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ORIGINAL ARTICLE

N-Acetyl cysteine does not improve repeated intense endurance cycling performance of well-trained cyclists

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Abstract

Purpose To evaluate the effect of antioxidant supplementation on intense endurance exercise performance and the physiologic exercise response acutely and in early recovery.

Methods Well-trained cyclists ($n = 11$, peak VO_2: 69 ± 7 ml/min/kg) completed two identical standardized 20-min warm-up periods (WU-1 and WU-2) prior to two performance tests (PT) with a duration of ~4 min representing a qualifying (PT-1) and final race (PT-2) on the same day separated by 90 min. Subjects were supplemented orally with placebo (PLA) and *N*-acetyl cysteine (NAC; 20 mg/kg) before exercise in a double-blinded crossover design.

Results Mean power during PT-1 did not differ ($P = 0.39$) between PLA (400 ± 44 W) and NAC (401 ± 44 W) as was the case during PT-2 with similar performance ($P = 0.74$) between PLA (401 ± 43 W) and NAC (400 ± 42 W). Subjective "readiness" was lowered by prior exhaustive exercise from PT-1 to PT-2 ($P = 0.012$) in both PLA and NAC. Plasma total antioxidant capacity was not affected by supplementation and prior exhaustive exercise (respective main effects: $P = 0.83$ and $P = 0.19$) which also was observed for peak VO_2 at ~5 L/min ($P = 0.84$ and $P = 0.30$). In WU-1 and WU-2, both cycling economy at ~20% ($P = 0.10$ and $P = 0.21$) and plasma potassium at ~5 mmol/L ($P = 0.46$ and $P = 0.26$) were unaffected by supplementation and prior exercise.

Conclusions Athletes executing maximal efforts of a ~4-min duration twice daily, as seen in track cycling, appear to gain no benefit from oral NAC supplementation on acute and subsequent performance following short-term recovery. Moreover, well-trained cyclists exhibit rapid recovery from a single bout of intense endurance cycling.

Keywords NAC · Gross efficiency · TAC · Cycling · ROS · VO_2-max

Abbreviations

°C	Degrees celsius
GE	Gross efficiency
iPPO	Incremental-test peak power output
kg	Kilogram
kJ	Kilojoule
L	Liters
m	Meters
mg	Milligram
min	Minute
mmol	Millimole
NAC	*N*-Acetyl cysteine
PLA	Placebo
P:O	Phosphate/oxygen ratio
PT	Performance test
ROS	Reactive oxygen species
s	Second
TAC	Total antioxidant capacity
VO_2	Pulmonary oxygen uptake
WU	Warm-up
W	Watt

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Introduction

The competition format in many endurance sports such as track cycling, swimming and cross-country skiing entails athletes to perform maximally more than once during a day

in events lasting ~ 1–5 min characterized by high anaerobic and aerobic energy turnover (Spencer and Gastin 2001). The reason for this is either because an athlete competes in numerous events, or because qualifying and a final race in a single event are scheduled on the same day. Data from the world championships in track cycling revealed a general ~ 1% decrease in performance (race speed) from qualifier to finals in 4000-m individual pursuit (~ 4 min) indicative of incomplete recovery or tactical differences (Christensen and Bangsbo 2016). Based on cycling physics formulas (Christensen et al. 2017) and assuming a mean power of 500 W in the first bout, combined with typical values for resistive forces in elite track cyclists, this would represent a reduction in mean power of ~ 2.5%. Likewise, Nielsen et al. (1996) in a laboratory setting had well-trained rowers perform three 6-min max tests separated by 4 h each, and relative to the first test reported a non-significant 1.5% and a significant 2.2% decrement in mean power for the second and third tests, respectively. Thus, intense exercise lasting 4–6 min can exert negative influence on subsequent performance a few hours later.

Muscle fatigue development during intense endurance exercise is complex. Perturbations of hydrogen, potassium and calcium ions in the contractile filaments has been associated with muscle fatigue, and an increase in reactive oxygen species (ROS) as a consequence of high aerobic metabolism may negatively affect potassium handling and calcium sensitivity (Hostrup and Bangsbo 2017). Moreover, the addition of ROS to isolated mitochondria from human muscle increases the oxygen requirement for metabolism (decrease in P:O ratio) (Tonkonogi et al. 2000). This is considered detrimental for endurance performance, since a large aerobic contribution to the total energy turnover is seen in events lasting more than 1 min (i.e., 85% for events lasting ~ 4 min) (Craig and Norton 2001). Consequently, any decrease in P:O ratio would lower total energy turnover for a given aerobic metabolism (i.e., a total VO_2 of 18 L for a duration of 4 min for a cyclist with a VO_2-max of ~ 5 L/min; Christensen and Bangsbo 2015).

ROS can be scavenged by the antioxidant system which as defined by Powers and Jackson (2008) can be characterized as any substance that delays or prevents the oxidation of a substrate. Since the 1990s, *N*-acetyl cysteine (NAC) has been studied for antioxidative properties during exercise and whether it has an ergogenic effect (Reid 2016). Oral intake of NAC is legal in a sporting context and side effects are rare and if present appears mostly centered around the gastrointestinal system with bloating or diarrhea in mild form (Cobley et al. 2011; Corn and Barstow 2011; Michailidis et al. 2013; Slattery et al. 2014). A recent systematic meta-analysis could not demonstrate any clear performance effect in endurance type exercise with NAC supplementation from the seven studies extracted for analysis (Rhodes and Braakhuis 2017). The groups included in the analysis constituted both sick, untrained and trained individuals and various dosing regimens and exercise modalities with and without preloading were used. In turn, marked individual differences appears present with regard to improving the exercise capacity (Bailey et al. 2011; Medved et al. 2004a) with improved potassium handling following NAC being one candidate mechanism (McKenna et al. 2006). Thus, as highlighted by Rhodes and Braakhuis (2017), more studies are warranted to obtain a stronger foundation to evaluate whether NAC has acute effects on endurance performance. In turn, within the intense exercise time domain, closed-end performance tests in a recovered state are few (Nielsen et al. 2001), since the majority of previous studies used open-ended time-to-exhaustion protocols (Bailey et al. 2011; Corn and Barstow 2011; Zembron-Lacny et al. 2010) frequently with preloads (Medved et al. 2003, 2004a, b) which has shortcomings in resembling most athletic competitions and holds greater variation than time trials or fixed work/time protocols (Christensen et al. 2017).

