Effect of *N*-acetylcysteine on Cycling Performance after Intensified Training

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ABSTRACT

SLATTERY, K. M., B. DASCOMBE, L. K. WALLACE, D. J. BENTLEY, and A. J. COUTTS. Effect of *N*-acetylcysteine on Cycling Performance after Intensified Training. *Med. Sci. Sports Exerc.*, Vol. 46, No. 6, pp. 1114–1123, 2014. **Purpose**: This investigation examined the ergogenic effect of short-term oral *N*-acetylcysteine (NAC) supplementation and the associated changes in redox balance and inflammation during intense training. **Methods**: A double-blind randomized placebo-controlled crossover design was used to assess 9 d of oral NAC supplementation (1200 $mg \cdot d^{-1}$) in 10 well-trained triathletes. For each supplement trial (NAC and placebo), baseline venous blood and urine samples were taken, and a presupplementation cycle ergometer race simulation was performed. After the loading period, further samples were collected preexercise, postexercise, and 2 and 24 h after the postsupplementation cycle ergometer race simulation. Changes in total antioxidant capacity, ferric reducing ability of plasma, reduced glutathione, oxidized glutathione, thiobarbituric acid-reactive substances, interleukin 6, xanthine oxidase, hypoxanthine, monocyte chemotactic protein 1, nuclear factor κB, and urinary 15-isoprostane F_{2t} concentration were assessed. The experimental procedure was repeated with the remaining supplement after a 3-wk washout. Eight participants completed both supplementation trials. **Results**: NAC improved sprint performance during the cycle ergometer race simulation ($P < 0.001$, $\eta_p^2 = 0.03$). Supplementation with NAC also augmented postexercise plasma total antioxidant capacity ($P = 0.005$, $\eta_p^2 = 0.19$), reduced exercise-induced oxidative damage (plasma thiobarbituric acid-reactive substances, $P = 0.002$, $\eta_p^2 = 0.22$; urinary 15-isoprostane F_{2t} concentration, $P = 0.010$, $\eta_p^2 = 0.431$), attenuated inflammation (plasma interleukin 6, $P = 0.002$, $\eta_p^2 = 0.22$; monocyte chemotactic protein 1, $P = 0.012$, $\eta_p^2 = 0.17$), and increased postexercise nuclear factor κB activity ($P < 0.001$, $\eta_p^2 = 0.21$). **Conclusion**: Oral NAC supplementation improved cycling performance via an improved redox balance and promoted adaptive processes in well-trained athletes undergoing strenuous physical training. **Key Words:** ANTIOXIDANT, OXIDATIVE STRESS, INFLAMMATION, NUCLEAR FACTOR κB

The oxidant–antioxidant balance during exercise has been shown to influence skeletal muscle contractile capacity (35) and adaptation to exercise (17). Moderate exercise-induced elevations in reactive oxidative species (ROS) within skeletal muscle can enhance contractile function and play an important role in the reduction–oxidation (redox) activation of numerous cellular signaling cascades, which promote skeletal muscle gene expression and integrity (32). However, during unaccustomed, high-intensity, prolonged, or strenuous exercise, the antioxidant defense system may be unable to buffer the oxidant concentration within skeletal muscle. This may lead to an accumulation of ROS, which has been shown to inhibit excitation coupling and contribute to the development of fatigue in skeletal muscle (35). It has been proposed that the consumption of exogenous antioxidant compounds may increase the total antioxidant capacity (TAC) of the intra- and extracellular milieu. This improved antioxidant status may then assist in the maintenance of an optimal redox balance during exercise and in turn promote skeletal muscle performance.

N-acetylcysteine (NAC) is a thiol containing compound that acts to minimize the exercise-induced oxidative insult through its actions as a cysteine donor in the maintenance of glutathione homeostasis and via direct scavenging of ROS (10). Several studies have demonstrated acute improvements in both aerobic and resistance-based performance tasks when participants were supplemented either orally or by infusion with the antioxidant NAC (7,22,26,35). Experimental evidence suggests that NAC supplementation exerts an acute ergogenic effect through the minimization of oxidant interference in the activity of key ion transporters and ion channel proteins (25) and through the improved regulation of calcium release within contracting myofibers (1). Recent *in vivo* research on the

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force-frequency characteristics of mouse diaphragm fiber bundles has also demonstrated that NAC treatment promotes fatigue resistance by delaying the slowing of cross-bridge detachment (14). In addition, NAC may improve hemodynamics via a secondary vasodilatory effect during exercise to promote blood flow and muscle perfusion (35). NAC has also been shown to have a role in the modulation of hematological parameters and erythropoietin secretion, which would contribute to an improved performance capacity (40). For instance, an 8-d supplementation period with NAC lead to significant increases in plasma erythropoietin (+26%) and hemoglobin (+9%) in eight healthy males compared with a control group (40). These data suggest that supplementation with NAC can induce several physiological changes that would enhance competitive performance. However, a beneficial effect of NAC during exercise has not been consistently demonstrated (24). Further research is required to firmly establish the ability of NAC to improve athletic performance in a practical sports setting.

