N-acetylcysteine infusion alters blood redox status but not time to fatigue during intense exercise in humans

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Numerous studies utilizing isolated animal muscle preparations have demonstrated that ROS accelerates, whereas antioxidants attenuate, muscle fatigue. Studies that induced increased ROS demonstrated accelerated fatigue in diaphragm and limb muscles (13, 37), which was inversely related to the amount of ROS produced (45). Antioxidant compounds have reduced fatigue in diverse experimental preparations, including canine diaphragm (52) and gastrocnemius muscle (6), rat diaphragm bundles (43), human diaphragm (53), voluntary exercise in mice (39) and humans (28), as well as electrical stimulation of limb muscles in humans (46). Although most studies in humans utilized oral vitamins A, C, and/or E as antioxidants and typically found no exercise performance enhancement (1, 4, 40), positive effects have been found by using pharmacological antioxidants (42). In a landmark study, Reid and colleagues (46) found that the decline in force during fatiguing electrical stimulation of tibialis anterior muscle in humans was attenuated by intravenous infusion of the antioxidant compound N-acetylcysteine (NAC). However, numerous serious adverse reactions were reported, including conjunctival irritation, dysphoria, vomiting, diarrhea, nausea, and loss of coordination, thus precluding NAC infusion during voluntary exercise (46). These adverse reactions may have been related to a high peak NAC concentration ([NAC]) resulting from the large bolus NAC dose, but this cannot be determined because [NAC] was not reported. On the basis of pharmacokinetic data (41), we estimate their peak [NAC] at ~500 mg/l, which would have then declined rapidly during the experimental period (46). Therefore, their muscle stimulation experiments (46) were performed both at unknown and uncontrolled [NAC]. Interpretation of their data is further confounded because subjects were pretreated with the antihistamine drug diphenhydramine to blunt the adverse reactions to NAC. The effects of diphenhydramine on muscle function were not re-

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ported. Others (50) have given oral NAC, but this approach is limited due to the low bioavailability of oral NAC (20), and blood [NAC] was again not reported (50). Thus the effects of NAC infusion on human muscle function are worthy of further exploration.

The first aim of this study was to develop a modified NAC infusion protocol designed to obviate serious adverse reactions, thus allowing whole body exercise. Specifically, we aimed to 1) avoid an excessive peak [NAC], 2) attain a stable [NAC] during the exercise period, and 3) include measurements of blood and plasma [NAC] for the first time during exercise. On the basis of this modified NAC infusion protocol, the second aim was to investigate the effects of intravenous NAC on fatigue during voluntary whole body exercise in healthy individuals.

Changes in glutathione redox state are often used as a sensitive measure of tissue oxidative stress (25). Exercise results in an increase in GSSG and a decline in GSH concentrations in blood (16). Thus the third aim was to investigate the effects of NAC infusion on potassium (K⁺) regulation during intense exercise, since impaired K⁺ regulation has been linked with muscle fatigue (15, 38, 48). We have recently found that intense fatiguing muscle contractions impaired skeletal muscle Na⁺-K⁺-ATPase activity (15). Although the mechanisms for this effect are unknown, an increase in ROS was suggested as a possibility (14, 15). The muscle Na⁺-K⁺-ATPase activity was inversely correlated to an index of plasma K⁺ regulation during exercise, the ratio of rise in plasma K⁺ per work done (15). Consequently, the final aim was to investigate NAC effects on plasma K⁺ regulation during exercise.

The three hypotheses tested were that NAC infusion would 1) attenuate the decrease in GSH and rise in GSSG during exercise; 2) reduce the rise in plasma K⁺-to-work ratio during exercise; and 3) enhance time to fatigue during voluntary, high-intensity, intermittent exercise.

METHODS

Subjects

Eight male subjects (age: 22.5 ± 2.4 yr; body mass: 77.81 ± 10.30 kg; height: 177.6 ± 1.6 cm; mean ± SD) volunteered for the study after being informed of all risks and giving written, informed consent. Subjects refrained from vigorous activity and avoided ingesting caffeine, alcohol, or other drugs in the 24 h before their visits to the laboratory. Ethical approval was obtained from the Victoria University of Technology Human Research Ethics Committee.

Overview of Trials

Subjects attended the laboratory on six separate occasions, separated by a 5- to 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 peak oxygen consumption (V̇O₂peak). Subjects then completed a total of five high-intensity, intermittent exercise trials. The first trial was for familiarization purposes, the second and third trials were to determine the within-subject variability of the time to fatigue, and the final two trials were the NAC (Parvolex, Faulding Pharmaceuticals) or placebo (Con) trials. The last two trials were conducted in a double-blind, randomized, counterbalanced design to determine the effects of NAC or Con infusion on exercise performance and blood redox status. For ethical reasons, the attending medical practitioner was nonblinded.

V̇O₂peak

Subjects were seated at a comfortable seat height that was kept constant for all trials. Subjects performed four 4-min submaximal workloads at 60, 90, 120, and 150 W, cycling at a pedal cadence of 80 rpm. After a 10-min rest period, with water consumed ad libitum, subjects recommenced cycling at 150 W, with increments of 25 W each minute until fatigue, which was defined as the inability to maintain pedal cadence above 60 rpm. The highest V̇O₂peak over a 30-s period was defined as V̇O₂peak. A regression equation of oxygen consumption (V̇O₂) vs. power output was derived from the four submaximal workloads and was used to determine the power output corresponding to 130% V̇O₂peak. All equipment and calibration procedures for V̇O₂ measurements were as previously described (29).