Whereas the results of NAC during acute exercise are somewhat mixed, there appears to be a stronger evidence for NAC to improve recovery in the hours following intense exhaustive exercise in humans, albeit few studies with this focus are published. Over a 5-day period, moderately trained subjects performed a 60-min standardized intermittent running protocol followed by a Yo–Yo intermittent recovery test on days 1, 3 and 5. One group was orally supplemented with placebo and another group with NAC, with the latter group experiencing much faster recovery in performance relative to their baseline level (Cobley et al. 2011). Two hours following exhaustive eccentric strength-type exercise, orally consumed NAC relative to placebo lowered markers of oxidative stress and eccentric strength was higher 24 and 48 h after exercise (Michailidis et al. 2013). Collectively, the former two studies do highlight that ROS appear causally linked with reduced strength and intermittent endurance performance in recovery, and that oral NAC intake can reduce the detrimental effect of prior exhaustive exercise. Whether oral NAC supplementation can improve recovery from less muscle damaging exercise such as cycling is unknown and represented the primary aim of the present study. Gejl et al. (2016) found a lowering of plasma total antioxidant capacity in well-trained skiers following a single 4-min max work bout and Slattery et al. (2014) reported a drop in plasma total antioxidant capacity in trained cyclists at the end of a simulated short road-cycling race with great range in intensity lasting ~ 2 h, denoting that exercise with little eccentric load of both short and long duration can induce oxidative stress. Interestingly oral NAC supplementation counteracted the drop seen with placebo and improved repeated 5- to 15-s sprinting during the protocol, but not 2- to 5-min time trials (Slattery et al. 2014). In contrast, 10-min time

trial performance just after 6 × 5 min intervals at ~ 90% VO_2-max was lowered with NAC in trained cyclists together with increased reliance on fat as a substrate (Trewin et al. 2013). Clearly, more studies are needed to clarify whether NAC has any impact on recovery from cycling exercise. Focusing on the influence on aerobic metabolism from prior exhaustive exercise, repeated ~ 2-min intervals lowered the P:O ratio in muscle biopsy samples obtained 2 h after cycling exercise (Tonkonogi et al. 1999) which is considered detrimental for endurance performance. Nevertheless, cycling economy, as an in vivo proxy of P:O ratio, was not impaired in well-trained cyclists in a simulated competitive setting for 4000-m track cycling pursuit, consisting of two ~ 4-min maximal exercise bouts separated by 3 h of recovery (Christensen and Bangsbo 2016). In the same study, VO_2-peak, maximal blood lactate and blood potassium was neither affected negatively by prior intense exercise, indicating the capacity for a high power production was not lowered by prior exercise suggesting that 3 h was sufficient for recovery in the settings of that study.

Thus, the aim of the present study was to evaluate the effect of oral NAC supplementation in well-trained cyclists on intense endurance exercise performance (~ 4 min) and the physiologic exercise response acutely and after 90 min of recovery.

It was hypothesized that subjects with NAC relative to placebo—as a consequence of acute intense exercise and short recovery—would experience less reduction in total antioxidant capacity, no worsening of cycling economy and potassium handling leading to subsequent improved performance.

Methods

Subjects

Eleven well-trained male cyclists completed the study (Table 1). During the course of the study, one participant dropped out due to illness not related to the study protocol. Study procedures were approved by the local ethical committee of the capital region of Copenhagen (Region Hovedstaden, H-15003164). All subjects received written and oral information about the study procedures and gave their written informed consent to participate in the study in accordance with the Helsinki Declaration.

Table 1 Mean (± SD) characteristics of subjects ($n = 11$)

Age (years)	28 ± 7
Height (cm)	183 ± 7
Body mass (kg)	73 ± 10
Peak VO_2 incremental test (L/min)	5.05 ± 0.48
Peak VO_2 incremental test (mL/min/kg)	69 ± 7

Study design

Each cyclist was tested on four separate days. On the first day, an incremental test (see description later) was carried out to determine peak VO_2 and incremental-test peak power output (iPPO). On the second day, a familiarization trial was carried out by having subjects complete the protocol used for the experimental days with minor modifications (see later). Briefly, two performance tests (PT) each lasting 4 min was executed, separated by 90 min, simulating a competition format with a qualifying and final race placed on the same day.

On the third and fourth day, the experiment was carried out by having cyclists orally supplement with placebo (PLA) or NAC in a randomized double-blind crossover study design (see Fig. 1). The PLA and NAC trial was separated by 6 ± 2 days.

Supplementation

PLA (dextrose) and NAC (20 mg/kg ~ 1500 mg total; Fagron BV, the Netherlands) were provided in identical capsules for acute supplementation. The dose was chosen based on studies displaying ergogenic effects and reduced markers of oxidative stress in the first 24 h after an exhaustive eccentric strength protocol (Michailidis et al. 2013) and intermittent high-intensity cycling (Slattery et al. 2014), albeit chronic supplementation for 9 days preceded the later protocol. The capsules were ingested with tap water 60 min prior to the performance test, since NAC bioavailability of such dose peaks around that time after intake (Borgström et al. 1986; Pendyala and Creaven 1995). PLA and NAC did not differ in smell. Adverse reactions such as gastrointestinal distress (Rhodes and Braakhuis 2017) were not reported by the subjects.