A reduction in oxidant release during skeletal muscle contractions observed with NAC supplementation may also impact the inflammatory response to exercise and alter the expression of redox-sensitive gene transcription factors (30). Previously, NAC supplementation has been shown to promote the up-regulation of anti-inflammatory cytokines and to minimize skeletal muscle injury after fatiguing contractile activity (31). This reduction in oxidative damage to contractile proteins within the myofibers may therefore prevent the decline in force production associated with exercise-induced muscle damage and increase the capacity for athletes to continue to perform strenuous physical training (33). However, a decrease in exercise-induced skeletal injury and/or inflammation has not been consistently shown with NAC supplementation (6,7). Several investigations have reported elevations in inflammatory measures and muscle damage with antioxidant supplementation (6,37). This has led to the hypothesis that antioxidant supplementation may suppress the activation of the inflammatory cascade after exercise-induced muscle damage and cause interference with the subsequent repair and remodeling of skeletal tissue (37).

The redox-sensitive transcription factor, nuclear factor κB (NF-κB), plays a key role in the regulation of genes involved both in the inflammatory cascade (i.e., interleukin 6 [IL-6] and monocyte chemotactic protein-1 [MCP-1]) and within the antioxidant enzyme system (20). Studies have reported elevations in NF-κB activity after both isolated muscle contractions and whole-body exercise (11,17). Because of the large number of genes that are up-regulated via the NF-κB pathway, it has been proposed that NF-κB may be a central mediator of the physiological adaption to exercise stimuli (17,32). Indeed, the inhibition of NF-κB phosphorylation with supplementation (i.e., allopurinol [18] and NAC [13]) has been shown to blunt positive exercise-induced physiological changes from occurring. However, not all investigations have reported an inhibition of NF-κB phosphorylation with antioxidant supplementation (30). These conflicting results on NF-κB activation highlight the complexity of exercise-induced redox-sensitive signaling pathways. Although transient activation of the NF-κB pathway is required to regulate physiological adaptation, chronic increases in NF-κB activation have been associated with unwanted skeletal muscle atrophy and insulin resistance (20). However, the amount of exercise-induced oxidative perturbation that leads to this chronic activation of NF-κB remains unclear. Further research is required to establish the effects of NAC supplementation on the NF-κB redox signaling pathway during a block of intense physical training in well-trained athletes.

Previous research has identified a delicate balance between training-induced oxidant production and antioxidant protection. During strenuous periods of physical training, considerable stress is placed upon the antioxidant defense system, which may be unable to prevent the occurrence of excessive exercise-induced oxidative damage and inflammation. For example, a 4-wk overtraining period was shown to induce a state of oxidative stress and impair duathlon performance in nine male triathletes (27). Athletes undergoing heavy training periods may require increased antioxidant support via exogenous supplementation (28). However, the excessive consumption of antioxidant compounds has also been demonstrated to have a negative effect on the up-regulation of redox-mediated adaptive processes to training stimuli (17). It is therefore important to determine an appropriate match between additional antioxidant requirements and increased training demands to promote exercise-induced physiological adaptations.

The aims of the current investigation were to establish the ergogenic potential of oral NAC supplementation on athletic performance during a cycle ergometer race simulation in well-trained triathletes and to examine the impact of NAC supplementation on oxidative stress parameters, antioxidant capacity, physiological adaptation (measured via changes in NF-κB p65 activity), and blood-borne markers of inflammation during a block of intensive training. It was anticipated that the results of the current investigation would demonstrate that supplementation with NAC can enhance antioxidant capacity and improve performance in a specific bout of exercise. However, prolonged supplementation with NAC may suppress the oxidative and immune response to exercise and hamper the adaptive processes in athletes.

METHODS

Participants. Ten well-trained male triathletes volunteered to participate in the investigation (age = 23.6 ± 3.2 yr, height = 179.8 ± 4.4 cm, body mass = 70.5 ± 7.2 kg, $\dot{V}O_{2peak}$ = 63.3 ± 4.8 $mL \cdot kg^{-1} \cdot min^{-1}$). The athletes regularly completed 15–25 h of swimming, cycling, and running each week. Before the study, participants were informed of the testing requirements and potential risks involved in the study and gave written and verbal consent. The study was approved by the University of Technology, Sydney (UTS) Human Ethics Committee (trial no. 2010/0254 HREC 2009/164), and the use of oral NAC

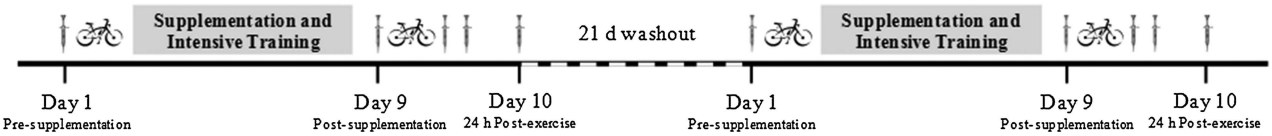

FIGURE 1—Schematic diagram of the crossover experimental design. Performance measures (cycle ergometer race simulation) were completed before and after each supplementation period (NAC and placebo). Baseline venous blood and urinary samples were assessed before the presupplementation cycle ergometer race simulation. Resting venous and urine samples were taken before the postsupplementation cycle ergometer race simulation. Postexercise venous and urinary measures were assessed immediately after and 2 and 24 h following the postsupplementation cycle ergometer race simulation.

was approved by the Therapeutic Goods Administration of Australia.