Intermittent, High-Intensity Exercise Protocol

The intermittent sprint test comprised four exercise bouts (EB) at a power output corresponding to 130% V̇O₂peak. The first three EB lasted 45 s and were separated by a 135-s passive recovery period on the ergometer (work-to-rest ratio: 1:3), whereas the last EB was continued to volitional fatigue, defined as an inability to maintain pedal cadence above 60 rpm. High-intensity exercise was chosen because this results in a markedly enhanced rate of production of ROS (51).

Experimental Trials

The two experimental trials investigated the effects of intravenous NAC during voluntary, high-intensity, intermittent exercise. On arrival at the laboratory, subjects were weighed and then, while supine on a couch, a 20-gauge catheter was inserted into a dorsal hand vein for subsequent arterialized venous blood sampling. A 22-gauge catheter was then inserted into a superficial median forearm vein for infusion of either NAC or saline. Subjects sat on a chair of comparable height immediately adjacent to the cycle ergometer for 10 min with their hand sheathed in a waterproof glove placed in a 45°C water bath. After an initial control blood sample, the infusion of NAC or saline was commenced and continued until the cessation of the exercise trial. After 25 min, subjects were transferred from the chair to the cycle ergometer, where they remained for a further 10 min before the onset of the exercise period to normalize any postural changes in plasma volume. After a total of 35 min of preinfusion, subjects began to exercise, with blood sampled prior to and in the final seconds of each EB. Blood samples were also taken at 1, 2, 5, 10, and 30 min into the recovery period. Expired gases were measured 2 min before exercise and continued until the cessation of the exercise trial.

NAC Infusion

The NAC infusion protocol was modified from previous rapid, non-steady-state bolus infusion protocols (41, 46). An initial loading dose of 125 mg·kg⁻¹·h⁻¹ for 15 min was used to achieve the peak plasma [NAC], followed by a constant
infusion of 25 mg·kg⁻¹·h⁻¹ that continued until the end of exercise. Our modifications aimed to avoid the initial high dose and thus minimize the many adverse reactions previously reported (46). We also aimed to induce a constant plasma [NAC] before commencement of exercise and, finally, to maintain this concentration throughout the exercise trial. An anesthesia infusion pump was used for all infusions (Grassey 3400, Grassey Medical, Watford, UK).

Blood Processing

Two blood samples were drawn in rapid succession at each sample point. The first 1-ml sample was taken by using a blood-gas syringe that contained lithium heparin (RapidLyte, Chiron Diagnostics); air bubbles were immediately expelled, and the syringe was placed on ice for immediate plasma gas, pH, and electrolyte analyses (Ciba Corning 865pH/blood-gas analyzer, Bayer). A second 5-ml sample was used for measurement of reduced and total thiols in blood and plasma HB concentration ([Hb]), Hct, and plasma concentrations of 

Fluid shifts and K⁺. The decline in plasma volume from rest with exercise was calculated from changes in [Hb] and Hct (18), with [Hb] and Hct measured in triplicate by using an automated analyzer (K-800, Sysmex Kobe, Japan). The ratio of the rise in plasma [K⁺] (Δ[K⁺]) per work output was calculated for each EB ([Δ[K⁺]/work ratio, nmol·l⁻¹·J⁻¹) and was used to represent the net plasma K⁺ accumulation per EB (33).

Red blood cell NAC, cysteine, and cystine. Red blood cell NAC ([NAC]rbc), cysteine, and cysteine concentrations were calculated by using an equation (8) modified to exclude the correction of Hct for trapped plasma

where rbc, wb, and p represent erythrocytes, whole blood, and plasma, respectively.

Statistical Analyses

Anthropometric data are presented as means ± SD, with all other data reported as means ± SE. Single comparisons were analyzed by using a paired Student’s t-test. For NAC, a one-way ANOVA with repeated measures was used. All other blood analyses were analyzed by using a two-way (treatment × time) ANOVA with repeated measures on both factors. Significance was accepted at P < 0.05. Post hoc analyses used the Newman-Keuls test. Individual coefficients of variation were calculated for all subjects within the exercise protocol and averaged to obtain an overall coefficient of variation (24).

RESULTS

Exercise Performance Variability and Effects of NAC

Incremental exercise. V0₂ peak was 43.0 ± 6.0 ml·kg⁻¹·min⁻¹, and the work rate corresponding to 130% V0₂ peak was 327 ± 41 W.

Intermittent exercise. Excellent reproducibility was seen in the time to fatigue in the fourth EB during the two variability trials, with a coefficient of variation of 2.4 ± 0.6% (range: 0.6–5.5%; Table 1). No differences
were seen in time to fatigue (NAC: 103 ± 18 s; Con: 106 ± 19 s) or total work (NAC: 33.2 ± 4.6 kJ; Con: 34.1 ± 5.7 kJ) during the final EB.

NAC and Adverse Reactions

Total NAC content infused was 3.45 ± 0.16 g. In contrast to previous studies, no severe or even moderate adverse reactions requiring treatment or causing discomfort were observed in any of the subjects by using our modified infusion protocol (Table 2).

Plasma NAC. During the 15-min loading infusion phase, [NAC]tp increased progressively until a peak at 15 min (P < 0.05; Fig. 1). During the maintenance infusion phase, [NAC]tp then decreased immediately before exercise (P < 0.05) with no further changes during exercise. In recovery, [NAC]tp had decreased from fatigue at 30 min (P < 0.05) but remained elevated above preinfusion (P < 0.05).

A similar pattern was found for [NAC]rp, which peaked at the end of the loading phase (P < 0.05), then declined slightly during the maintenance phase (P < 0.05), with no subsequent differences during exercise. No differences were observed in [NAC]rp at 30 min recovery compared with fatigue levels, but [NAC]rp remained elevated above preinfusion levels (P < 0.05). No NAC was detected in any preinfusion blood or plasma sample during Con trials.

