Effects of intravenous *N*-acetylcysteine infusion on time to fatigue and potassium regulation during prolonged cycling exercise

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Medved, Ivan, Malcolm J. Brown, Andrew R. Bjorksten, and Michael J. McKenna. Effects of intravenous *N*-acetylcysteine infusion on time to fatigue and potassium regulation during prolonged cycling exercise. *J Appl Physiol* 96: 211–217, 2004. First published September 5, 2003; 10.1152/japplphysiol.00458.2003.—The production of reactive oxygen species in skeletal muscle is linked with muscle fatigue. This study investigated whether the antioxidant compound *N*-acetylcysteine (NAC) augments time to fatigue during prolonged, submaximal cycling exercise. Seven men completed a double-blind, crossover study, receiving NAC or placebo before and during cycling exercise, comprising 45 min at 70% of peak oxygen consumption ($\dot{V}O_{2\,peak}$) and then to fatigue at 90% $\dot{V}O_{2\,peak}$. NAC was intravenously infused at 125 mg·kg^{-1}·h^{-1} for 15 min and then 25 mg·kg^{-1}·h^{-1} for 20 min before and throughout exercise, which was continued until fatigue. Arterialized venous blood was analyzed for NAC concentration, hematology, and plasma electrolytes. NAC induced no serious adverse reactions and did not affect hematology, acid-base status, or plasma electrolytes. Time to fatigue was reproducible in preliminary trials (coefficient of variation $7.4 \pm 1.2\%$) and was not augmented by NAC (NAC 14.6 ± 4.5 min; control 12.8 ± 5.4 min). However, time to fatigue during NAC trials was correlated with $\dot{V}O_{2\,peak}$ ($r = 0.78$; $P < 0.05$), suggesting that NAC effects on performance may be dependent on training status. The rise in plasma K$^+$ concentration at fatigue was attenuated by NAC ($P < 0.05$). The ratio of rise in K$^+$ concentration to work and the percentage change in time to fatigue tended to be inversely related ($r = -0.71$; $P < 0.07$). Further research is required to clarify a possible training status-dependent effect of NAC on muscle performance and K$^+$ regulation.

reactive oxygen species; muscle fatigue; sodium-potassium-adenosine triphosphatase; training status

SKELETAL MUSCLE PRODUCES reactive oxygen species (ROS) at low rates under resting conditions (17, 24, 36), but this rate is greatly enhanced during muscular contraction (4, 11, 35). Endogenous antioxidants, including the enzymes superoxide dismutase, catalase, and glutathione peroxidase, and thiol compounds such as glutathione and cysteine, are present in skeletal muscle and protect against the harmful effects of ROS (34). However, during exercise the endogenous antioxidant system is overwhelmed, and an increased ROS concentration occurs (4, 9, 35).

In animal models, ROS are known to accelerate muscular fatigue (5, 11, 28, 35), which is attenuated by antioxidants (5, 37, 40, 41). Importantly, studies implicating ROS in muscular fatigue have generally involved low-frequency muscle-stimulation protocols. In rat diaphragm bundles stimulated at 30 Hz, force was 20% greater after treatment with superoxide dismutase, catalase, and dimethyl sulfoxide (35). Similarly, superoxide dismutase increased tension by ~20% in canine diaphragm stimulated at 15 Hz (28). *N*-acetylcysteine (NAC), a thiol-containing compound, also attenuated skeletal and diaphragm muscle fatigue (10, 19, 40). NAC attenuated fatigue in rat diaphragm when stimulated at 20 Hz for 4 min (10) and in rabbit diaphragm when stimulated at 20 Hz for 20 min (40). In humans, NAC attenuated diaphragm muscle fatigue induced by loaded breathing (43) and reduced by 15% the force loss with fatigue induced by electrical stimulation of tibialis anterior muscle at 10 Hz (38). However, the adverse reactions reported in the latter study, including loss of coordination, vomiting, diarrhea, and nausea, would preclude voluntary exercise in humans (38).

We recently reported that a modified NAC-infusion protocol, without antihistamine pretreatment, was free of severe adverse reactions and was well tolerated by healthy humans during voluntary high-intensity, intermittent exercise (27). We found no fatigue-sparing effects of NAC during intense voluntary cycling exercise in humans (27), but this was consistent with a lack of an effect of NAC during high-frequency (40 Hz) stimulation in human muscle (38). Given the apparent involvement of ROS in fatigue induced by low-frequency muscle stimulation in animal and human models, it is likely that a similar involvement may be evident in prolonged submaximal exercise, with scavenging of ROS (3) and supporting glutathione synthesis as a possible mechanism (8). No studies have investigated the effects of intravenous NAC infusion on performance in healthy humans during prolonged exercise. We therefore hypothesized that intravenous NAC infusion would prolong the time to fatigue during exhaustive, submaximal exercise.