Experimental design. The study used a double-blind randomized placebo-controlled crossover design to investigate the effects of NAC supplementation on cycling performance and adaptation to physical training. In the 2 wk before the data collection period, participants were familiarized with the cycle ergometer race simulation (38) and completed a cycling power profile test to determine $\dot{V}O_{2peak}$ (34). All cycle tests throughout the study were performed on an SRM ergometer (Schoberer Rad Meßtechnik SRM GmbH, Jülich, Germany) under standardized laboratory conditions. On day 1 of the investigation, baseline venous blood and urine samples were taken, and a presupplementation cycle ergometer race simulation was completed. Participants then began a 9-d supplementation period of either NAC or placebo. On day 9 of the study, venous blood and urine samples were collected before, immediately after, and 2 and 24 h after the postsupplementation cycle ergometer race simulation. Time course changes were assessed in plasma for TAC, ferric reducing ability of plasma (FRAP), reduced glutathione (GSH)-to-oxidized glutathione (GSSG) ratio (GSH:GSSG), thiobarbituric acid-reactive substances (TBARS), xanthine oxidase (XO), hypoxanthine, IL-6, and MCP-1. In addition, changes in NF-κB within mononuclear cell extracts and urinary 15-isoprostane F_{2t} concentration (F_2-isoprostane) were also assessed at the same time points. After a 3-wk washout period, the experimental procedure was repeated with the remaining supplement (for schematic view of experimental design, see Fig. 1).

Supplementation. Participants refrained from consuming additional antioxidant supplements for at least 1 month before and throughout the investigation. Participants were randomly assigned in a double-blind manner to receive either 1200 mg·d^{-1} of NAC (2 × 600 mg capsules) or 700 mg·d^{-1} of a placebo (lactose; 2 × 350 mg capsules). NAC was purchased in powdered form (Batch: 254709, The Melbourne Food Ingredient Depot, Victoria, Australia), weighed on electronic scales (A&D Weighing, Seven Hills, NSW, Australia), and placed into capsules for the participants to consume. Capsules for both NAC and placebo were identical in size and appearance. An additional dose of NAC or placebo was also consumed 2 h before the postsupplementation cycle ergometer session. Plasma NAC levels were not measured. Instead, plasma GSH and TAC were used as surrogate measures to assess the effectiveness of NAC supplementation to increase antioxidant capacity. Participants completed 4-d food records during each testing period to ensure a similar dietary intake. The food records were analyzed using Foodworks software (version 6, 2009; Xyris, Qld, Australia). Participants were contacted on a daily basis during the supplementation period to ensure the supplements had been taken.

Cycle ergometer race simulation. After familiarization, participants performed the cycle ergometer session (Schoberer Rad Meßtechnik SRM GmbH) before and after each supplementation period. The exercise was similar to the 105-min fatigue-inducing cycle protocol described previously (38) (Table 1). This protocol was chosen to mimic the demands of a cycling race and allow for changes in both anaerobic and aerobic performance capacity to be assessed. The typical error of this protocol has been reported as 17.1 W and 2.1% (38). Participants were instructed to perform each sprint maximally and to complete as much work as possible during each steady-state time trial effort. HR (Suunto dual belt, Vantaa, Finland) and power data were recorded every second. Each session was analyzed using the SRM software program (Schoberer Rad Meßtechnik SRM GmbH) to determine average power output, work (kJ), and HR for each effort.

Physical training and daily analysis of life demands of athletes. Physical training was recorded during the experimental period using the session-RPE method,

TABLE 1. Cycle ergometer race simulation protocol.

Cycle Ergometer Race Simulation	
Warm-up	10 min with 3 × 3-s efforts
Set 1	12 × 5 s efforts with 30-s rest
Rest	5 min easy
Set 2	12 × 5 s efforts with 15-s rest
Rest	5 min easy
Set 3	12 × 5 s efforts with 5-s rest
Rest	5 min easy
Time trial 1	2 min
Rest	5 min easy
Set 4	6 × 10 s efforts with 60-s rest
Rest	5 min easy
Set 5	6 × 10 s efforts with 30-s rest
Rest	5 min easy
Set 6	6 × 10 s efforts with 10-s rest
Rest	5 min easy
Time trial 2	2 min
Rest	5 min easy
Set 7	4 × 15 s efforts with 90-s rest
Rest	5 min easy
Set 8	4 × 15 s efforts with 45-s rest
Rest	5 min easy
Set 9	4 × 15 s efforts with 15-s rest
Rest	5 min easy
Time trial 3	5 min
Rest	5 min easy

which is calculated as the product of training duration (min) and the mean training intensity (rating of perceived exertion CR-10) (16). The participants were asked to replicate a similar training program during each experimental period, which was supervised by the investigators or each athlete's respective coach. Likewise, participants recorded their training load in 3 wk preceding the first trial and replicated this program during the 3-wk washout period. Participants also completed the Daily Analyses of Life Demands for Athletes (DALDA) questionnaire at the same time each day to assess general stress levels and to determine stress-reaction symptoms of the participants (36). An elevated number of "worse than normal" responses were used to determine when an athlete was in a state of stress.

Biochemistry measures. Venous blood and urine samples were collected at five time points during each intervention. On day 1, a resting baseline sample was collected. Then after the 9-d supplementation period, additional samples were collected at rest, immediately after, and 2 and 24 h after the postsupplementation cycle ergometer race stimulation. Blood samples were collected into 2 × 6 mL EDTA lined vacutainer tubes and 1 × 8 mL cell preparation tube with sodium heparin (Becton Dickson, Franklin Lakes, NJ), which were centrifuged at 3000*g* for 15 min immediately after collection at 4°C. Plasma, supernatant containing mononuclear cells, and urine were transferred into 500-μL aliquots, frozen in liquid nitrogen, and stored at −80°C until assayed.