Whole blood NAC. During the loading infusion phase, [NAC]tb increased progressively until a peak at 15 min (P < 0.05) and then decreased during the maintenance infusion phase (P < 0.05; Fig. 2). No significant changes were seen in [NAC]tb during exercise.

Table 1. Individual time to fatigue during preexperimental, high-intensity, intermittent exercise trials

<table>
<thead>
<tr>
<th>Subject</th>
<th>Fatigue Time, s</th>
<th>Familiarization trial</th>
<th>Variability trial 1</th>
<th>Variability trial 2</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>89</td>
<td>91</td>
<td>92</td>
<td>0.8</td>
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<tr>
<td>2</td>
<td>206</td>
<td>208</td>
<td>215</td>
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<tr>
<td>3</td>
<td>119</td>
<td>126</td>
<td>125</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>51</td>
<td>56</td>
<td>59</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>71</td>
<td>79</td>
<td>77</td>
<td>1.8</td>
<td></td>
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<td>75</td>
<td>89</td>
<td>87</td>
<td>1.6</td>
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<tr>
<td>7</td>
<td>55</td>
<td>53</td>
<td>49</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>110</td>
<td>120</td>
<td>125</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Means ± SE</td>
<td>97 ± 18</td>
<td>103 ± 18</td>
<td>104 ± 19</td>
<td>2.4 ± 0.6</td>
<td></td>
</tr>
</tbody>
</table>

Each trial comprised three 45-s bouts, followed by a final bout to fatigue, each at 130% peak oxygen consumption (V̇O₂peak). Coefficient of variance (CV) was calculated from variability trial 1 and 2.

Table 2. Lack of severe or moderate adverse reactions with NAC or saline infusion before and during high-intensity, intermittent 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>Vomiting</td>
<td>NAC</td>
<td>Con</td>
<td>NAC</td>
<td>Con</td>
</tr>
<tr>
<td>Erythema</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Swelling</td>
<td>1</td>
<td>8</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Flushing</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Rash</td>
<td>6</td>
<td>7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Coughing</td>
<td>8</td>
<td>7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Altered moods</td>
<td>6</td>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Results are from n = 8 subjects. NAC, N-acetylcysteine; Con, saline (control).

Fig. 1. Plasma total (●) and reduced (▼) N-acetylcysteine (NAC) concentration ([NAC]) before, during, and after high-intensity, intermittent exercise. Shaded bars represent exercise bout (EB) at 130% peak O₂ consumption (V̇O₂ peak); EB1–3 were 45 s in duration, whereas EB4 was continued to fatigue (F). Values are means ± SE of n = 8 subjects, except EB1 where n = 7 subjects. Significant time main effect (P < 0.005); all times greater than preinfusion (P < 0.05).

Fig. 2. Blood total (●) and reduced (▼) [NAC] before, during, and after high-intensity, intermittent exercise. Shaded bars represent EB at 130% V̇O₂ peak. EB1–3 were 45 s in duration, whereas EB4 was continued to fatigue. Values are means ± SE of n = 8 subjects, except EB1 where n = 7 subjects. Significant time main effect (P < 0.005); all times greater than preinfusion (P < 0.05).
cise, but [NAC]_{rbc} decreased from fatigue at 30 min after infusion cessation (P < 0.05) but remained elevated above preinfusion levels (P < 0.05).

Similarly, [NAC]_{rbc} peaked at the end of the loading phase (P < 0.05), declined slightly during the maintenance phase (P < 0.05), with no differences during exercise and until 30 min of recovery, where [NAC]_{rbc} remained elevated above preinfusion levels (P < 0.05).

Red blood cell NAC. Total red blood cell [NAC] ([NAC]_{rbc}) increased from preinfusion levels at pre-EB1 (P < 0.001; Table 3), was then unchanged during exercise, decreased from fatigue at 30 min of recovery (P < 0.005), but remained elevated above preinfusion levels (P < 0.05). Similarly, reduced [NAC]_{rbc} increased at pre-EB1 (P < 0.001), with no further changes during exercise, decreased from fatigue at 30 min of recovery (P < 0.005), but remained elevated above preinfusion levels (P < 0.005; Table 3).

Whole Blood Glutathione

Total. Whole blood [GSH] was elevated above preinfusion levels at pre-EB2 and remained elevated thereafter during exercise and until 1 min of recovery (P < 0.05; Fig. 3). No differences were found in [TGSH] between treatments at any time.

Reduced. Whole blood [GSH] was not significantly changed during the preexercise infusion, but at EB1 had declined from preinfusion levels (P < 0.05) and remained lower during the exercise and recovery periods (P < 0.05; Fig. 4). Although no differences were found in [GSH] between NAC and Con during the preinfusion period, [GSH] was higher in NAC than in Con at EB1, during subsequent exercise (P < 0.005), and throughout recovery (P < 0.05).

Oxidized. The [cGSSG] was unchanged during preinfusion, increased during exercise, and remained elevated above preinfusion levels at 30 min of recovery (P < 0.05; Fig. 5). No differences between NAC and Con were found in [cGSSG] from rest to pre-EB1. However, from pre-EB2 to 30 min of recovery, [cGSSG] was lower in NAC compared with Con (P < 0.05; Fig. 6).