A further possible mechanism may include effects on skeletal muscle Na$^+$-K$^+$-ATPase. Maximal Na$^+$-K$^+$-ATPase activity in human skeletal muscle is depressed with exhaustive dynamic (14) and isometric contractions (13) and in rat muscle after prolonged running and recovery (12), with one proposed causal mechanism being ROS accumulation (20, 39). Furthermore, several studies have demonstrated a decline in muscle intracellular K$^+$ with prolonged exercise and suggested a link with fatigue (see references in Ref. 25). Hence, the ROS

Table 1. *Lack of severe adverse reactions with NAC or saline infusion, before and during prolonged, submaximal exercise in 8 healthy subjects*

	Reaction Frequency and Severity							
	None		Mild		Moderate		Severe	
Reaction	Con	NAC	Con	NAC	Con	NAC	Con	NAC
Vomiting	8	8	0	0	0	0	0	0
Erythema	4	2	4	6	0	0	0	0
Swelling	8	2	0	6	0	0	0	0
Flushing	8	7	0	1	0	0	0	0
Rash	7	8	1	0	0	0	0	0
Coughing	8	8	0	0	0	0	0	0
Nausea	8	7	0	0	0	1	0	0

NAC, *N*-acetylcysteine; Con, saline control.

scavenging effects of NAC might alleviate Na^+-K^+-ATPase inactivation, enhance muscle K^+ regulation, and thereby improve prolonged exercise performance. However, we recently demonstrated converse effects, that NAC infusion in untrained humans impaired plasma K^+ regulation during intense exercise (27). The mechanism is unknown, but this possible conflict is of potential importance and requires resolution. During prolonged submaximal exercise, K^+ fluxes are expected to be markedly less compared with our earlier intense exercise study (27). Therefore, we investigated whether NAC would improve plasma K^+ regulation during prolonged submaximal exercise. We hypothesized that NAC would enhance K^+ regulation, as evidenced by a decreased rise in plasma K^+ concentration ($[K^+]$) and a lesser ratio of rise in K^+ concentration to work ($\Delta[K^+]$/work). Finally, because NAC infusion attenuated plasma H^+ concentration ($[H^+]$) during intense intermittent exercise (27), we also explored the effects of NAC on plasma $[H^+]$ and other electrolytes during prolonged, submaximal exercise.

METHODS

Subjects

Eight male subjects (age 21.3 ± 2.3 yr, body mass 77.81 ± 10.50 kg, height 179.4 ± 4.4 cm; means $\pm$ SD) volunteered for the study

Table 2. *Individual time to fatigue during preexperimental familiarization and variability prolonged, submaximal cycling exercise trials*

	Fatigue Time, min			
Subject No.	Familiarization trial	Variability trial 1	Variability trial 2	CV, %
1	9.6	11.6	12.6	5.8
2	11.2	15.2	14.1	4.9
3	19.3	24.6	25.6	2.8
4	9.8	13.7	12.4	6.8
5	23.2	21.9	18.5	11.8
6	8.6	13.0	14.2	6.2
7	12.3	17.4	15.1	9.9
8	3.6	5.30	6.2	11.1
Means $\pm$ SE	12.2 ± 2.1	15.3 ± 2.1	14.8 ± 1.9	7.4 ± 1.1

Each exercise trial comprised 45 min at 70% of peak oxygen consumption ($\dot{V}O_{2peak}$) and then 90% $\dot{V}O_{2peak}$ continued to fatigue. Time to fatigue at 90% $\dot{V}O_{2peak}$ was used as an index of performance. Coefficient of variation (CV) was calculated from variability *trials 1* and *2*.
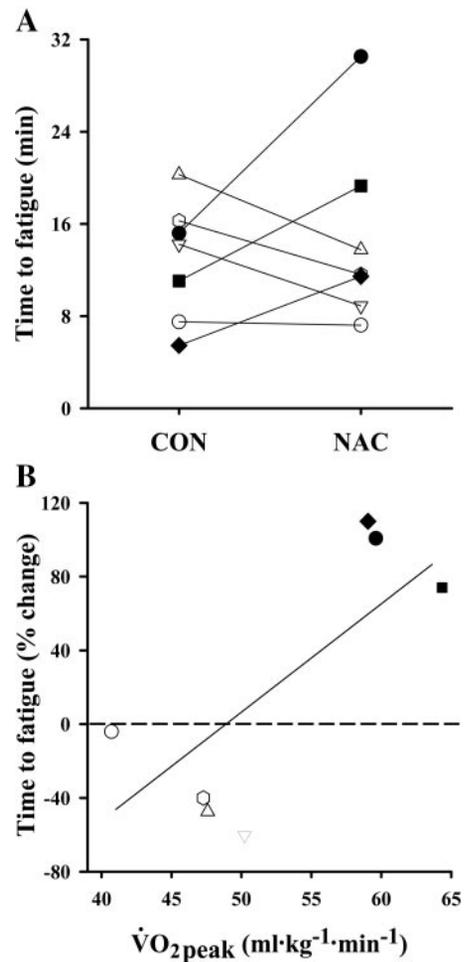