Enzyme-linked immunosorbent assay (ELISA) kits were used to measure the plasma concentration of MCP-1 (Quantikine Immunoassay; R&D Systems, Minneapolis, MN; intra-assay coefficient of variation [CV] = 7.4%), IL-6 (Quantikine HS ELISA, R&D Systems; intra-assay CV = 7.6%), TAC (Cayman Chemical Company, Ann Arbor, MI; intra-assay CV = 5.4%), TBARS (Oxford Biomedical Research, Rochester Hills, MI; intra-assay CV = 5.7%), XO, and hypoxanthine (Amplex Red Xanthine/Xanthine Oxidase Assay Kit; Invitrogen, Carlsbad, CA; intra-assay CV: XO = 5.4%, hypoxanthine = 2.2%). NF-κB p65 DNA binding activity was measured in nuclear extracts obtained from peripheral mononuclear cells using a nuclear extraction kit (Cayman Chemical Company) and analyzed with an NF-κB p65 Transcription Factor Assay Kit (Cayman Chemical Company; intra-assay CV = 6.3%). An ELISA kit was also used to measure the urinary F_2-isoprostane concentration (Oxford Biomedical Research), which was normalized against urinary creatinine concentration (Oxford Biomedical Research). All assay kits were completed according to the manufacturer's instructions on a Bio-Rad plate reader (Hercules, CA).

GSH and GSSG were measured in plasma treated with 3% 5-sulfosalicyclic acid and analyzed enzymatically according to the method of Baker et al. (3). Briefly, GSH concentration was determined by assessing the rate of enzymatic recycling using 5,5′-dithiobis 2-nitro-benzoic acid, glutathione reductase, and NADPH at 405 nm on a Bio-Rad plate reader (intra-assay CV = 7.3%). GSSG was measured using the same protocol after the removal of GSH from the plasma through the addition of 2-vinylpyridine and triethanolamine to each sample (intra-assay CV = 3.9%). Standard curves from stock solutions of GSH and GSSG were created using serial dilutions with 3% 5-sulfosalicyclic acid. The GSH:GSSG ratio was calculated as [(GSH − (2GSSG)] / GSSG.

The FRAP assay was conducted using a modification of the original method described by Benzie and Strain (4). Plasma samples and FRAP reagent (300 mM acetate buffer, 10 mM 2,4,6-tripyridyl-*s*-triazine, 40 mM hydrochloric acid, and 20 mM Iron (III) chloride hexahydrate) were added to a microtitre plate and incubated for 4 min at 37°C. Absorbance was measured on a Bio-Rad plate reader at 593 nm and compared with an Iron (II) sulfate standard curve (intra-assay CV = 2.4%). All reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Statistical analyses. Before using parametric statistical procedures, the assumption of sphericity was verified. Statistica 8.0 software (StatSoft Inc., Tulsa, OK) was used for all calculations. An *a priori* sample analysis revealed that eight pairs of subjects is the minimum required in a matched pair design to be able to reject the null hypothesis that this response difference is zero with probability (power) 0.8. The type I error probability associated with this test of this null hypothesis is 0.05 (G*Power version 3.1.3; Universität Kiel, Germany). Changes in biochemical parameters were analyzed using a repeated-measures factorial two-way ANOVA by condition (NAC and placebo supplementation) and time (presupplementation and postsupplementation preexercise, immediately postexercise, and 2 and 24 h postexercise). Performance variables during the cycle ergometer test were also analyzed with a repeated-measures factorial ANOVA by condition (NAC and placebo supplementation) and time (presupplementation and postsupplementation). If significant main effects were observed, a Scheffe *post hoc* analysis was used to locate the source of the differences. The magnitude of partial eta squared effects was interpreted as trivial (<0.001), small (0.001–0.089), moderate (0.09–0.25), and large (>0.25), respectively (8). A paired-samples *t*-test was used to determine differences in training load, DALDA, and nutrient intake between the experimental periods. Statistical significance was accepted at $P < 0.05$. Data are presented as mean ± SD.

RESULTS

Of the 10 participants, 8 completed both the NAC and the placebo trials. One participant withdrew due to injury, the other due to illness during the second supplementation period. Data from these participants were not included in the analysis. No adverse events or side effects were reported by participants for either the NAC or the placebo supplements. The participants completed a similar amount of training duration (NAC = 20.1 ± 3.7 h, placebo = 19.6 ± 2.8 h, $P = 0.72$) and intensity (RPE, NAC = 5.9 ± 1.4, placebo = 6.3 ± 0.7, $P = 0.74$). A significant increase ($P = 0.05$) was observed in the number of "worse than normal" responses reported during the placebo period (57.0 ± 54.3) than when the NAC

