratios were found with either exercise or NAC (data not shown).

Blood Thiols

Cysteine and cystine. No change in total and reduced Cys concentration ([Cys]) or cystine from preinfusion levels occurred in Con at any time (Tables 3 and 4). Before infusion, no differences were found between NAC and Con in whole blood, plasma, or RBC for total and reduced cysteine ([Cys]), Table 3 or cystine (Table 4). However, NAC increased total and reduced [Cys] and cystine in whole blood, plasma, and RBCs by up to threefold above preinfusion levels (P < 0.005). Consequently, NAC increased total and reduced [Cys] and cystine in whole blood, plasma and RBC immediately before and throughout the exercise and recovery periods (P < 0.005).

Glutathione. In contrast to muscle, whole blood TGSH concentration ([TGSH]) was unchanged from preexercise infusion, at any time during exercise and recovery (Fig. 5). Whole blood GSH concentration ([GSH]) was also unchanged during the preexercise infusion, but [GSH] had declined at 15 min during exercise (P < 0.05) and remained lower during the subsequent exercise and recovery periods (P < 0.05). Whole blood cGSSG concentration ([cGSSG]) was unchanged during preinfusion, increased during exercise, and remained elevated above preinfusion levels at 30 min of recovery (P < 0.05; Fig.
No differences between treatments were found for blood TGSH, GSH, or cGSSG (Fig. 5).

The whole blood GSH-to-TGSH ratio (data not shown) did not change during the preinfusion period, but it decreased at 15 min exercise and remained lower throughout the remainder of exercise and throughout the recovery period compared with preinfusion (P < 0.05). No differences in the GSH-to-TGSH ratio were observed at any time between Con or NAC.

**Plasma Glutathione**

Plasma glutathione levels were too low to be reliably detected consistent with other studies (14, 26).

**DISCUSSION**

This study provides several novel findings in regard to muscle glutathione and performance in humans. First, we observed a dramatic enhancement of submaximal exercise performance with NAC infusion, with time to fatigue increased by 26%. Second, an increase in NAC was detected in human skeletal muscle with NAC infusion. We also report for the first time the effects of NAC infusion on whole blood TGSH, GSH, and Cys during prolonged exercise in endurance-trained individuals. Finally, we provide a potential mechanism for this ergogenic effect, with an increased muscle Cys, TGSH, and GSH availability observed with NAC.

**Increased Muscle NAC and Increased Muscle Performance**

Our data clearly demonstrate that NAC infusion substantially enhanced performance in well-trained individuals, with a 26% increase in time to fatigue during prolonged exercise. This finding confirms our earlier preliminary observation based on only a few athletes (27) and provides the first evidence of attenuated fatigue by NAC during voluntary, whole body exercise in humans. We show, for the first time, that NAC infusion increased NAC content in skeletal muscle. It is probable that a small portion of this NAC detected in muscle was due to contamination by blood. However, given the magnitude of increase in muscle NAC, it is highly likely that muscle intracellular [NAC] was elevated. Furthermore, we demonstrate here and in previous studies (26, 27) that NAC infusion increases RBC [NAC]. Together these indicate that an elevation in muscle intracellular NAC is highly likely.

**Muscle Cys, Glutathione, and Exercise**

Because the proportion of oxidized to total muscle glutathione was ~15%, it is possible that some ex vivo oxidation may have occurred, perhaps due to the small delay in freezing muscle samples. However, this is an inevitable limitation when obtaining muscle samples via needle biopsy, and our proportion of muscle GSSG to TGSSG is consistent with others (45). We show that muscle TGSH content decreased during submaximal cycling exercise, which differs from other studies (35, 45). The reason for this discrepancy is unclear, but it may be related to the fact that our subjects were more highly trained than subjects used in previous studies (35, 45). However, our findings of a tendency for a decrease in muscle GSH (P = 0.06) and no change in muscle GSSG during submaximal exercise are in agreement with others (35, 45). More importantly, we demonstrate for the first time that NAC was able to increase both muscle TGSH and GSH during whole body exercise. The maintenance of GSH is dependent on regeneration from GSSG by glutathione reductase (40). However, this seems unlikely as a mechanism responsible for the elevation in GSH, because there was no change in muscle GSSG during submaximal cycling exercise in agreement with others (35, 45). More importantly, we demonstrate for the first time that NAC was able to increase both muscle TGSH and GSH during whole body exercise. The maintenance of GSH is dependent on regeneration from GSSG by glutathione reductase (40). However, this seems unlikely as a mechanism responsible for the elevation in GSH, because there was no change in muscle GSSG during exercise or NAC, therefore implicating another mechanism. This was probably due to increased availability of the glutathione precursor, Cys, as evidenced by increases in each of muscle, RBC, and plasma.