Testing procedures

All testing was carried out on a mechanically braked bike ergometer controlled by an external computer (Monark 839E) with modifications for cyclists in terms of race handlebar, saddle and pedals. Cyclists were instructed to follow their usual dietary routines and to have a rest day or only low-intensity short duration (< 60 min) training the day before testing. The last meal was to be consumed 3 h prior to testing and any caffeine intake was restricted to low dose (one cup of coffee) in the morning. In the 2-day lead up to the first experiment (day 3), subjects kept a food and training diary, which they were instructed to replicate for the final test day (day 4). Body mass (kg) before trials with PLA (74.1 ± 9.7) and NAC (74.5 ± 9.5) was similar ($P = 0.34$, t

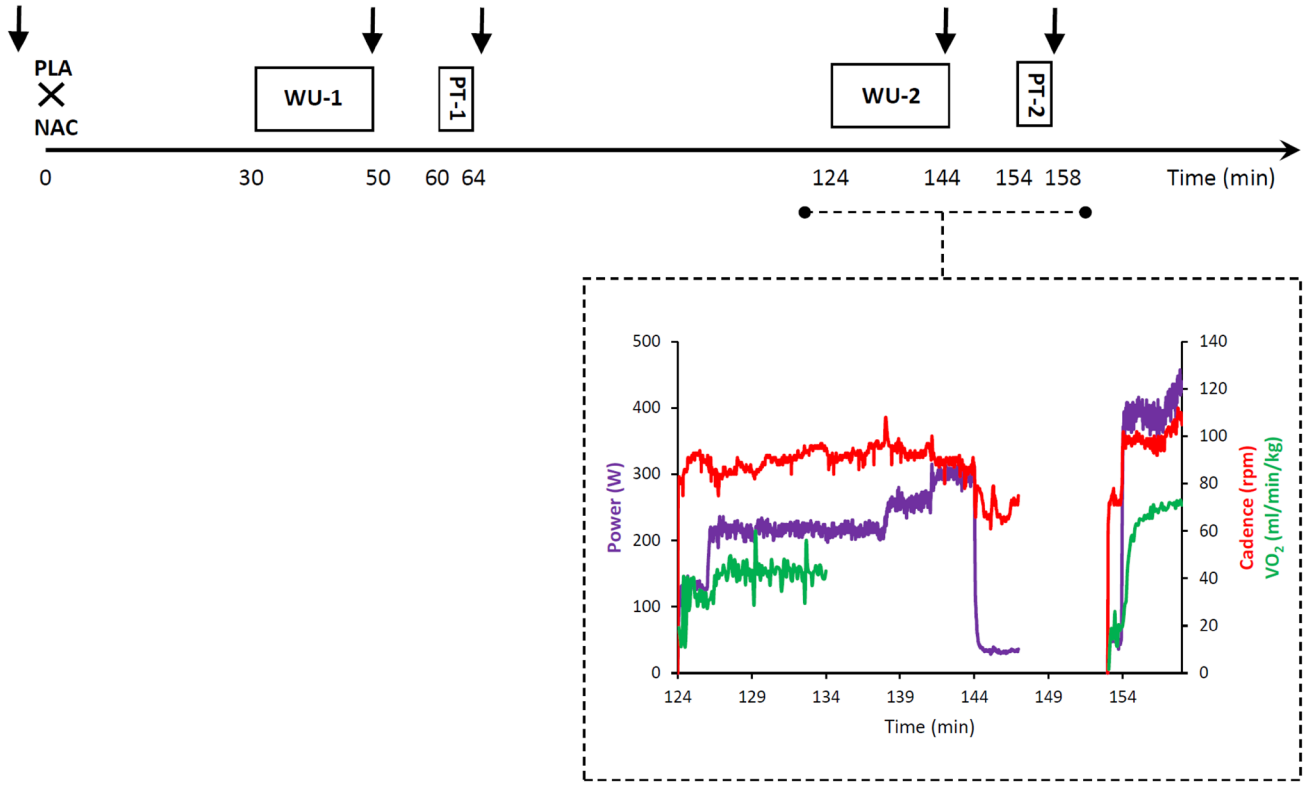

Fig. 1 Design overview for a simulated competitive setting encompassing two identical standardized 20-min warm-up periods (WU-1 and WU-2) prior to two maximal endurance performance tests (~4 min) representing a qualifying (PT-1) and final race (PT-2) separated by 90 min with pre-exercise placebo (PLA) or *N*-acetyl cysteine (NAC; 20 mg/kg) supplementation in well-trained cyclists. Arrows denote time of venous blood sampling. The graphs in the hatched box display recordings of power and cadence every 1 s and pulmonary VO_2 every 5 s from a representative subject during WU-2 and PT-2 in the NAC condition

test). The experiments were initiated at the same time of day for each subject. In turn, tests were only carried 72 h or longer after any competition.

Pulmonary gas exchange was measured (Jaeger Oxycon Pro) continuously during testing. For subsequent analysis, data were extracted in 5-s intervals.

The bike ergometer and pulmonary gas exchange system were calibrated prior to all testing sessions in accordance with the manufacturer's instructions. An example of data recording from the bike ergometer and VO_2 system is displayed in Fig. 1. Stable environmental settings were present in the laboratory during testing, with recorded temperature and humidity being similar in PLA (21.8 ± 1.2 °C and 34 ± 9%) and NAC (21.4 ± 1.5 °C and 39 ± 9%). A mechanical fan placed behind the subjects ensured air cooling.

Incremental test

From a 5-min baseline at 100 W, increments of 25 W/min was applied until subjects stopped pedaling or cadence dropped below 70 rpm for more than 3 s. To calculate incremental-test peak power output (iPPO) the following formula was used:

$$\text{iPPO (W)} = \text{last fully completed increment(W)} + ((\text{s at exhaustive increment}/60\ \text{s}) \times 25\ \text{W}).$$

Performance testing

A fixed load (given in newton; N) on the bike for the performance test (PT) was calculated (40 ± 4 N) based on iPPO and cadence from the incremental test as in our previous studies (Christensen and Bangsbo 2015, 2016):

$$\text{Load (N)} = (\text{iPPO}/11) + (\text{average cadence incremental test}/-10) + (10).$$

Accordingly, power output on the bike ergometer in PT was determined by cycling cadence from which mean power was calculated.