GSH-to-TGSH ratio. No differences in the GSH-to-TGSH ratio were found before or during the 35-min preinfusion period in either trial. Exercise decreased the GSH-to-TGSH ratio (P < 0.005), which was also

### Table 3. Calculated [NAC]_{rbc} during high-intensity, intermittent exercise before, during, and after NAC infusion

<table>
<thead>
<tr>
<th></th>
<th>Preinfusion</th>
<th>Pre-EB1</th>
<th>EB1</th>
<th>EB2</th>
<th>EB3</th>
<th>Fatigue</th>
<th>30-min Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.00</td>
<td>29.77 ± 4.87</td>
<td>44.95 ± 4.92</td>
<td>33.44 ± 14.61</td>
<td>39.60 ± 8.20</td>
<td>47.12 ± 7.19</td>
<td>13.17 ± 2.80</td>
</tr>
<tr>
<td>Reduced</td>
<td>0.00</td>
<td>16.21 ± 4.78</td>
<td>13.26 ± 6.34</td>
<td>23.59 ± 8.68</td>
<td>18.37 ± 5.64</td>
<td>16.94 ± 6.19</td>
<td>3.84 ± 0.93</td>
</tr>
</tbody>
</table>

Values are means ± SE (in mg/l); n = 6 subjects for preinfusion, exercise bout (EB) 3, and 30-min recovery; n = 5 subjects for EB1, EB2, and fatigue. [NAC]_{rbc}, red blood cell NAC concentration. All times significantly different from preinfusion (P < 0.001).

![Fig. 3. Effect of NAC (•) and placebo (○) infusion on blood total (reduced + oxidized) glutathione (TGSH) before, during, and after high-intensity, intermittent exercise. Shaded bars represent EB at 130% \( \dot{V}O_2 \text{peak} \). EB1–3 were 45 s in duration, whereas EB4 was continued to fatigue. Values are means ± SE of n = 8 subjects, except EB1 where n = 7 subjects and 1- to 10-min recovery where n = 6 subjects. *Significant time main effect greater than preinfusion (P < 0.005).

![Fig. 4. Effect of NAC (•) and Con (○) infusion on blood reduced glutathione concentration ([GSH]) before, during, and after high-intensity, intermittent exercise. Shaded bars represent EB at 130% \( \dot{V}O_2 \text{peak} \). EB1–3 were 45 s in duration, whereas EB4 was continued to fatigue. Values are means ± SE of n = 8 subjects, except EB1 where n = 7 subjects and 1- to 10-min recovery where n = 6 subjects. †Significant time main effect lower than preinfusion (P < 0.005). ‡NAC significantly greater than Con (P < 0.05).](http://jap.physiology.org/ Downloaded from japp Physiol.org by 142.105.3.5 on June 9, 2017)
attenuated by NAC (P < 0.005, data not shown). At 30 min of recovery, GSH-to-TGSH ratio remained lower than preinfusion levels in both trials (P < 0.05) but was still higher in NAC compared with Con.

**Plasma Glutathione**

Plasma glutathione levels were too low to be reliably detected.

**Cysteine and Cystine**

Before infusion, no differences in [CYS] (Table 4) or cystine concentration (Table 5) were found between NAC and Con, in whole blood, plasma, or red blood cells, either in total or reduced forms. No change in [CYS] or cystine from preinfusion levels occurred in Con at any time (Tables 4 and 5). However, NAC increased [CYS] and cystine in whole blood, plasma, and red blood cells, in both total and reduced forms, compared with preinfusion levels (P < 0.05). Hence, [CYS] and cystine were higher in NAC than Con at all times during exercise and recovery (P < 0.05; Tables 4 and 5).

**Fluid Shifts**

Both [Hb] and Hct were higher than preinfusion levels at EB2, during subsequent exercise, and until 30 min of recovery, where each returned to preinfusion levels (P < 0.05; Table 6). Plasma volume declined at EB1, during exercise, and until 30 min of recovery (P < 0.05; Table 6), where it had returned to preinfusion levels. No differences between NAC and Con were found for [Hb], Hct, or plasma volume.

**Plasma [K+] and Electrolyte Concentrations**

K⁺. Plasma [K⁺] increased above preinfusion levels in each EB, declined from fatigue at 1 and 2 min of recovery, and fell below preinfusion levels at 5 and 10 min of recovery (P < 0.05; Fig. 6A). No significant difference between NAC and Con was found for plasma [K⁺].

The rise in plasma [K⁺] (Δ[K⁺]) did not differ between EB1–3 but was increased in the final bout to fatigue (P < 0.001). Plasma Δ[K⁺] was higher in NAC than Con during EB2 and EB3 (P < 0.05), with no differences found between treatments during EB1 and EB4.

Similarly, the Δ[K⁺]-to-work ratio did not differ between EB1–3, but was greater in EB4 (P < 0.005; Fig. 6B). The Δ[K⁺]-to-work ratio was higher in NAC during EB2 and EB3 (P < 0.05; Fig. 6B).

[Na⁺], [Cl⁻], and [Ca²⁺]. Plasma [Na⁺] increased above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatigue (P < 0.05; Table 7), remained elevated above preinfusion levels at EB2, EB3, and fatig...
Table 4. Cysteine concentrations (μmol/l) in whole blood, plasma, and red blood cells during high-intensity, intermittent exercise before, during, and after NAC and Con infusion