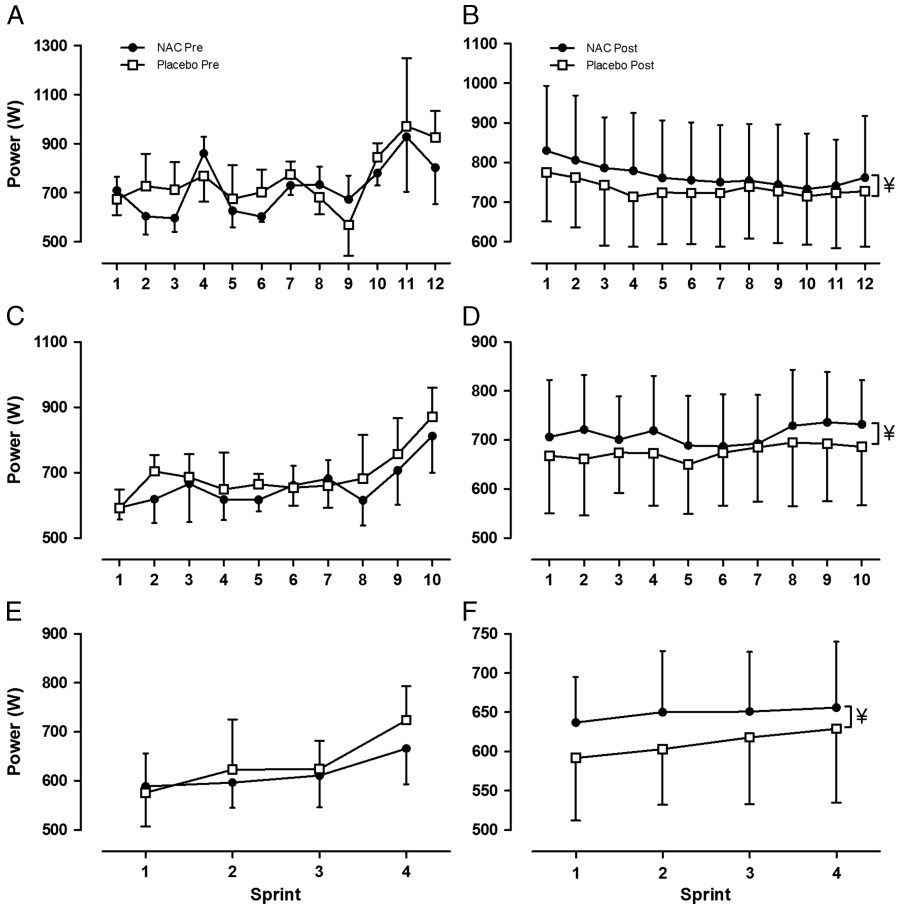

FIGURE 2—Changes in combined mean power during each effort across all repetitions during the presupplementation 5 s (A), 10 s (C), and 15 s efforts (E) and postsupplementation 5 s (B), 10 s (D), and 15 s (F) cycle ergometer race simulation (mean ± SD). ¥Significant main effect for condition ($P < 0.001$).

supplement (25.0 ± 19.7) was consumed. No significant difference was observed in the macro- or micronutrient intake of the participants during the two supplementation periods.

Presupplementation performance measures (power output, work, and HR) during the cycle ergometer race simulation were similar in both the NAC and placebo condition. Repeated sprint performance during the postsupplementation cycle ergometer race simulation, compared with the presupplementation cycle ergometer race simulation, was significantly improved ($P < 0.001$, $\eta_p^2 = 0.03$) during the 5-, 10-, and 15-s efforts when athletes consumed NAC as opposed to the placebo (Fig. 2). No change was observed in mean HR, total work, or mean power during each steady-state time trial effort between the postsupplementation NAC and placebo trials (Table 2). No significant differences were shown in lactate or RPE measures taken throughout the cycle sessions (data not shown).

Participants began each experimental trial in a similar state. No significant differences were observed in any of the measured biochemical variables before commencing each supplementation period (NAC and placebo). After the 9-d NAC supplementation, plasma antioxidant capacity increased as observed via a significant interaction effect in plasma TAC ($P = 0.005$, $\eta_p^2 = 0.19$) (Fig. 3A) and a greater plasma GSH concentration by condition (NAC = 13.16 ± 0.98 μM, placebo = 10.19 ± 0.98 μM, $P = 0.036$, $\eta_p^2 = 0.06$) (Fig. 3B). This finding was not reflected in plasma FRAP measures (Fig. 3E). However, a significant effect was observed in plasma FRAP for time with an overall increase from pre to postexercise ($P = 0.016$, $\eta_p^2 = 0.16$), without an interaction or condition main effect. No change was observed in the plasma GSSG or GSH:GSSG ratio for time or condition (Figs. 3C and 3D).

TABLE 2. Time trial performance parameters before and after supplementation with both NAC and placebo (mean ± SD).

		Time Trial 1		Time Trial 2		Time Trial 3	
		Pre	Post	Pre	Post	Pre	Post
Mean power (W)	Placebo	405 ± 31	402 ± 41	375 ± 46	384 ± 34	327 ± 36	338 ± 39
	NAC	390 ± 46	399 ± 38	392 ± 42	399 ± 46	327 ± 40	345 ± 40
Mean HR (bpm)	Placebo	166 ± 9	166 ± 10	161 ± 9	162 ± 7	165 ± 9	167 ± 8
	NAC	161 ± 7	158 ± 9	161 ± 8	157 ± 8	164 ± 9	163 ± 10
Total work (kJ)	Placebo	44 ± 7	45 ± 5	44 ± 7	45 ± 5	98 ± 11	101 ± 11
	NAC	47 ± 5	47 ± 6	47 ± 5	47 ± 6	97 ± 12	102 ± 12