On the familiarization day, the subjects completed a 4-min time trial with subjects instructed to complete as much work as possible within the 4 min. To synchronize bike ergometer and the gas exchange system, a 1-min period at 6 N with low cadence (~60 rpm ~40 W) was undertaken before test start. Cyclists had no visual info about cadence (i.e., actual power output) and received no verbal support.

To facilitate pacing, time points were given every 30 s for the first 3 min, and thereafter every 15 s. This particular performance test has a low coefficient of variance of 1.6% (Christensen and Bangsbo 2015).

In the main experiment (PLA vs. NAC), the first performance test was a modified version of the 4-min max test (PT-1), followed 90 min later by the second performance test (PT-2) in the form of the 4-min time trial as used on the day of test familiarization. The modified version for PT-1 was chosen to avoid any influence from large differences in pacing between the two test days, given the main focus of the present study was to evaluate the influence of NAC on recovery (i.e., important that the subjects recover from a comparable effort). Thus, a pacing scheme was calculated based on the best 4-min max PT obtained during familiarization from which a total workload was calculated (122 ± 33 kcal) based on the linear relationship between power output and energy turnover on the ergometer. For the first 3.5 min in PT-1, the cyclists were to exercise at 98% of the best mean power, and thereafter attain the total workload as fast as possible. In practice, the actual pacing profile on day 3 was noted and to be replicated on day 4 for the first 3.5 min. A technical error precluded calculation of mean power for one subject; hence, data for acute performance effects (PT-1) from NAC represent ten subjects.

The perceived readiness to perform maximally just prior to PT-1 and PT-2 was assessed on a 1 (lowest) to 10 (highest) scale.

Standardized warm-up and energy intake routines

A standardized 20-min warm-up (WU) period and 10-min recovery period was undertaken before PT-1 (WU-1) and PT-2 (WU-2) and also before max tests on the day of familiarization. As part of the 10-min recovery period, 3 min at 30 W followed the warm-up and the 1-min low-intensity synchronization period before PT-1 and PT-2, described previously. The warm-up period consisted of 2-min low-intensity cycling at 30% iPPO, 12-min moderate-intensity cycling at 50% iPPO, followed by gradual higher intensity for 3 min at 60% and 70% iPPO. Cyclists were free to choose their preferred cadence and were instructed to keep this in a stable range (± 5 rpm), 94 ± 6 and 95 ± 7 rpm in PLA and 94 ± 5 and 92 ± 7 rpm in NAC for WU-1 and WU-2, respectively.

Standardized energy intake was provided to each subject just after PT-1 (53 g carbohydrates and 7 g protein) from 330 mL of soda (Faxe Kondi Soda with negligible caffeine content; Unibrew, Denmark) and 200 ml of chocolate milk (Lille Lise, Arla Foods, Denmark).

Physiologic measurements

Peak VO_2 during max testing was calculated as the highest 30-s value.

Cycling economy determined as gross efficiency (GE)—as a proxy of muscle P:O ratio—was calculated for a 2-min period averaged from 8 to 10 min during moderate-intensity cycling in WU-1 and WU-2 using the formula:

$$\mathrm{GE}\,(\%) = \text{ergometer power output}\,(\mathrm{kJ/min})/\text{whole body energy turnover}\,(\mathrm{kJ/min}).$$

The actual power recorded on the bike ergometer was used (219 ± 18 and 219 ± 20 W in PLA and 220 ± 18 and 220 ± 19 W in NAC for WU-1 and WU-2, respectively) and whole body energy turnover was calculated from oxygen uptake accounting for the respiratory exchange ratio (RER) using 19.6 and 21.1 kJ/L O_2 for pure fat (RER = 0.7) and carbohydrate (RER = 1.0) oxidation.

In the PLA and NAC trials, a catheter was placed in an antecubital vein upon arrival for blood sampling. Approximately, 4 mL of blood was drawn at rest, after WU-1, PT-1, WU-2 and PT-2. Samples was obtained 15 s before termination of high-intensity exercise in WU-1 and WU-2 and 1 min after PT-1 and PT-2 (Fig. 1). Within 30 min of sampling, analysis on whole blood was carried out for lactate and potassium concentrations (ABL720, Radiometer). From plasma, total antioxidant capacity (TAC) was measured in accordance with the guidelines provided by the manufacturer (Cayman Chemical). Briefly, blood was centrifuged directly after sampling for 10 min at 840*g* at 4 °C. Plasma was stored at − 80 °C until analyzed in duplicates. Technical difficulties with blood sampling and analysis means data for potassium ($n = 9$), lactate and TAC ($n = 8$) do not represent all 11 subjects.

Statistics

A paired *t* test was used to evaluate if differences existed in performance (mean power) between PLA and NAC separately for PT-1 and PT-2. All data were normally distributed (Shapiro–Wilk test).

A two-way analysis of variance for repeated measures was used to test for differences for the remaining variables with fixed factors being supplementation (PLA vs. NAC) and previous exhaustive exercise (WU-1 vs. WU-2 for submaximal exercise, PT-1 vs. PT-2 for maximal exercise, and all time points for TAC).

If a significant main effect or interaction was present a Student–Newman–Keuls post hoc analysis was applied to localize where interventions differed. Data are reported as the mean ± standard deviation.

Results

Performance and maximal exercise response

Supplementation did not affect performance (Fig. 2) as there was no difference in mean power in PT-1 (PLA: 400 ± 44 vs. NAC: 401 ± 44 W; $P = 0.39$) or in PT-2 (PLA: 401 ± 43 vs. NAC: 400 ± 42 W; $P = 0.74$).