<table>
<thead>
<tr>
<th>Variable (blood)</th>
<th>Treatment</th>
<th>Preinfusion</th>
<th>Pre-EB1</th>
<th>EB1</th>
<th>EB2</th>
<th>EB3</th>
<th>Fatigue</th>
<th>30-min Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Cys]wb NAC</td>
<td>51.0 ± 8.4†</td>
<td>161.3 ± 19.9‡</td>
<td>157.9 ± 17.6‡</td>
<td>150.2 ± 19.0‡</td>
<td>165.3 ± 18.4‡</td>
<td>284.4 ± 17.9‡</td>
<td>122.3 ± 13.6‡</td>
<td></td>
</tr>
<tr>
<td>[Cys]wb Con</td>
<td>49.1 ± 7.6</td>
<td>51.6 ± 11.6</td>
<td>55.6 ± 13.1</td>
<td>51.1 ± 12.0</td>
<td>52.6 ± 14.8</td>
<td>56.6 ± 9.6</td>
<td>43.6 ± 9.1</td>
<td></td>
</tr>
<tr>
<td>[Cys]tp NAC</td>
<td>7.6 ± 1.9‡</td>
<td>42.9 ± 4.4‡</td>
<td>40.0 ± 3.4‡</td>
<td>39.9 ± 2.4‡</td>
<td>37.1 ± 2.0‡</td>
<td>38.8 ± 3.0‡</td>
<td>22.15 ± 3.0‡</td>
<td></td>
</tr>
<tr>
<td>[Cys]tp Con</td>
<td>7.1 ± 2.7</td>
<td>10.4 ± 3.4‡</td>
<td>11.3 ± 2.4</td>
<td>11.2 ± 3.1</td>
<td>11.6 ± 4.3</td>
<td>11.1 ± 2.9</td>
<td>8.8 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>[Cys]rp NAC</td>
<td>81.4 ± 8.9</td>
<td>270.9 ± 15.4‡</td>
<td>286.4 ± 16.3‡</td>
<td>269.6 ± 15.2‡</td>
<td>288.7 ± 17.5‡</td>
<td>284.4 ± 17.9‡</td>
<td>208.7 ± 15.1‡</td>
<td></td>
</tr>
<tr>
<td>[Cys]rp Con</td>
<td>79.0 ± 6.3</td>
<td>84.3 ± 14.8</td>
<td>87.3 ± 13.4</td>
<td>86.2 ± 14.6</td>
<td>87.0 ± 12.1</td>
<td>96.5 ± 16.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Cys]rb NAC</td>
<td>9.7 ± 3.5‡</td>
<td>71.8 ± 7.8‡</td>
<td>68.4 ± 5.3‡</td>
<td>66.5 ± 5.0‡</td>
<td>60.9 ± 4.2‡</td>
<td>65.3 ± 4.1‡</td>
<td>35.1 ± 2.8‡</td>
<td></td>
</tr>
<tr>
<td>[Cys]rb Con</td>
<td>8.8 ± 2.1</td>
<td>15.2 ± 4.8</td>
<td>15.4 ± 4.6</td>
<td>16.4 ± 5.5</td>
<td>16.9 ± 5.1</td>
<td>15.4 ± 5.9</td>
<td>11.7 ± 5.9</td>
<td></td>
</tr>
<tr>
<td>[Cys]rtrbc NAC</td>
<td>15.5 ± 2.1‡</td>
<td>35.2 ± 3.1‡</td>
<td>35.3 ± 2.1‡</td>
<td>41.0 ± 2.0‡</td>
<td>41.0 ± 3.0‡</td>
<td>37.7 ± 2.4‡</td>
<td>22.1 ± 2.0‡</td>
<td></td>
</tr>
<tr>
<td>[Cys]rtrbc Con</td>
<td>14.1 ± 2.6</td>
<td>13.5 ± 2.6</td>
<td>19.0 ± 3.0</td>
<td>12.8 ± 2.9</td>
<td>17.7 ± 2.6</td>
<td>17.8 ± 2.6</td>
<td>13.8 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>[Cys]rb NAC</td>
<td>5.1 ± 1.2†</td>
<td>9.6 ± 1.1†</td>
<td>8.6 ± 1.0†</td>
<td>12.1 ± 0.9†</td>
<td>13.1 ± 2.0†</td>
<td>12.9 ± 1.0†</td>
<td>8.3 ± 0.7†</td>
<td></td>
</tr>
<tr>
<td>[Cys]rb Con</td>
<td>5.2 ± 0.8</td>
<td>4.5 ± 0.9</td>
<td>6.7 ± 1.1</td>
<td>5.6 ± 1.1</td>
<td>6.01 ± 1.1</td>
<td>6.8 ± 1.4</td>
<td>5.1 ± 1.3</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 subjects, except EB1 where n = 7 for [Cys]wb, [Cys]tp, [Cys]rp, and [Cys]rb. For [Cys]rtrbc, n = 6 for preinfusion, EB3, and 30-min recovery, and n = 5 for EB1, EB2, and fatigue. [Cys]wb, whole blood total cysteine; [Cys]tp, plasma total cysteine; [Cys]rp, plasma reduced cysteine; [Cys]rb, calculated red blood cell cysteine. Significant main effect for treatment NAC > Con: †P < 0.05; ‡P < 0.005. *Significant main effect for time: different from preinfusion (P < 0.005).

Acid-Base Status

Plasma [H+] was increased, whereas plasma PCO₂ and HCO₃⁻ concentration fell during EB2 and remained lower during exercise and recovery, compared with preinfusion levels (P < 0.05; Table 7). A slightly lower [H+] was found in NAC compared with Con (P < 0.05), whereas no differences were found for plasma HCO₃⁻ concentration or PCO₂.

DISCUSSION

This study investigated the effects of an antioxidant (NAC) drug infusion on blood redox state and intense intermittent exercise performance in healthy humans. Our first important observation was that our modified NAC infusion protocol was free of serious adverse reactions and well tolerated under resting and vigorous exercise conditions. Although NAC altered blood redox status, plasma K⁺ regulation was also impaired. Finally, NAC infusion did not enhance performance during voluntary, high-intensity, intermittent cycling exercise in humans.