Fig. 1. Effect of *N*-acetylcysteine (NAC) and saline (Con) infusion on time to fatigue during prolonged exercise comprising 45 min at 70% of peak oxygen consumption ($\dot{V}O_{2peak}$), then to fatigue at 90% $\dot{V}O_{2peak}$ in healthy humans. *A*: individual time to fatigue at 90% $\dot{V}O_{2peak}$. *B*: scatterplot showing percentage change in time to fatigue at 90% $\dot{V}O_{2peak}$ with NAC relative to Con. A positive correlation was found between percentage change with NAC and $\dot{V}O_{2peak}$ ($y = 6.3x + 307.3$; $r = 0.78$; $P < 0.05$). $n = 7$ Subjects.

after being informed of all risks and giving written, informed consent. The subjects comprised four recreationally active team sports participants who trained or competed one to two times per week and four endurance-trained cyclists who trained four to five times per week. Subjects refrained from vigorous activity and avoided ingesting caffeine, alcohol, or other drugs in the 24 h before exercise 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, Netherlands).

$\dot{V}O_{2\ peak}$. Subjects first completed an incremental exercise test to determine their peak oxygen consumption ($\dot{V}O_{2\ peak}$), with all equipment, calibration, and procedures as previously detailed (22, 27).

Prolonged, submaximal exercise protocol. Subjects completed a total of five prolonged, submaximal exercise trials. The first trial was for familiarization purposes, the second and third trials were to determine the within-subject variability of their time to fatigue, whereas the final two trials were the NAC (Parvolex, Faulding
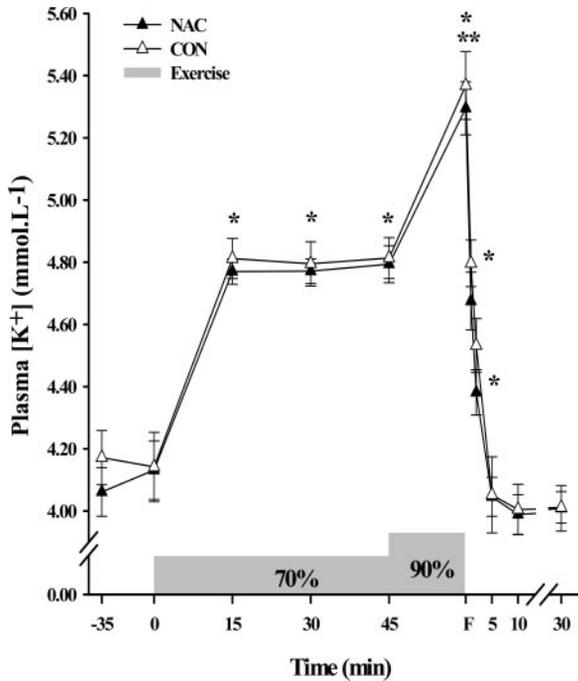
Fig. 2. Effect of NAC (▲) and Con (△) infusion on plasma potassium concentration ($[K^+]$) during prolonged exercise. Shaded bar denotes exercise comprising 45 min at 70% $\dot{V}_{O_2peak}$, then to fatigue (F) at 90% $\dot{V}_{O_2peak}$. *Significant time main effect; greater than preinfusion (-35 min, $P < 0.005$). **Significantly different from 45 min ($P < 0.05$). Values are means $\pm$ SE; $n = 7$ subjects.

Pharmaceuticals) or saline (Con) infusion trials (27). The prolonged, submaximal cycling exercise test comprised an initial 45 min at a work rate corresponding to 70% $\dot{V}_{O_2 peak}$, followed by exercise at 90% $\dot{V}_{O_2 peak}$, with subjects cycling at 100 rpm until fatigue. Fatigue was defined as an inability to maintain pedal cadence above 60 rpm. The time to fatigue at 90% $\dot{V}_{O_2 peak}$ was used as an index of performance. Prolonged exercise was chosen because NAC attenuates fatigue during repetitive, low-frequency electrical stimulation of limb muscle and diaphragm in humans (38, 43).