Peak VO_2 during PT-1 and PT-2 (Fig. 3) was unaffected by both supplementation ($P = 0.84$) and prior exhaustive exercise ($P = 0.30$) with similar values reached in PT-1 (PLA: 5.10 ± 0.51 vs. NAC: 5.15 ± 0.54 L/min) and PT-2 (PLA: 5.09 ± 0.46 vs. NAC: 5.08 ± 0.57 L/min). Peak lactate (Fig. 3) was also unaffected by both supplementation ($P = 0.38$) and prior exhaustive exercise ($P = 0.90$) reaching ~ 12 mmol/L after exercise.

Readiness

A significant main effect of prior exhaustive exercise ($P = 0.012$) but not supplementation ($P = 0.656$) was present for readiness (Fig. 4) being higher before PT-1 (PLA: 7.4 ± 1.6 vs. NAC: 7.6 ± 1.3 in NAC) than before PT-2 (PLA: 6.6 ± 2.0 vs. NAC: 6.7 ± 1.3).

Submaximal exercise

Gross efficiency (Fig. 5) calculated during the standardized moderate-intensity exercise in the warm-up was not significantly affected by either supplementation ($P = 0.10$) or prior exhaustive exercise ($P = 0.21$) with similar values reached in WU-1 (PLA: 19.8 ± 0.6 vs. NAC: 20.0 ± 0.6%) and WU-2 (PLA: 19.6 ± 0.7 vs. NAC: 19.9 ± 1.1%). Plasma potassium was neither influenced by prior exercise or NAC with values

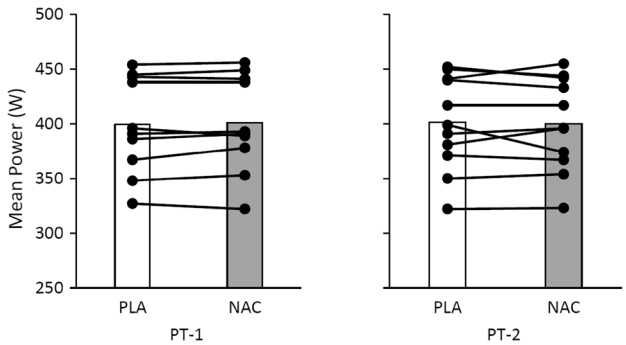

Fig. 2 Mean power in two maximal endurance performance tests (~ 4 min) representing a qualifying (PT-1; $n = 10$) and final race (PT-2; $n = 11$) separated by 90 min in a simulated competitive setting with pre-exercise placebo (PLA) or *N*-acetyl cysteine (NAC) supplementation in well-trained cyclists. Bars display average values and lines display individual values

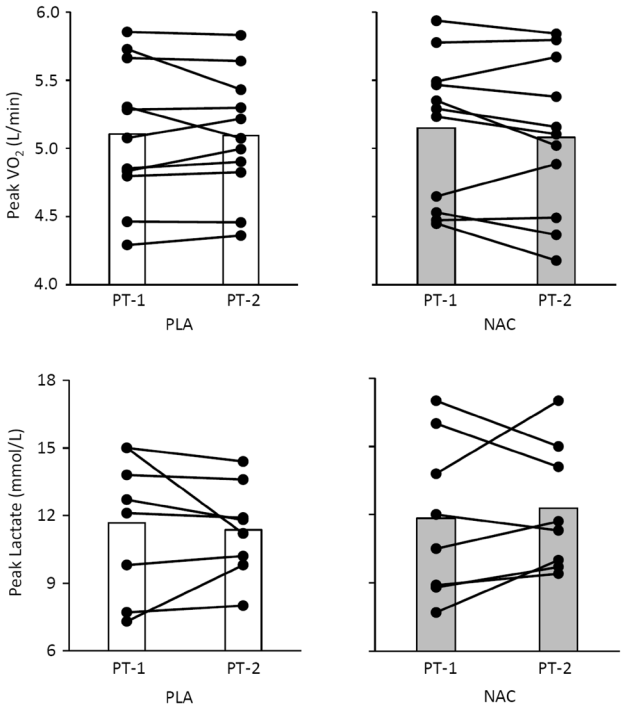

Fig. 3 Peak VO_2 (TOP; $n = 11$) and peak lactate (BOTTOM; $n = 8$) in two maximal endurance performance tests (~ 4 min) representing a qualifying (PT-1) and final race (PT-2) separated by 90 min in a simulated competitive setting with pre-exercise placebo (PLA) or *N*-acetyl cysteine (NAC) supplementation in well-trained cyclists. Bars display average values and lines display individual values

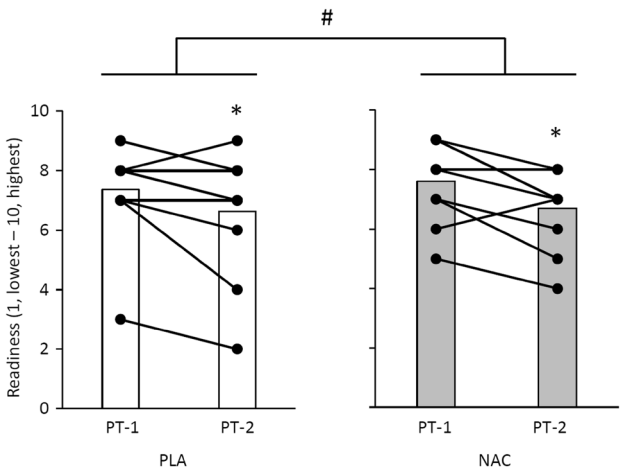

Fig. 4 Subjective readiness ($n = 11$) before two maximal endurance performance tests (~ 4 min) representing a qualifying (PT-1) and final race (PT-2) separated by 90 min in a simulated competitive setting with pre-exercise placebo (PLA) or *N*-acetyl cysteine (NAC) supplementation in well-trained cyclists. Bars display average values and lines display individual values (please note that some subjects have identical values in PT-1 and PT-2). Hash: main effect for previous exhaustive exercise (PT-1 vs PT-2; $P = 0.012$). Asterisk: WU-2 different from WU-1 in PLA ($P = 0.025$) and NAC ($P = 0.013$)