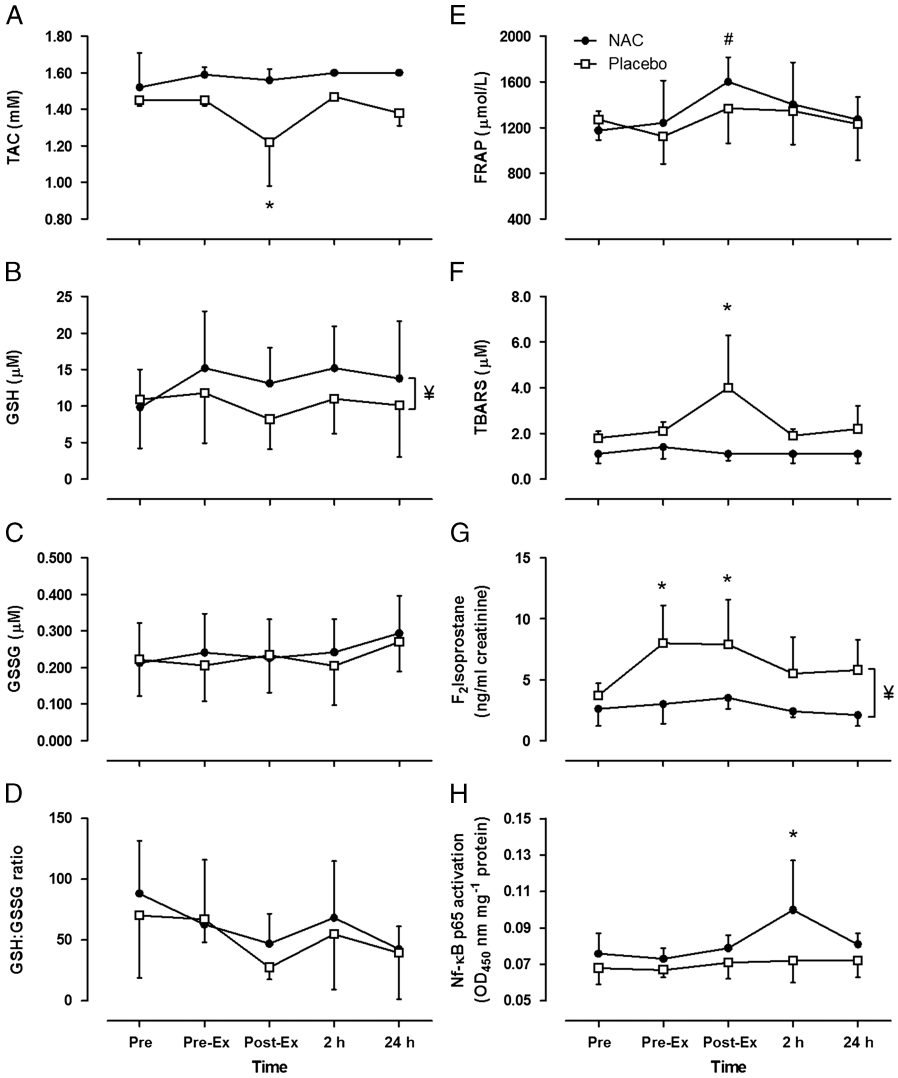

FIGURE 3—Time course of changes in plasma total antioxidant capacity (TAC) (A), glutathione (GSH) (B), reduced glutathione (GSSG) (C), GSH:GSSG ratio (D), ferric reducing ability of plasma (FRAP) (E), plasma thiobarbituric acid-reactive substances (TBARS) (F), urinary 15-isoprostane F_{2t}-concentration (F_2-isoprostane) (G), and nuclear factor κB (NF-κB) activation (H). Pre (presupplementation and preexercise), Pre-ex (preexercise after supplementation with NAC and placebo), Postex (postexercise), 2 h (2 h postexercise), and 24 h (24 h postexercise) (mean ± SD). *Significant interaction effect ($P < 0.01$). #Significant effect for time ($P < 0.05$). ¥Significant main effect for condition ($P < 0.01$).

Reductions in plasma and urinary markers of oxidative damage after the cycle ergometer simulation were shown with NAC supplementation. A significant interaction effect was observed in plasma TBARS ($P = 0.002$, $\eta_p^2 = 0.22$) (Fig. 3F) with *post hoc* analysis revealing a significant reduction in plasma TBARS immediately postexercise in the NAC supplementation intervention. No significant difference in plasma TBARS was observed at any other time point between conditions. *Post hoc* analysis showed a significantly greater urinary F_2-isoprostane concentration both before and immediately after the cycle ergometer race simulation when the placebo was consumed ($P = 0.010$, $\eta_p^2 = 0.431$) (Fig. 3G).

NAC supplementation significantly blunted the exercise-induced increases in plasma concentration of both IL-6 ($P = 0.002$, $\eta_p^2 = 0.22$) (Fig. 4C) and MCP-1 ($P = 0.012$, $\eta_p^2 = 0.17$) (Fig. 4D), which were observed in the placebo condition. No time or condition effect was observed for plasma XO ($P = 0.71$, $\eta_p^2 = 0.03$) (Fig. 4A). The cycle ergometer simulation induced significant increases in plasma hypoxanthine concentration ($P < 0.001$, $\eta_p^2 = 0.43$) (Fig. 4B). However, there was no difference between conditions ($P = 0.57$, $\eta_p^2 = 0.04$). A significant time ($P = 0.002$, $\eta_p^2 = 0.21$) and condition effect ($P < 0.001$, $\eta_p^2 = 0.21$) was observed in NF-κB activation with a greater increase in activity at 2 h post exercise when NAC supplementation was consumed as opposed to the placebo (NAC = 0.100 ± 0.027, OD = 450 nm·mg^{-1} protein; placebo = 0.072 ± 0.012, OD = 450 nm·mg^{-1} protein) (Fig. 3H).