Cys can be actively transported into cells (4) and increased intracellular Cys availability enhances intracellular GSH (40). Therefore, the increased extracellular Cys consequent to NAC infusion increases the intracellular availability of this amino acid. This increased availability of Cys is consistent with an increased muscle TGSH and GSH content observed with NAC.
Table 3. Total and reduced cysteine concentrations in whole blood, plasma, and erythrocytes during prolonged, submaximal exercise before, during, and after NAC and placebo infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Preinfusion</th>
<th>Preexercise</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>Fatigue</th>
<th>Recovery 30 min</th>
</tr>
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<tbody>
<tr>
<td>Blood total</td>
<td>Con</td>
<td>37.85 ± 2.25</td>
<td>36.61 ± 3.72</td>
<td>41.38 ± 5.02</td>
<td>38.56 ± 4.88</td>
<td>37.71 ± 3.48</td>
<td>41.75 ± 2.30</td>
<td>35.89 ± 3.41</td>
</tr>
<tr>
<td></td>
<td>NAC</td>
<td>29.80 ± 3.58</td>
<td>138.08 ± 19.41†</td>
<td>139.24 ± 17.31†</td>
<td>134.93 ± 17.66†</td>
<td>138.55 ± 17.98†</td>
<td>148.01 ± 25.75†</td>
<td>74.08 ± 6.42†</td>
</tr>
<tr>
<td>Blood reduced</td>
<td>Con</td>
<td>8.37 ± 1.79</td>
<td>9.26 ± 1.46</td>
<td>9.21 ± 1.28</td>
<td>10.74 ± 1.21</td>
<td>10.38 ± 1.18</td>
<td>10.10 ± 0.95</td>
<td>9.40 ± 2.05</td>
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<tr>
<td></td>
<td>NAC</td>
<td>7.12 ± 0.57</td>
<td>51.34 ± 6.15†</td>
<td>45.50 ± 3.83†</td>
<td>44.64 ± 3.89†</td>
<td>49.32 ± 5.43†</td>
<td>41.57 ± 2.60†</td>
<td>31.49 ± 6.77†</td>
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<tr>
<td>Plasma total</td>
<td>Con</td>
<td>51.33 ± 2.65</td>
<td>49.41 ± 4.52</td>
<td>55.14 ± 4.66</td>
<td>53.25 ± 3.32</td>
<td>49.54 ± 3.66</td>
<td>59.31 ± 5.09</td>
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<tr>
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<td>NAC</td>
<td>44.97 ± 8.00</td>
<td>204.93 ± 24.52†</td>
<td>195.13 ± 39.04†</td>
<td>181.98 ± 26.83†</td>
<td>199.26 ± 27.96†</td>
<td>175.00 ± 24.99†</td>
<td>116.02 ± 12.54†</td>
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<tr>
<td>Plasma reduced</td>
<td>Con</td>
<td>6.59 ± 0.80</td>
<td>8.98 ± 0.62</td>
<td>10.93 ± 0.97</td>
<td>12.69 ± 1.49</td>
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<td>NAC</td>
<td>7.73 ± 1.22</td>
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<td>74.81 ± 6.16†</td>
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<tr>
<td>RBC total</td>
<td>Con</td>
<td>22.78 ± 3.18</td>
<td>21.93 ± 4.20</td>
<td>25.36 ± 6.13</td>
<td>21.21 ± 4.44</td>
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<td>30.09 ± 5.43†</td>
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<td>RBC reduced</td>
<td>Con</td>
<td>11.17 ± 3.39</td>
<td>9.83 ± 3.51</td>
<td>6.56 ± 2.99</td>
<td>7.34 ± 2.59</td>
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<td>16.13 ± 9.73†</td>
<td>19.30 ± 7.90†</td>
<td>14.59 ± 4.90†</td>
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<td>5.18 ± 9.90†</td>
</tr>
</tbody>
</table>

Values are means ± SE (in μmol/l); n = 8 subjects for total and reduced plasma and n = 5 subjects for total and reduced whole blood and red blood cells (RBC). [Cys], cysteine concentration. *Significant interaction effect greater than preinfusion, P < 0.005. †Significant interaction effect NAC > Con (P < 0.005).