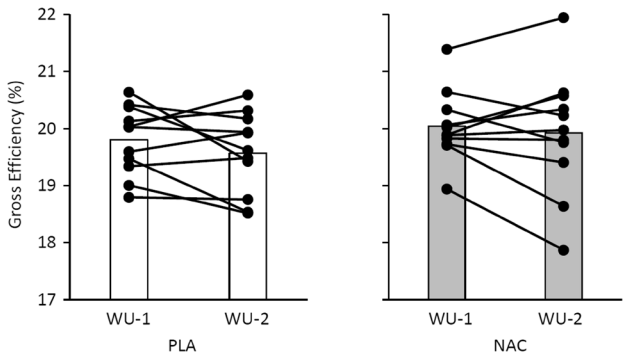

Fig. 5 Gross efficiency (cycling economy; $n=11$) in a simulated competitive setting encompassing two identical standardized 20-min warm-up periods (WU-1 and WU-2) prior to two maximal endurance performance tests (~4 min) representing a qualifying and final race separated by 90 min with pre-exercise placebo (PLA) or *N*-acetyl cysteine (NAC) supplementation in well-trained cyclists. Bars display average values and lines display individual values

of ~5 mmol/L (Table 2). The remaining results from the warm-up are presented in Table 2.

Total antioxidant capacity

Plasma total antioxidant capacity was not altered by supplementation ($P=0.83$) or prior exhaustive exercise ($P=0.19$) with values of ~2 mmol/L (Fig. 6).

Correlations

Individual change in performance with NAC relative to PLA was not associated with relative VO_2-max (mL/min/kg) in PT-1 ($r^2=0.007$) or PT-2 ($r^2=0.028$). Likewise, resting levels of TAC did not correlate to differences in performance between NAC and PLA in PT-1 ($r^2=0.004$) or PT-2 ($r^2=0.27$).

Discussion

The primary findings in the present study were that supplementation with NAC (20 mg/kg) did not improve intense endurance cycling performance (~4 min), or recovery, as performance was similar in a simulated competitive setting consisting of two performance tests separated by 90 min as seen in track cycling, swimming and cross-country skiing. These findings were obtained in a group of well-trained cyclists in a randomized placebo-controlled double-blind crossover study design.

Influence of NAC on intense endurance performance without prior exhaustive exercise

The unaltered performance in NAC relative to PLA in a setting without influence from prior exhaustive exercise (PT-1) is in line with the conclusion reached in a recent meta-analysis combining various types of tests, dosing regimens and subject groups (Rhodes and Braakhuis 2017). Having a strict focus on closed-end performance tests (i.e., fixed work or time) in a similar time domain as in the present study, oral NAC did not improve 6-min max performance in trained rowers (Nielsen et al. 2001). Likewise, some studies report unaltered exercise capacity with NAC in time-to-exhaustion protocols lasting 2–4 min (Corn and Barstow 2011; Medved et al. 2003) and 12–15 min (Bailey et al. 2011; Medved et al. 2004a), but in tests lasting 5–7 min, improvements have been reported (Corn and Barstow 2011; Medved et al. 2004b). Apparently, NAC supplementation prior to endurance tests

Table 2 Mean (±SD) pulmonary (VO_2 and RER; $n=11$) and blood lactate ($n=8$) and potassium($n=9$) values during submaximal intensity exercise normalized to incremental test peak power output (iPPO) in a simulated competitive setting encompassing two identical standardized 20-min warm-up periods (WU-1 and WU-2) prior to two maximal endurance performance tests (~4 min) representing a qualifying and final race separated by 90 min with pre-exercise placebo (PLA) or *N*-acetyl cysteine (NAC) supplementation in well-trained cyclists

	PLA		NAC		2 Way ANOVA RM		
	WU-1	WU-2	WU-1	WU-2	Supp.	Time	Supp.×Time
Moderate intensity 50% iPPO @ 8–10 min							
VO_2 (L/min)	3.19±0.28	3.23±0.3	3.18±0.29	3.20±0.31	0.44	0.20	0.51
RER	0.94±0.04	0.93±0.02	0.92±0.04	0.92±0.02	0.062	0.86	0.48
End high-intensity exercise 70% iPPO @ 20 min							
Lactate (mmol/L)	3.8±0.6	3.9±0.8	3.6±1.0	4.1±1.1[a]	0.89	0.027[b]	0.05
Potassium (mmol/L)	5.1±0.2	5.1±0.3	5.0±0.2	5.1±0.3	0.46	0.26	0.92

Main effects for supplementation (PLA vs. NAC) and previous exhaustive exercise (WU-1 vs. WU-2) and interactions are provided

[a]WU-2 different from WU-1 in NAC ($P=0.004$)

[b]Main effect for previous exhaustive exercise (WU-1 vs. WU-2)