No Severe Adverse Reactions With NAC Infusion

We developed and utilized here a modified NAC infusion protocol, with major improvements on previous studies (41, 46, 53). Specifically, we attained relatively stable concentrations during exercise and completed the experiments without antihistamine pre-treatment. The peak plasma [NAC] was ~240 mg/l (~1.5 mmol/l) at the end of the NAC loading phase and ~191 mg/l before exercise, in contrast to the estimated peak [NAC] of ~500 mg/l (~3 mmol/l) in Reid and colleagues (46). The unchanged [NAC] during the exercise period allowed us to examine the effects of a relatively stable [NAC] during exercise, in contrast to the expected rapidly declining [NAC] during the experimental period in a previous study (46).

An important finding is that our NAC infusion protocol was free of any major adverse reactions in these eight healthy volunteers. Consistent with this, we have recently completed a second study in a further nine subjects in whom no serious adverse reactions to NAC infusion were found (Medved, Brown, Bjorksten, and McKenna, unpublished results). The most common

Table 5. Calculated cystine concentrations (μmol/l) in whole blood, plasma, and red blood cells during high-intensity, intermittent exercise before, during, and after NAC and Con infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Preinfusion</th>
<th>Pre-EB1</th>
<th>EB1</th>
<th>EB2</th>
<th>EB3</th>
<th>Fatigue</th>
<th>30-min Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma NAC</td>
<td>71.7 ± 9.9‡</td>
<td>199.1 ± 16.9‡</td>
<td>200.1 ± 15.9‡</td>
<td>203.1 ± 15.9‡</td>
<td>203.1 ± 15.9‡</td>
<td>227.7 ± 15.9‡</td>
<td>219.0 ± 18.9‡</td>
<td>173.6 ± 15.6‡</td>
</tr>
<tr>
<td>Whole Blood NAC</td>
<td>43.4 ± 4.9‡</td>
<td>118.4 ± 16.6‡</td>
<td>117.8 ± 17.6‡</td>
<td>118.0 ± 16.6‡</td>
<td>128.1 ± 15.9‡</td>
<td>126.0 ± 16.6‡</td>
<td>100.2 ± 13.9‡</td>
<td></td>
</tr>
<tr>
<td>RBC NAC</td>
<td>10.3 ± 2.3†</td>
<td>25.6 ± 3.9†</td>
<td>26.7 ± 2.0†</td>
<td>28.9 ± 2.6†</td>
<td>27.8 ± 2.6†</td>
<td>24.8 ± 3.6†</td>
<td>13.7 ± 2.9†</td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>8.8 ± 2.6</td>
<td>8.8 ± 2.9</td>
<td>12.3 ± 2.3</td>
<td>7.2 ± 2.6</td>
<td>11.7 ± 3.8</td>
<td>11.1 ± 2.6</td>
<td>8.0 ± 2.1</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects for preinfusion, EB3, and 30-min recovery, and n = 5 for EB1, EB2, and fatigue. Cystine calculated from total cysteine – reduced cysteine. RBC, red blood cell. Significant main effect for treatment (NAC > Con): †P < 0.05; ‡P < 0.005. *Significant main effect for time: different from preinfusion (P < 0.005).
side effect resulting from our NAC infusion was erythema at the site of infusion, but this was also evident during some Con trials. Of the side effects unique to thema at the site of infusion, but this was also evident side effect resulting from our NAC infusion was erythema, diarrhea, angiodema, tachycardia, and dyspepsia. Furthermore, our protocol avoided other reported adverse reactions to NAC infusion, including loss of coordination, nausea, bronchospasm, conjunctival irritation, diarrhea, angiodema, tachycardia, and dyspepsia (30, 41, 46). Therefore, our modified NAC infusion protocol may be utilized in future studies investigating NAC effects in healthy humans.

NAC Modulates Exercise Effects on Blood Redox State

Our results demonstrate two important findings with respect to intense exercise and NAC effects on blood redox state. First, intense intermittent exercise decreased whole blood [GSH], with a concomitant increase in whole blood [cGSSG]. Together with our finding of unchanged whole blood [TGSCH], this demonstrates a shift in whole blood redox status during exercise. We were unable to detect glutathione in plasma, consistent with others (16).

The reported effects of high-intensity exercise on [GSSG] and [GSH] in humans are inconsistent. Blood [GSSG] after graded exercise to exhaustion was increased in two studies (47, 50) but unchanged in another (16), whereas blood [GSH] was decreased (47) or unchanged (16, 50). These discrepancies may in part reflect the subjects’ differing training status, which influence the response of the glutathione system (35). However, it would be expected that both a decline in the GSH together with a rise in GSSG would occur, yet only one of the three above studies reported this (47). We observed a clear effect of intense exercise on blood [GSH] and [cGSSG], indicating that exercise directly modulates blood redox state. Whole blood glutathione oxidation reflects defense against ROS formation (47) and is often used as a sensitive marker of oxidative stress (25). Therefore, the rise in [cGSSG] indicates increased extramuscular accumulation of ROS during intermittent, high-intensity exercise, consistent with ROS efflux from contracting leg muscles during exercise (5). Because plasma glutathione was undetectable, our results indicate increased oxidative stress within red blood cells.

Our second major finding was that NAC attenuated both the decline in blood [GSH] and rise in blood [cGSSG] during intense, intermittent exercise. A previous study found that oral NAC attenuated the rise in [GSSG] during high-intensity exercise, although, surprisingly, they reported no effect of exercise or NAC on GSH, and blood [NAC] was also not reported (50).

Cysteine is a precursor to glutathione synthesis (10, 20, 41) and meets the intracellular needs for synthesis of GSH (10). NAC infusion increased reduced [Cys] and increased the ratio of reduced [Cys] to oxidized [Cys] and may therefore have spared glutathione oxidation. This likely explains the attenuated decline in [GSH] and increased [cGSSG]. However, NAC is rapidly deactylated to cysteine, which is itself a ROS scavenger (10), and this could also be a factor for the attenuated decrease and increase in [GSH] and [cGSSG], respectively.