Experimental trials. The two experimental trials investigated the effects of intravenous NAC (27) during voluntary, prolonged, submaximal exercise. These trials were conducted in a double-blind, randomized, counterbalanced design to determine the effects of NAC or saline infusion on exercise performance and K^+ regulation. For
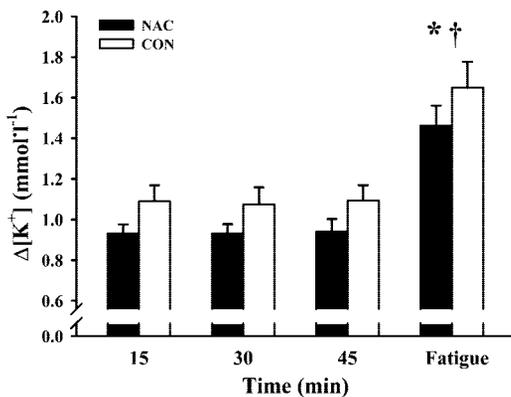

Fig. 3. Effect of NAC and Con infusion on the rise in plasma potassium ($\Delta[K^+]$) during prolonged submaximal exercise. *Significant time main effect; greater than 15 min ($P < 0.05$); †NAC < Con ($P < 0.05$). Values are means $\pm$ SE; $n = 7$ subjects.
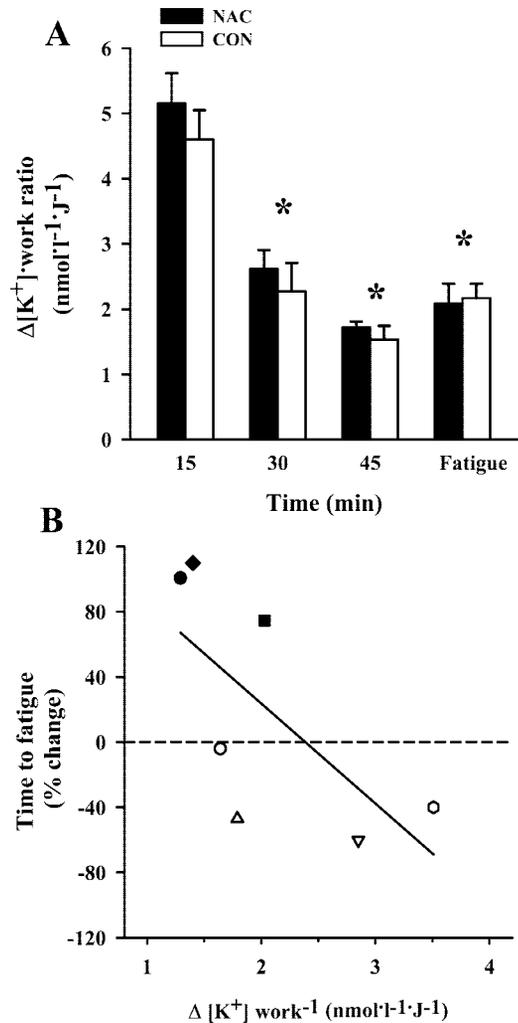

Fig. 4. *A*: effect of NAC and Con infusion on ratio of rise in K^+ concentration to work ($\Delta[K^+]$/work) during prolonged exercise. *B*: scatterplot showing percentage change in time to fatigue at 90% $\dot{V}_{O_2peak}$ with NAC vs. $\Delta[K^+]$/work ($y = -61.3x + 146.1$; $r = -0.71$; $P = 0.07$). *Significant time main effect; less than 15 min ($P < 0.005$). Values are means $\pm$ SE; $n = 7$ subjects.

ethical reasons, the attending medical practitioner was nonblinded. Arterialized venous blood was sampled from a dorsal hand vein (14) at rest, during exercise at 15, 30, 45 min and fatigue, and at 1, 2, 5, 10, and 30 min of recovery. Expired gases were measured over 5-min periods at 10-min intervals during exercise. Subjects also consumed standard food packages for 24 h before their two experimental trials.

NAC Infusion

The NAC intravenous infusion protocol was as previously described in healthy subjects (27). Briefly, an initial loading dose of 125 mg·kg^{-1}·h^{-1} was used for 15 min to increase plasma NAC concentration ([NAC]), followed by a constant infusion of 25 mg·kg^{-1}·h^{-1} to achieve a plateau in [NAC]. This was continued for 20 min before exercise and continued throughout exercise until fatigue. We have shown that this protocol considerably reduced the initial high [NAC] and avoided the associated adverse reactions compared with previously reported bolus NAC infusion protocols (27). Furthermore, this protocol preserves blood redox status during exercise, by attenuating the decline of reduced glutathione and the rise in oxidized glutathione in blood during exercise (27).