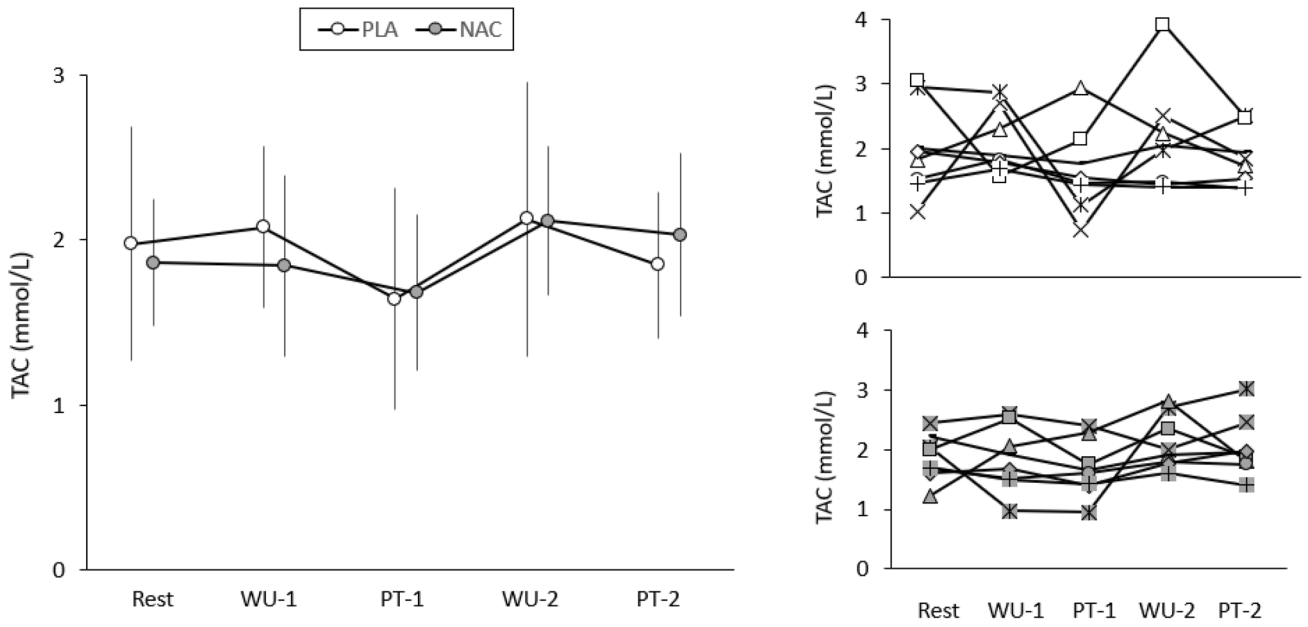

Fig. 6 Plasma total antioxidant capacity (TAC; $n=8$) displayed as group average (±SD) (LEFT) and individual values (RIGHT) in a simulated competitive setting encompassing two identical standardized 20-min warm-up periods (WU-1 and WU-2) prior to two maximal endurance performance tests (~4 min) representing a qualifying (PT-1) and final race (PT-2) separated by 90 min with pre-exercise placebo (PLA) or *N*-acetyl cysteine (NAC) supplementation in well-trained cyclists

lasting 2–15 min has none or modest ergogenic properties. This in turn implies that ROS in this time domain likely contributes little to fatigue development and that the antioxidant system is not a limiting factor for performance and exercise capacity. As with other supplements, individual differences regarding ergogenic impact may exist due to diet, gender and training status among others (Christensen et al. 2017). NAC is no exception (Medved et al. 2004a; Bailey et al. 2011). Despite that training is known to increase antioxidant capacity in muscle and blood (Gliemann et al. 2014), training status is likely not a single important factor as Medved et al. (2004b) observed longer group time to exhaustion with NAC in well-trained cyclists with an average VO_2-max of 66 mL/min/kg. Rather, glutathione levels (with NAC being involved in glutathione metabolism) may be a better discriminator. This is based on a recent study in which moderately trained subjects with low and moderate glutathione levels improved their performance during 30-s sprinting and 15-min time trialing after 30 days with oral NAC supplementation, whereas high level glutathione subjects observed a decrease in 15-min performance (Paschalis et al. 2018).

Influence of NAC for recovery

In previous published studies, NAC supplementation improved short-term recovery (1–2 days) from exercise with high eccentric loads (Cobley et al. 2011; Michailidis et al. 2013). The present study was designed to evaluate if a similar effect would be observed with concentric loading. Based on earlier findings, any large oxidative stress following PT-1 were expected to reduce cycling economy (Tonkonogi et al. 1999, 2000) and potassium handling (McKenna et al. 2006) in subsequent exercise. However, this was not the case since submaximal plasma lactate and potassium (Table 2) and cycling economy (Fig. 5) were not influenced by prior intense exhaustive cycling (~4 min) in PLA as well as peak VO_2 and peak plasma lactate (Fig. 3) and plasma TAC (Fig. 6). Moreover, performance in PT-2 did not differ between PLA and NAC. The only significant observation caused by NAC was a slightly higher submaximal lactate in WU-2. Thus, well-trained cyclists appear to exhibit rapid recovery from a ~4-min max bout of cycling. This lends support to a well-developed antioxidative system in trained individuals as expected based on training studies (Gliemann et al. 2014). In line herewith, maximal exercise did not lower plasma total antioxidant capacity (TAC) in PLA or NAC (Fig. 6).

Influence of NAC on metabolism

NAC tended to lower RER during the moderate-intensity part of the warm-up exercise prior to both PT-1 and PT-2 (Table 2). This is in line with a finding during intense exercise (Trewin et al. 2013). For athletes competing in endurance events where preservation of muscle glycogen is a priority, NAC may, therefore, have an ergogenic potential since

the lower RER indicates a higher fat oxidation. Lactate was higher at the end of WU-2 relative to WU-1 in NAC with no apparent explanation (Table 2). Collectively, NAC appears to have affected metabolism during submaximal exercise, but the physiologic implications for intense endurance athletes are probably limited, as cycling economy, performance and maximal exercise values were similar.

Methodological considerations

The lack of effect from NAC in the present study is not considered to be a result of suboptimal dosing or timing. The NAC dosage of 20 mg/kg, corresponding to ~ 1500 mg total, ingested 1 h before PT-1 is similar to the daily dose ingested after an exhaustive eccentric strength protocol, where recovery of force was improved 24 h later relative to placebo with markers of oxidative stress being lowered also 2 h following the exercise (Michailidis et al. 2013). Interestingly, the daily dose in the latter study was spilt into three implying that even lower concentrations than the one used in the present study can exert antioxidative effects. Likewise, a daily dose of 1200 mg for 8 days combined with intake also 2 h before exercise maintained plasma TAC levels at resting level after maximal intermittent cycling whereas a drop was observed with placebo (Slattery et al. 2014), but the chronic and acute effects cannot be discerned. Timing of supplementation is also considered optimal in the present study. Plasma NAC peaks ~ 1 h after oral intake (Borgström et al. 1986; Pendyala and Creaven 1995), coinciding with PT-1 where a large oxidative stress was expected. The standardized energy intake provided between PT-1 and PT-2 was chosen to replicate "real-world settings" where most athletes consume carbohydrate and protein between the exercise bouts. Cow milk has a cysteine content around ~ 10 mg/g of protein (Rafiq et al. 2016) amounting to a total of ~ 70 mg consumed as chocolate milk, which is not considered to significantly affect cysteine levels in the PLA trial, and is much lower than the ~ 1500 mg received in the NAC trial.