Lack of Ergogenic Effect

This is the first study to investigate possible ergogenic effects of NAC infusion in healthy humans undertaking voluntary whole body exercise. The intra-subject variability during the intermittent exercise protocol was 2.4%, well within the typical variation for high-intensity exercise (~5%; Ref. 32). NAC did not increase time to fatigue during high-intensity, intermittent exercise, in contrast to the attenuation of fatigue in stimulated muscle contractions with NAC (46) or in diaphragm fatigue induced by inspiratory loading (53).

The lesser rise in [cGSSG] during exercise with NAC indicates enhanced glutathione synthesis and greater protection against ROS formation. Thus it is unlikely that the lower [NAC] in this study compared with Reid and colleagues (46) can account for our lack of performance enhancement. Our finding is consistent with the lack of effect of NAC on muscle fatigability during high-frequency electrical stimulation in human tibialis anterior muscle (46). Together, these suggest that NAC does not affect contractile performance during heavy muscular contractions. The attenuated fatigue with NAC in human tibialis anterior muscle during low-frequency muscle stimulation (46) and in the diaphragm during inspiratory loading (53) suggests that NAC may be more effective in modulating performance during low-frequency fatigue protocols (42). Therefore,

---

Table 6. Hematology and calculated fluid shifts during high-intensity, intermittent exercise before, during, and after NAC and Con infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Preinfusion</th>
<th>Pre-EB1</th>
<th>EB1</th>
<th>EB2</th>
<th>EB3</th>
<th>Fatigue</th>
<th>30-min Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Hb], g/dl</td>
<td>NAC</td>
<td>15.3 ± 0.5</td>
<td>15.6 ± 0.4</td>
<td>15.7 ± 0.5</td>
<td>16.2 ± 0.5</td>
<td>16.6 ± 0.5</td>
<td>16.8 ± 0.4</td>
<td>15.3 ± 0.4</td>
</tr>
<tr>
<td>Con</td>
<td>15.4 ± 0.4</td>
<td>15.6 ± 0.4</td>
<td>15.8 ± 0.4</td>
<td>16.2 ± 0.2</td>
<td>16.3 ± 0.5</td>
<td>17.1 ± 0.3</td>
<td>15.0 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Hct, %</td>
<td>NAC</td>
<td>46.1 ± 1.1</td>
<td>46.5 ± 1.1</td>
<td>47.4 ± 1.4</td>
<td>48.8 ± 1.0</td>
<td>49.8 ± 0.9</td>
<td>50.7 ± 1.2</td>
<td>46.3 ± 0.9</td>
</tr>
<tr>
<td>Con</td>
<td>46.1 ± 1.0</td>
<td>46.2 ± 0.9</td>
<td>46.4 ± 1.1</td>
<td>47.8 ± 1.1</td>
<td>48.3 ± 1.0</td>
<td>50.5 ± 1.5</td>
<td>44.4 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>ΔPV, %</td>
<td>NAC</td>
<td>-7.6 ± 1.1</td>
<td>-10.5 ± 2.4</td>
<td>-14.4 ± 1.1</td>
<td>-16.1 ± 3.5</td>
<td>-18.7 ± 2.9</td>
<td>0.5 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>-6.4 ± 0.6</td>
<td>-11.5 ± 2.1</td>
<td>-12.7 ± 2.6</td>
<td>-18.7 ± 2.9</td>
<td>2.1 ± 3.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 (NAC), n = 5 (Con). [Hb], Hb concentration; ΔPV, change in plasma volume. *Significant main effect for time: different from preinfusion (P < 0.05).
research into NAC effects on prolonged exercise performance appears warranted.

**NAC, Intracellular Actions, and Muscle Performance**

The capacity of NAC to act as an effective antioxidant during exercise may depend on whether it influences intracellular processes in skeletal muscle. An important clinical application of NAC is the treatment of paracetamol overdose, which relies on NAC’s reducing capacity within liver cells (10). Tissues such as bladder, bone marrow, erythrocytes, and liver all take up NAC and/or its reduced cysteine derivatives (11, 34), with the exact mechanism unknown. We demonstrate that NAC infusion increased [NAC]_{rbc} in healthy humans, indicating that NAC penetrates healthy cell membranes and suggesting that NAC may also permeate the sarcolemma. However, we cannot find any studies that have investigated this possibility. Nonetheless, several studies demonstrate that NAC directly affects skeletal muscle contractile function (19, 26). Nevertheless, several studies demonstrate that NAC directly affects skeletal muscle contractile function (19, 26).