Assessing reaction severity. Adverse reactions to NAC were continually assessed throughout the experimental trials. Reactions were

graded as either none (no adverse effects were observed); mild (adverse events observed but not causing discomfort to subject and/or interruption of exercise protocol); moderate (adverse effects causing discomfort to subject and interruption of exercise protocol, but no active treatment after stopping infusion); or severe (adverse effects causing discomfort to subject, interruption of the exercise protocol, and active treatment after stopping infusion). Subjects were also monitored for more serious adverse reactions including angioedema, tachycardia, bronchospasm, dyspepsia, and conjunctival irritation, as reported in previous studies (see references in Ref. 27).

Blood Processing and Analyses

Two blood samples were drawn in rapid succession at each sample point. The first 1-ml sample was taken by using a syringe containing lithium heparin (RapidLyte, Chiron Diagnostics), for immediate plasma pH, gas, and electrolyte analyses, including sodium ($[Na^+]$), chloride ($[Cl^-]$), and calcium ($[Ca^{2+}]$) concentrations, by using an automated analyzer (Ciba Corning 865, Bayer). A second 5-ml sample was used for measurement of blood hemoglobin concentration ($[Hb]$) and hematocrit (Hct) using an automated analyzer (Sysmex, K-800, Kobe, Japan), plasma K^+ concentration ($[K^+]$), and reduced and total thiols in blood and plasma. All blood and plasma were processed as previously reported (27). NAC concentration was determined by HPLC (Waters Associates), with fluorescence detection (Hitachi, Tokyo, Japan) (27). Glutathione and cysteine unfortunately could not be analyzed because of a laboratory freezer failure.

Calculations

The decline in plasma volume from rest with exercise was calculated from changes in $[Hb]$ and Hct (16). The rise in plasma $[K^+]$ above rest ($\Delta[K^+]$) was calculated for each exercise value. The ratio of $\Delta[K^+]$ divided by cumulative work output during exercise ($\Delta[K^+]$/work, $nmol \cdot l^{-1} \cdot J^{-1}$) was calculated as an index of plasma K^+ regulation (16, 26). Red blood cell NAC concentration was calculated as described earlier (27).

Statistical Analyses

All data are presented as means ± SE, except anthropometric data. Single comparisons (e.g., time to fatigue) were analyzed by use of a paired Student's t-test. A one-way ANOVA with repeated measures was used for [NAC]. All other blood analyses were analyzed via a two-way (treatment, time) ANOVA with repeated measures on both factors. Post hoc analyses used the Newman-Keuls test. Correlation analyses used least square linear regression. Significance was accepted at $P < 0.05$. 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).

RESULTS

NAC and Adverse Reactions

Total NAC content infused was 5.09 ± 0.23 g. One subject suffered nausea during NAC infusion and did not complete the exercise trial. With cessation of the NAC infusion, no treatment was required. Importantly, no severe adverse reactions were observed (Table 1).

Exercise Performance Variability and Effects of NAC

The subjects' $\dot{V}O_{2\,peak}$ was 52.3 ± 2.8 $ml \cdot kg^{-1} \cdot min^{-1}$, and their work rates corresponding to 70 and 90% $\dot{V}O_{2\,peak}$ were 156 ± 12 and 230 ± 18 W, respectively. Good reproducibility was seen in the time to fatigue at 90% $\dot{V}O_{2\,peak}$ during the two variability trials, with a CV of 7.4 ± 1.2% (Table 2). No significant differences were found between trials for time to

fatigue at 90% $\dot{V}O_{2\,peak}$ (NAC 14.68 ± 1.72 vs. Con 12.5 ± 2.06 min) or total work (NAC 627.7 ± 69.8 vs. Con 588.2 ± 30.9 kJ). However, individual time-to-fatigue data showed tremendous variability in responsiveness to NAC, with a CV of 31.8 ± 16.1% (Fig. 1A). This was clearly at odds with their performance stability in the trials used to determine variability (Table 2). To attempt to explain this result, time to fatigue with NAC for each individual was expressed as a percentage change relative to Con trials and then plotted against aerobic power (Fig. 1B). A strong positive correlation was found between percentage change in time to fatigue during NAC trials and $\dot{V}O_{2\,peak}$ ($r = 0.78$; $P < 0.05$; Fig. 1B), indicating a different responsiveness to NAC in subjects with divergent $\dot{V}O_{2\,peak}$.