With the results in hindsight, it is worth considering if PT-1 in combination with the recovery period induced sufficient oxidative stress to have any detrimental physiologic effects later. A lowering of TAC was reported in well-trained cohorts after 105 min of intermittent cycling exercise (Slattery et al. 2014) and ~ 4-min max cross-country skiing (Gejl et al. 2016). Moreover, in less trained subjects, a reduction in P:O ratio was observed with repeated ~ 2–3 min bouts (Tonkonogi et al. 1999) likely mediated by ROS (Tonkonogi et al. 2000). Thus, exercise mode (little eccentric load) and exercise duration per se are not considered the causes for lack of detrimental effects. Subjects attained the same peak VO_2 in PT-1 (Fig. 3) as in the incremental test (Table 1) and a significant reduction in readiness from PT-1 to PT-2 (Fig. 4) demonstrating that the cyclists were under the influence of the prior bout of exercise. Still, the total load in the present study may have been too low to induce large oxidative stress or other detrimental effects, in the well-trained cohort with an expected highly developed antioxidant system (Gliemann et al. 2014). The subjects in the present study had an average VO_2-max of 69 mL/min/kg, which is higher than studies of well-trained athletes (VO_2-max: ~ 64 mL/min/kg) who experienced a drop in TAC (Slattery et al. 2014; Gejl et al. 2016). The involvement of the upper body muscle groups may also explain why cross-country skiers experienced a lowering of TAC in the study by Gejl et al. (2016) contrary to the cyclists in the present study with a similar exercise duration. Accordingly, in sedentary subjects the shoulder muscle (m. deltoideus) relative to leg muscle (m. vastus lateralis) recently was reported to have a lower expression of antioxidant enzymes, and a smaller increase in antioxidant enzymes in response to a training period with high-intensity swimming (Mohr et al. 2017). Thus, future studies may evaluate whether NAC supplementation has a higher ergogenic effect in exercise modalities dominated by recruitment of the upper body muscle groups (e.g., swimming, kayak, and double pooling in skiing) compared to exercise with the lower body (e.g., running and cycling).

Performance was evaluated with two tests differing slightly in form. PT-1 consisted of a 3.5-min preload and a ~ 30-s closed-end test, whereas PT-2 was a 4-min closed-end test, with the latter having a small coefficient of variance (Christensen and Bangsbo 2015). As in our previous study (Christensen and Bangsbo 2016), we chose to standardize the majority of the first test for subjects to recover from a comparable exercise stress (i.e., avoid large differences in pacing between days in the first max test which potentially could impact subsequent performance in the second max test and consequently any influence NAC might have). This allowed to evaluate how NAC influenced recovery. Studies have used a protocol similar to PT-1 and have been able to detect changes in performance with caffeine supplementation (Wiles et al. 1992; Doherty et al. 2004). Thus, we do believe PT-1 and PT-2 both are sensitive tests suitable for the detection of any difference in performance caused by NAC supplementation.

It is worth considering if the lack of change in performance with NAC and unchanged TAC following intense exercise relates to low statistical power. The number of subjects ($n = 11$) in the present study was in the same range as in studies observing an improved performance or exercise capacity following oral NAC supplementation with sample sizes of 7 (Corn and Barstow 2011), 8 (Slattery et al. 2014), 10 (Michailidis et al. 2013) and 12 (Cobley et al. 2011). In turn, the performance test used following previous exercise (PT-2) has a low CV of 1.6%, and a 3% reduction in mean power following different warm-up routines has been reported at $P < 0.01$ significance level (Christensen and

Bangsbo 2015). Thus, if NAC had an effect, we would likely have detected it (e.g., $P = 0.74$ for PT-2). Likewise, studies reporting a lowered TAC in trained athletes had sample sizes of 8 (Slattery et al. 2014) and 11 (Gejl et al. 2016), relative to 8 in the present study. Collectively, we cannot exclude a large sample size would have changed the outcome of the study, but considering the results from the aforementioned studies we believe the sample size was adequate to detect any relevant changes if present.

Perspectives

Future studies with NAC may benefit from measures of glutathione to evaluate whether "low" and "high" glutathione level athletes obtain a performance improvement or decrement, respectively, as reported in a recent study (Paschalis et al. 2018). Comparison of exercise modalities dominated by arm and leg muscles is also considered of interest, since arm muscles appear to have a lower antioxidative enzymatic profile than leg muscles (Mohr et al. 2017).

Conclusions

Oral ingestion of the antioxidant NAC in well-trained cyclists did not influence intense endurance performance when maximal cycling (~4 min) was performed twice separated by 90 min to simulate a typical competitive setting in events such as track cycling and swimming. Following the first maximal cycling bout, no change was observed in plasma total antioxidant capacity, and subsequent submaximal cycling economy and plasma potassium as well as peak VO_2 and lactate were not negatively influenced by prior intense exhaustive exercise. Therefore, on the basis of the present study, well-trained endurance athletes are not expected to gain any ergogenic effect from NAC supplementation during a single intense exercise bout in a time domain around 4 min. If prior maximal exercise does not cause a reduction in antioxidant capacity, no positive effect on intraday recovery and subsequent performance should be expected either with NAC supplementation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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