DISCUSSION

The purpose of this study was to examine the ergogenic potential of oral NAC supplementation on race-specific

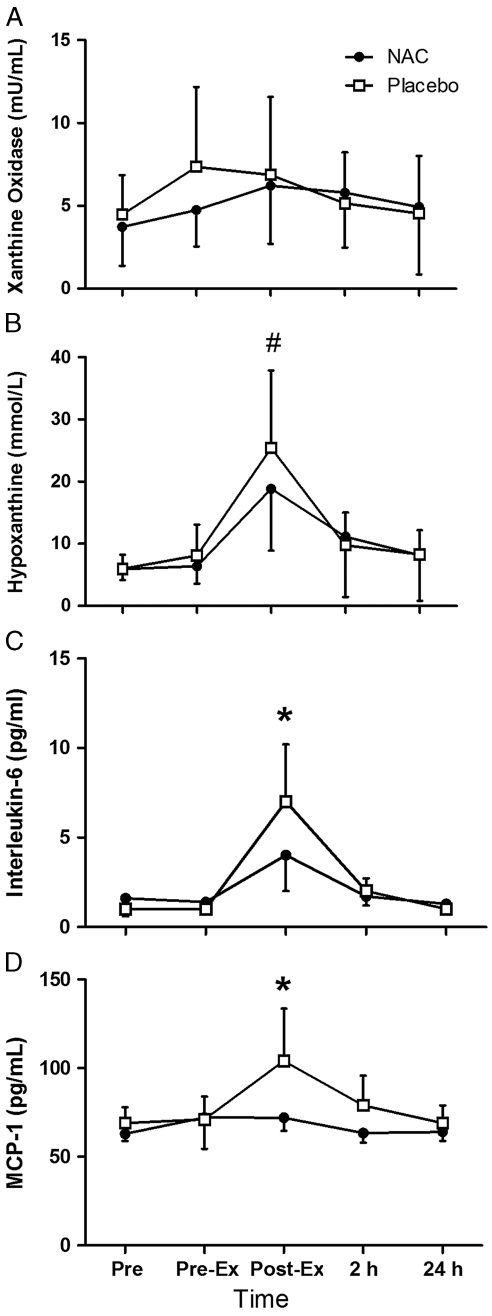

FIGURE 4—Time course of changes in plasma xanthine oxidase (XO) (A), plasma hypoxanthine (B), plasma interleukin 6 (IL-6) (C), and plasma monocyte chemoattractant protein-1 (MCP-1) (D). Pre (pre-supplementation and preexercise), Pre-ex (preexercise after supplementation with NAC and placebo), Postex (postexercise), 2 h (2 h postexercise), and 24 h (24 h postexercise) (mean ± SD). *Significant interaction effect ($P < 0.01$). #Significant effect for time ($P < 0.01$).

cycling performance and associated exercised-induced changes within the oxidative and inflammatory pathways in athletes during heavy training. Previous research has shown an ergogenic benefit of NAC on performance within a variety of exercise modalities. However, not all studies have shown benefits in cycle time trial performance (12), or in high-intensity cycle efforts (24). To further investigate the ergogenic effects of NAC, the cycle ergometer race simulation was chosen as it is able to quantify both aerobic and anaerobic performance within a single test. Similarly, as this performance test is designed to replicate a cycle race, it was though that the findings of the investigation would be more applicable within a practical sporting setting. The findings provide novel evidence that oral NAC supplementation induces a significant improvement in repeated cycle (anaerobic) sprint performance compared with a placebo in well-trained triathletes. This supports the findings of previous laboratory-based research that has identified the ability of NAC to promote fatigue resistance during strenuous exercise (23,26,35). Similar increases in performance have also recently been reported during repeated high-intensity intermittent shuttle runs in recreationally trained men after a 6-d loading period with a 50-mg·kg^{-1} dose of NAC (7). Collectively, these studies demonstrate that the usefulness of NAC supplementation extends past the laboratory and can improve athletic performance in a practical sporting environment.

Although the mechanisms underlying the sprint performance changes during exercise were not directly measured in this study, other research has identified potential physiological factors, which may explain the ergogenic effect of NAC (23,35). It has been suggested that NAC acts to minimize ROS accumulation within contractile tissue, which can assist in the maintenance of force production during fatiguing skeletal muscle contractions (35). Specifically, the elevated antioxidant capacity with NAC may reduce ROS interference within the sodium, potassium (Na^+,K^+)-pump activity (23), and calcium regulation (1) during the excitation contraction coupling process. This maintenance of muscle function has been demonstrated by Kelly et al. (19), who reported no change in respiratory muscle fatigue with NAC supplementation during heavy exercise. However, postexercise mean maximal inspiratory pressure reduced by ~14% in the placebo condition. Indeed, the observed performance and physiological benefits with NAC supplementation in the current study may reflect an enhanced ability to maintain an optimal redox balance during exercise.

When participants were supplemented with NAC, plasma TAC concentration remained elevated postexercise compared with the significant reduction observed in plasma TAC in the placebo condition. In addition, exercise-induced increases in markers of oxidative damage were completely blunted in the NAC condition. This finding is comparable with other investigations, whereby supplementation with antioxidants did not increase basal antioxidant capacity but did effectively counterbalance the oxidative insult during exercise (21,37). Similarly, the dosage of NAC used in the current study may have been insufficient to alter plasma glutathione status. For instance, a recent investigation demonstrated that oral NAC doses of 1200 mg did not affect the glutathione-based thiol concentration in human plasma (15). Nonetheless, it is apparent that NAC can exert a performance benefit irrespective of changes in plasma glutathione metabolism (22,26). The results of the present investigation suggest that NAC

supplementation can reduce exercise-induced redox perturbations and improve exercise performance. However, improvements in performance with NAC supplementation have not been consistently shown (2,24,25).