Plasma [K+]

Plasma $[K^+]$ increased above preinfusion levels throughout exercise, increased further at fatigue, and then declined during recovery ($P < 0.05$), returning to preinfusion levels at 10 min (Fig. 2). No significant differences were found between NAC and Con. $\Delta[K^+]$ did not differ between 15 and 45 min but was increased at fatigue ($P < 0.005$). The $\Delta[K^+]$ during exercise at
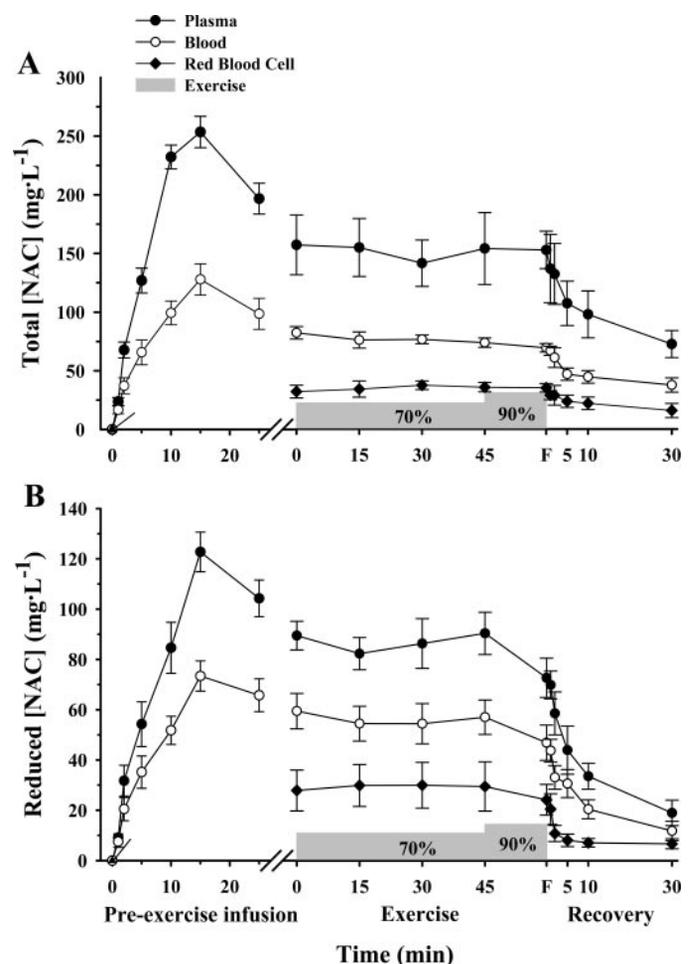
Fig. 5. Total (*A*) and reduced (*B*) NAC concentration ([NAC]) in plasma, blood, and red blood cells before, during, and after prolonged, submaximal exercise. Shaded bar denotes exercise comprising 45 min at 70% $\dot{V}O_{2peak}$, then to fatigue (F) at 90% $\dot{V}O_{2peak}$. Values are means ± SE; $n = 7$ subjects. Preexercise infusion lasted 35 min. Significant time main effect with all times greater than preinfusion ($P < 0.05$, asterisks not shown for clarity).

70% $\dot{V}_{O2\,peak}$ tended to be lower with NAC ($P < 0.07$) compared with Con and at fatigue was lower in NAC compared with Con ($P < 0.01$, Fig. 3).

The $\Delta[K^+]$/work decreased during exercise, being higher at 15 min than subsequent exercise times ($P < 0.05$), with no differences between NAC and Con (Fig. 4A). However, the $\Delta[K^+]$/work and percentage change in time to fatigue tended to be inversely related ($r = -0.71$; $P < 0.07$; Fig. 4B).

NAC

Plasma, blood, and red blood cell NAC. During the 15-min loading infusion phase, total plasma [NAC] ([NAC]$_{tp}$) increased progressively until a peak of 253.51 ± 34.94 mg/l at 15 min ($P < 0.05$, Fig. 5A). During the maintenance infusion phase, [NAC]$_{tp}$ decreased to 182.02 ± 29.70 mg/l immediately before exercise and then plateaued with no further changes during exercise. In recovery, [NAC]$_{tp}$ decreased rapidly from fatigue levels but remained higher than preinfusion at 30 min after stopping the infusion ($P < 0.05$). A similar pattern of change was found for reduced plasma [NAC] ([NAC]$_{rp}$) (Fig. 5B), except that the decline in [NAC]$_{rp}$ at 30 min of recovery was not significant compared with fatigue levels. An identical pattern of change was found for both total and reduced forms of NAC in whole blood and red blood cells during the loading, maintenance, exercise, and recovery periods (Fig. 5).