Table 4. Calculated cystine concentrations in whole blood and plasma during prolonged, submaximal exercise during, and after NAC and placebo infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Preinfusion</th>
<th>Preexercise</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>Fatigue</th>
<th>Recovery 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>Con</td>
<td>29.07 ± 2.90</td>
<td>27.32 ± 2.56</td>
<td>32.17 ± 4.38</td>
<td>28.36 ± 3.67</td>
<td>27.32 ± 1.96</td>
<td>31.65 ± 2.11</td>
<td>26.49 ± 3.87</td>
</tr>
<tr>
<td></td>
<td>NAC</td>
<td>22.68 ± 3.05</td>
<td>86.74 ± 15.48†</td>
<td>94.41 ± 21.00†</td>
<td>90.28 ± 16.22†</td>
<td>89.24 ± 15.23†</td>
<td>106.44 ± 25.89†</td>
<td>42.60 ± 9.51†</td>
</tr>
<tr>
<td>Plasma</td>
<td>Con</td>
<td>44.73 ± 2.52</td>
<td>40.50 ± 4.33</td>
<td>44.21 ± 4.24</td>
<td>39.99 ± 2.82</td>
<td>38.50 ± 2.91</td>
<td>47.54 ± 4.35</td>
<td>44.97 ± 4.84</td>
</tr>
<tr>
<td></td>
<td>NAC</td>
<td>36.70 ± 6.84</td>
<td>129.86 ± 19.99†</td>
<td>131.52 ± 31.69†</td>
<td>170.09 ± 24.47†</td>
<td>124.04 ± 23.72†</td>
<td>112.79 ± 23.84†</td>
<td>64.60 ± 15.99†</td>
</tr>
</tbody>
</table>

Values are means ± SE (in μmol/l); n = 8 subjects for plasma and n = 5 subjects for whole blood. *Significant interaction effect, different from preinfusion (P < 0.05). †Significant interaction effect NAC > Con (P < 0.005).
NAC blunts unpleasant sensations produced during exhaustive exercise (46). Whether this occurred in this study cannot be determined. However, this seems unlikely because of the large effect of NAC on muscle TGSH, GSH, and Cys, which were already evident at 45 min, where work was matched to Con trials.

It is possible that elevated muscle antioxidant capacity may have exerted a protective effect on key ion transporting or ion channel proteins in muscle, including the Na\(^+-\)K\(^+\)-ATPase enzyme, the sarcoplasmic reticulum Ca\(^{2+}\)-release channel (ryanodine receptor), and Ca\(^{2+}\)-ATPase enzyme, each of which is deleteriously affected by increased ROS production (20). Our laboratory has found that the activities of Na\(^+-\)K\(^+\)-ATPase and Ca\(^{2+}\)-ATPase and also the rate of Ca\(^{2+}\) release are depressed at fatigue in human muscles (6, 13, 24), with increased ROS a proposed mechanism. Animal models demonstrate that ROS deleteriously affects Na\(^+-\)K\(^+\)-ATPase activity (21, 39), ryanodine-receptor function (31, 32), and Ca\(^{2+}\)-ATPase activity (1, 47). Whether NAC blunts these effects in human skeletal muscle remains to be elucidated.

**Blood and Plasma Thiols**

The decreased whole blood [GSH] and increased [cGSSG] with prolonged exercise is consistent with other human studies (14, 15, 38). However, the lack of modification with NAC contrasts our laboratory’s previous findings during high-intensity, intermittent exercise (26), where our group found a clear effect of NAC on blood thiol status during exercise. This may reflect differences in the training status of subjects and the exercise intensity and duration. Our laboratory’s previous study utilized untrained individuals (26), whereas this study utilized endurance-trained individuals.
have increased blood antioxidant activity compared with sedentary subjects (25) and training increases blood antioxidant enzyme activity (28), which may reduce the overall oxidative stress in RBCs (19). Increasing exercise intensity results in increased blood glutathione oxidation (36) and ROS production (3), which might also explain an effect of NAC on blood thiol status during intense (26) but not in submaximal exercise. More profound changes in blood glutathione may have occurred in blood draining the exercising muscles, and hence NAC effects on GSH and cGSSG may be greater at these sites.

In conclusion, we demonstrate for the first time that NAC infusion during prolonged, submaximal exercise increased muscle NAC and skeletal muscle cysteine, cystine, and glutathione availability during exercise and that it substantially enhanced performance in well trained individuals.

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REFERENCES