Table 7. Plasma acid-base variables and electrolyte concentrations during high-intensity, intermittent exercise before, during, and after NAC and Con infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Preinfusion</th>
<th>Pre-EB1</th>
<th>EB1</th>
<th>EB2</th>
<th>EB3</th>
<th>Fatigue</th>
<th>30-min Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>[H⁺], nmol/l</td>
<td>NAC†</td>
<td>38.9 ± 0.3†</td>
<td>37.4 ± 0.6†</td>
<td>39.0 ± 2.0++</td>
<td>46.2 ± 1.4+</td>
<td>48.5 ± 0.7++</td>
<td>54.7 ± 1.7++</td>
<td>44.6 ± 2.8+</td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>39.1 ± 0.4</td>
<td>39.1 ± 0.6</td>
<td>38.6 ± 0.5a</td>
<td>48.5 ± 0.7a</td>
<td>48.5 ± 0.7*</td>
<td>55.7 ± 2.3a</td>
<td>48.4 ± 2.6a</td>
</tr>
<tr>
<td>[HCO₃⁻], nmol/l</td>
<td>NAC</td>
<td>28.7 ± 0.5</td>
<td>25.5 ± 0.7</td>
<td>25.1 ± 1.2</td>
<td>21.6 ± 2.0*</td>
<td>17.6 ± 0.5*</td>
<td>14.7 ± 0.5*</td>
<td>16.5 ± 1.3*</td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>25.5 ± 0.4</td>
<td>24.3 ± 0.5</td>
<td>25.2 ± 0.5</td>
<td>19.6 ± 0.5*</td>
<td>17.4 ± 0.5*</td>
<td>14.9 ± 0.7*</td>
<td>17.9 ± 1.6*</td>
</tr>
<tr>
<td>Pco₂, Torr</td>
<td>NAC</td>
<td>43.2 ± 0.7</td>
<td>39.5 ± 0.8</td>
<td>40.3 ± 0.4</td>
<td>38.4 ± 1.2*</td>
<td>38.8 ± 1.4*</td>
<td>34.0 ± 1.4*</td>
<td>30.1 ± 2.5*</td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>41.3 ± 0.6</td>
<td>39.7 ± 0.5</td>
<td>40.5 ± 0.4</td>
<td>39.1 ± 0.5*</td>
<td>35.9 ± 0.7*</td>
<td>34.7 ± 1.4*</td>
<td>34.8 ± 1.0*</td>
</tr>
<tr>
<td>[Na⁺], nmol/l</td>
<td>NAC</td>
<td>141.0 ± 0.7</td>
<td>141.9 ± 0.7</td>
<td>141.8 ± 0.6</td>
<td>144.7 ± 0.4*</td>
<td>144.6 ± 1.0*</td>
<td>151.0 ± 0.8*</td>
<td>141.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>138.5 ± 1.4</td>
<td>142.6 ± 0.6</td>
<td>139.1 ± 1.7</td>
<td>144.9 ± 1.7*</td>
<td>143.8 ± 1.0*</td>
<td>147.1 ± 1.4*</td>
<td>141.2 ± 0.7</td>
</tr>
<tr>
<td>[Cl⁻], mmol/l</td>
<td>NAC</td>
<td>104.0 ± 0.5</td>
<td>103.9 ± 0.9</td>
<td>103.5 ± 0.7</td>
<td>102.1 ± 0.6</td>
<td>103.1 ± 0.7</td>
<td>105.1 ± 0.9</td>
<td>101.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>104.0 ± 0.3</td>
<td>103.2 ± 0.9</td>
<td>104.8 ± 1.4</td>
<td>103.0 ± 0.8</td>
<td>102.0 ± 1.3</td>
<td>107.7 ± 1.6</td>
<td>105.7 ± 2.7</td>
</tr>
<tr>
<td>[Ca²⁺], mmol/l</td>
<td>NAC</td>
<td>1.23 ± 0.01</td>
<td>1.22 ± 0.01</td>
<td>1.24 ± 0.01</td>
<td>1.25 ± 0.01</td>
<td>1.25 ± 0.01</td>
<td>1.31 ± 0.01*</td>
<td>1.20 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>1.21 ± 0.01</td>
<td>1.23 ± 0.01</td>
<td>1.23 ± 0.01</td>
<td>1.28 ± 0.01</td>
<td>1.27 ± 0.01</td>
<td>1.30 ± 0.02*</td>
<td>1.20 ± 0.02</td>
</tr>
<tr>
<td>Δ[K⁺], mmol/l</td>
<td>NAC</td>
<td>0.07 ± 0.1†</td>
<td>0.62 ± 0.15†</td>
<td>0.61 ± 0.04†</td>
<td>0.67 ± 0.04†</td>
<td>2.23 ± 0.21†</td>
<td>2.30 ± 0.20†</td>
<td>2.63 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>0.01 ± 0.09</td>
<td>0.58 ± 0.07</td>
<td>0.35 ± 0.07</td>
<td>0.45 ± 0.04</td>
<td>2.09 ± 0.33*</td>
<td>2.63 ± 0.45</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8, except EB1 where n = 7. Δ[K⁺], rise in plasma K⁺ concentration during each EB. †Significant main effect for treatment. *Significant main effect for time: different from preinfusion (P < 0.05).
tive muscle or other tissues. These changes might also be mediated by alterations in muscle blood flow because NAC is a potent vasodilator (2). Because muscle K⁺ regulation is linked with muscle performance (38), the impaired K⁺ regulation with NAC might also help to explain why performance was unaltered, despite a marked effect of NAC on blood redox state.

Finally, NAC attenuated plasma [H⁺] during exercise. This effect in plasma was small and likely to be of minor physiological significance. However, if NAC also lowers [H⁺] in skeletal muscle, this may have important implications, since a semi-quinone radical in the presence of hydrogen peroxide and a high [H⁺] may form the potent hydroxyl radical (23), which denatures the Ca²⁺-ATPase enzyme (55).

In conclusion, NAC did not induce any serious adverse reactions in healthy volunteers at rest or during or after vigorous exercise. NAC blunted the decline in [GSH] as well as the concomitant rise in [cGSSG] in whole blood during exercise, indicating its efficacy as a modulator of blood redox status during exercise. NAC failed to enhance performance during high-intensity, intermittent exercise, suggesting that ROS may not exert an important role in muscle fatigue under these exercise conditions. However, it is possible that the adverse effects of impaired K⁺ regulation with NAC counterbalanced any positive effects due to redox modulation. Further research is required to investigate the cellular actions of NAC and, in particular, the effects on prolonged exercise performance in humans.

REFERENCES

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