Fluid Shifts, Plasma Electrolyte Concentrations, and Acid-Base Status

Both [Hb] and Hct were higher than preinfusion levels, and thus plasma volume declined, during exercise and until 30 min of recovery ($P < 0.05$, Table 3). No differences between NAC and Con were found for [Hb], Hct, or decline in plasma volume. Plasma [Na$^+$] increased above preinfusion levels throughout the exercise period until 2 min of recovery ($P < 0.05$, Table 3). Plasma [Cl$^-$] did not differ during exercise or recovery, whereas plasma [Ca^{2+}] was increased above preinfusion levels at fatigue until 2 min of recovery ($P < 0.05$, Table 3). Compared with preinfusion levels, plasma [H$^+$] was increased, whereas plasma P_{CO_2} and bicarbonate ([HCO$_3^-$]) fell, throughout exercise and recovery ($P < 0.05$, Table 3). No differences between NAC and Con were found for these plasma electrolyte or acid-base variables.

DISCUSSION

Ergogenic Effects of NAC Are Dependent on $\dot{V}_{O2\,peak}$

We hypothesized a performance enhancement with NAC, on the basis of the findings that NAC infusion attenuated fatigue during low-frequency electrical stimulation of human tibialis anterior muscle (38). Although there was no effect on time to fatigue in the whole group, the markedly different responsiveness of individuals to NAC necessitated more careful evaluation. For the first time we show that the effects of NAC on prolonged exercise are dependent on $\dot{V}_{O2\,peak}$, with a tendency for time to fatigue to be increased in the fitter subjects. Further research in a larger group of subjects with homogenous $\dot{V}_{O2\,peak}$ is required to validate these intriguing preliminary findings.

Table 3. *Hematology, calculated fluid shifts, plasma acid-base variables, and electrolyte concentrations during prolonged, submaximal exercise before, during, and after NAC and Con infusion*

Variable and Treatment	Preinfusion	Preexercise	Exercise Time, min				Recovery, 30 min
			15	30	45	Fatigue	
[Hb], g/dl							
Con	14.6±0.8	14.8±0.2	15.5±0.1*	15.6±0.2*	15.5±0.2*	15.8±0.1*	14.4±0.2
NAC	14.7±0.3	14.7±0.3	15.6±0.3*	15.5±0.2*	15.5±0.2*	15.8±0.2*	14.4±0.2
Hct, %							
Con	44.5±0.6	45.0±0.6	47.2±0.7*	47.1±0.5*	47.0±0.6*	47.9±0.7*	43.8±0.4
NAC	44.5±0.8	44.8±0.8	47.4±0.7*	47.0±0.7*	46.7±0.5*	48.2±0.6*	43.2±0.2
ΔPV, %							
Con		−1.9±0.4	−10.4±0.8*	−10.6±0.5*	−9.7±0.7*	−13.7±0.9*	3.3±2.1*
NAC		−1.0±0.7	−10.9±0.9*	−9.4±1.2*	−9.0±1.6*	−13.3±1.4*	4.7±2.1*
[H$^+$], nmol/l							
Con	39.1±0.4	38.4±0.3	42.0±0.7*	40.8±0.8*	39.8±0.9*	45.2±1.4*	42.6±0.3*
NAC	39.1±1.0	37.8±1.1	42.4±0.5*	41.5±0.5*	40.7±0.5*	46.0±1.3*	42.0±0.6*
[HCO$_3^-$], mmol/l							
Con	26.4±0.5	25.4±0.6	23.1±0.4*	23.0±0.3*	23.9±0.3*	18.0±0.9*	23.1±1.4*
NAC	26.0±0.4	25.0±0.2	22.4±0.4*	22.4±0.6*	23.2±0.6*	18.0±0.9*	24.1±0.5*
P_{CO_2}, Torr							
Con	41.3±0.6	39.7±0.5	40.5±0.4	39.1±0.5*	35.9±0.7*	34.7±1.4*	34.8±1.0*
NAC	43.2±0.7	39.5±0.8	40.3±0.4	38.4±1.2*	38.8±1.4*	34.0±1.4*	30.1±2.5*
[Na$^+$], mmol/l							
Con	139.2±1.3	138.8±1.0	140.5±1.1*	141.5±0.7*	140.4±0.8*	143.0±0.8*	138.5±0.7
NAC	139.1±0.8	139.9±0.6	141.8±0.8*	141.8±0.8*	141.6±0.5*	143.4±0.7*	138.9±0.6
[Cl$^-$], mmol/l							
Con	104.0±0.3	103.2±0.9	104.8±1.4	103.0±0.8	102.0±1.3	107.7±1.6	105.7±2.7
NAC	104.0±0.5	103.9±0.9	103.5±0.7	102.1±0.6	103.1±0.7	105.1±0.9	101.1±0.8
[Ca^{2+}], mmol							
Con	1.21±0.01	1.23±0.01	1.23±0.01	1.28±0.01	1.27±0.01	1.30±0.02*	1.20±0.02
NAC	1.23±0.01	1.22±0.01	1.24±0.01	1.25±0.01	1.25±0.01	1.31±0.01*	1.20±0.01

Values are means ± SE, $n = 7$ NAC, $n = 8$ Con. [Hb], hemoglobin concentration; Hct, hematocrit; ΔPV, decline in plasma volume; brackets denote concentration. *Significant main effect for time: different from preinfusion $P < 0.05$.