In the current investigation, despite a significant improvement in repeat cycle sprint performance, there was no change in steady-state time trial (2 or 5 min) performance after the NAC supplementation period. These observations are in contrast to the current consensus that NAC is more effective in enhancing submaximal exercise performance (22) rather than anaerobic activity (9,24). Experimental evidence on the effects of NAC in isolated muscle fibers has previously demonstrated an inhibition of low-frequency contractile fatigue without a concomitant reduction in fatigue during high-frequency stimulation (35). Similarly, in whole-body exercise, NAC infusion failed to improve repeat sprint cycling performance (4 × 45-s maximal efforts) in eight untrained men (24). Alternatively, in a separate study by the same investigators, a significantly improved cycle time to exhaustion at 92% $\dot{V}O_{2peak}$ with NAC infusion was reported in eight well-trained men (26). These equivocal results regarding the ergogenic effect of NAC may be in part due to the training status of the participants, differing supplementation protocols and the varied metabolic demands of each exercise protocol. Further speculation on the underlying contributors to this observed difference in improvements during repeat sprint versus short duration steady-state time trial performance is beyond the scope of this investigation. Future research is therefore required to fully elucidate the respective dosage and mode of exercise whereby NAC potentiates an ergogenic effect for well-trained endurance athletes.

To the authors' knowledge, this was the first study to investigate the effects of a short-term (9 d) oral NAC supplementation protocol on the physiological responses to training stimuli, using a crossover experimental design. In comparison with previous studies investigating NAC supplementation, the present study administered a comparatively low dose of NAC (1200 $mg \cdot d^{-1}$). The supplementation protocol was chosen in a successful attempt to prevent the occurrence of unwanted side effects from consuming oral NAC capsules such as nausea, diarrhea, flatulence, and sleepiness (15). This lack of adverse reactions improves the efficacy of incorporating a short-term period of NAC supplementation into an athlete's preparation for competition or during an intensive period of physical training.

Another potential concern in the current investigation was that the consumption of NAC over a 9-d loading period would negate beneficial physiological adaptive responses from occurring. This was based on the findings of several prior studies, which have demonstrated the ability of antioxidant compounds to interfere with the gene transcription pathways and ameliorate redox-regulated adaptations to exercise in human participants (18,30). However, the results of the present study provide contrary evidence that suggests that NAC supplementation promoted physiological adaption after the cycle race simulation protocol, as indicated by the significantly greater 2 h postexercise increase in NF-κB activation with NAC compared with the placebo supplementation. This disparate finding may be due to the dosage and type of supplement used by previous researchers. Petersen et al. (30) observed a postexercise reduction in NF-κB activation when a high dose of NAC was infused intravenously (125 $mg \cdot kg^{-1} \cdot h^{-1}$ for 15 min, then 25 $mg \cdot kg^{-1} \cdot h^{-1}$ throughout the exercise) in eight healthy men during a bout of exhaustive cycling. This inhibition of redox signaling pathways was not observed with the lower dosage of NAC (1200 mg) used in the present study. Moreover, a blunted NF-κB activation was observed after a marathon when well-trained runners were supplemented with allopurinol, which is known to inhibit XO (18). XO-meditated increases in reactive oxygen species play an important role in the activation of the NF-κB pathway and the adaptive responses to physical training (18). Unlike allopurinol, supplementation with NAC did not affect the plasma levels of XO and facilitated the activation of the NF-κB. These findings suggest that a low dose of NAC can be effective in promoting fatigue resistance during high-intensity cycling without preventing the up-regulation of redox-sensitive transcription factors.

NAC supplementation also appeared to enhance the participant's ability to tolerate physical training. When consuming NAC, participants reported a significantly reduced number of "worse than normal responses" in the DALDA and reduced levels of basal oxidative damage as measured in urinary F_2-isoprostane compared with the placebo condition. In addition, NAC blunted exercise-induced increases in the cytokines IL-6 and MCP-1 after the cycle ergometer race simulation. These results are in agreement with previous research that has demonstrated no negative effects of antioxidant supplementation on physiological adaptive processes (5,39) and a greater ability to cope with periods of demanding physical training (28,41). However, the partial suppression of IL-6 is a potentially unwanted response to NAC supplementation. IL-6 released from contracting skeletal muscle exerts an autocrine/paracrine and endocrine role in regulating the metabolic response to exercise (29). The suppression of the IL-6 release with NAC may interfere with this important role. Consequently, short-term rather than habitual supplementation with NAC may be more effective.

There are a myriad of physiological, environmental, biomechanical, psychological, and lifestyle factors that contribute to an athlete's ability to complete physical training loads and produce optimal performance. The present findings show that short-term supplementation with the antioxidant NAC improves repeated sprint cycling performance and promotes the maintenance of an optimal redox balance during exercise. In addition, the relatively low dose (i.e., 1200 $mg \cdot d^{-1}$) of NAC did not appear to incur a negative impact on the adaptive responses to exercise. Instead, NAC supplementation enhanced the oxidant–antioxidant balance during exercise and provided a more conducive environment for muscle contraction and adaptation to occur in well-trained triathletes undergoing a period of intensive physical training. However, a 9-d supplementation period may not be of sufficient duration

to significantly impact training-induced physiological adaption. Further research is required to investigate the impact of prolonged NAC loading periods on performance and adaptive responses to exercise stimuli within the daily training environment.

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