NAC Improves K^+ Regulation During Exercise

An interesting finding was the reduced $\Delta[K^+]$ at fatigue, which is consistent with our hypothesis that NAC would enhance K^+ regulation during exercise. A possible underlying mechanism for such an effect might reflect ROS inhibition of Na^+-K^+-ATPase activity, as shown in cardiac muscle (44), sarcolemmal vesicle (21), and skeletal muscle-derived L6 cells (39). Importantly, acute exercise also depresses maximal Na^+-K^+-ATPase activity in skeletal muscle (12–14), which may also be consequent to increased ROS. Thus one possible explanation for the lesser $\Delta[K^+]$ during exercise with NAC is an attenuation of the ROS effect on skeletal muscle Na^+-K^+-ATPase activity. However, no studies have investigated the effects of ROS and/or antioxidants on human skeletal muscle Na^+-K^+-ATPase activity. The nonsignificant negative correlation between time to fatigue and $\Delta[K^+]$/work ($r = -0.71$, $n = 7$) does suggest a possible link between exercise performance and K^+ regulation with NAC. The lack of significance may reflect a type II error due to the small sample size. This possible relationship should be explored in further studies.

Other Potential Mechanisms

It is possible that several factors may underlie the observed relationship between percentage change in time to fatigue with NAC and $\dot{V}O_{2\,peak}$. We recently showed that this intravenous NAC infusion protocol had marked effects on blood redox status during intense, intermittent exercise, such that NAC attenuated the decline in reduced glutathione and rise in oxidized glutathione with exercise (27). Here we report that almost identical [NAC] changes in plasma, whole blood, and red blood cell occurred during prolonged, as in intense, exercise. Therefore, it is highly likely that NAC would have exerted similar effects on blood redox status in prolonged exercise. Exogenous glutathione supplementation enhanced glutathione biosynthesis and increased swimming endurance by up to 141% in rats (7, 29). Thus enhanced glutathione synthesis (27) is consistent with an apparent increased time to fatigue in fitter individuals with NAC infusion.

Fiber-type-specific effects may also underlie the observed positive relationship between percentage change in time to fatigue with NAC and $\dot{V}O_{2\,peak}$. Individuals with high $\dot{V}O_{2\,peak}$ possess a higher proportion of slow-twitch fibers (22), which show a greater increase in isometric force with the reducing agent dithiothreitol, than fast muscle (30). Slow-twitch fibers also have increased mitochondrial content (15), which is considered a major source of ROS (6). It is likely that individuals with a high $\dot{V}O_{2\,peak}$ produce a greater amount of ROS than subjects with a lower $\dot{V}O_{2\,peak}$, which would be exacerbated when cycling at a higher absolute exercise intensity (1, 23). This may be counterbalanced by increased skeletal muscle endogenous antioxidant enzyme activities with training (1). However, there is reduced protection of the mitochondria against ROS after endurance training (42). Thus NAC protection of susceptible oxidative fibers from ROS could underlie the relationship between percentage change in time to fatigue with NAC and $\dot{V}O_{2\,peak}$. Further studies should address possible different fiber-type effects.

Sarcoplasmic reticulum Ca^{2+} regulation is sensitive to redox modulation, with ROS enhancing ryanodine receptor opening, which may also diminish with prolonged exposure (33). Hydrogen peroxide also decreases myofibrillar Ca^{2+} sensitivity and force, which is reversed by the reducing agent dithiothreitol (2). This effect is more prominent in slow-twitch fibers and may be glutathione dependent (31). In addition, Ca^{2+} uptake is decreased with increased ROS concentration (32, 45). Whether these effects occur in human muscle remains to be elucidated and is worthy of further investigation. Finally, NAC had no effects on fluid shifts, acid-base status, or plasma electrolyte concentrations during exercise.

In conclusion, NAC infusion effects on fatigue during prolonged cycling exercise may be dependent on $\dot{V}O_{2\,peak}$. Furthermore, NAC improved K^+ regulation as evidenced by a decreased rise in plasma $[K^+]$. These intriguing findings demand further studies for verification and to determine possible mechanisms.

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