Pflugers Arch - Eur J Physiol (2014) 466:577–585
DOI 10.1007/s00424-013-1331-z

MUSCLE PHYSIOLOGY

Effects of *N*-acetylcysteine on isolated mouse skeletal muscle: contractile properties, temperature dependence, and metabolism

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Received: 6 March 2013 / Revised: 18 July 2013 / Accepted: 18 July 2013 / Published online: 3 August 2013

Abstract The effects of the general antioxidant *N*-acetylcysteine (NAC) on muscle function and metabolism were examined. Isolated paired mouse extensor digitorum longus muscles were studied in the absence or presence of 20 mM NAC. Muscles were electrically stimulated to perform 100 isometric tetanic contractions (300 ms duration) at frequencies resulting in ~85 % of maximal force (70–150 Hz at 25–40 °C). NAC did not significantly affect peak force in the unfatigued state at any temperature but significantly slowed tetanic force development in a temperature-dependent fashion (e.g., time to 50 % of peak tension averaged 35±2 ms [control] and 37±1 ms [NAC] at 25 °C vs. 21±1 ms [control] and 52±6 ms [NAC, $P<0.01$] at 40 °C). During repeated contractions, NAC maximally enhanced peak force by the fifth tetanus at all temperatures (by ~30 %). Thereafter, the effect of NAC disappeared rapidly at high temperatures (35–40 °C) and more slowly at the lower temperatures (25–30 °C). At all temperatures, the enhancing effect of NAC on peak force was associated with a slowing of relaxation. NAC did not significantly affect myosin light chain phosphorylation at rest or after five contractions (~50 % increase vs. rest). After five tetani, lactate and inorganic phosphate increased about 20-fold and 2-fold, respectively, both in control and NAC-treated muscles. Interestingly, after five tetani, the increase in glucose 6-P was ~2-fold greater, whereas the increase in malate was inhibited by ~75 % with NAC vs. control, illustrating the metabolic effects of NAC. NAC slightly decreased the maximum shortening velocity in early fatigue (five to seven repeated tetani). These data demonstrate that the antioxidant NAC transiently enhances muscle force generation by a mechanism that is independent of changes in myosin light chain phosphorylation and inorganic phosphate. The slowing of relaxation suggests that NAC enhances isometric force by facilitating fusion (i.e., delaying force decline between pulses). The initial slowing of tension development and subsequent slowing of relaxation suggest that NAC would result in impaired performance during a high-intensity dynamic exercise.

Keywords Muscle · Force · *N*-Acetylcysteine · Temperature · Metabolism

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Introduction

Reactive oxygen/nitrogen species are known to affect force generation and signaling in various metabolic pathways in skeletal muscle [12, 15, 24, 25, 27, 40]. An antioxidant that is frequently used in such studies is *N*-acetylcysteine (NAC).

Perusal of the literature showed that the effects of NAC on force and fatigue have been studied mainly in diaphragm muscle preparations [6, 13, 30, 35] and in humans [14, 19–21, 26, 37]. The effects of NAC appear to be task specific, sometimes enhancing force generation and delaying fatigue, sometimes yielding no effect, and sometimes even diminishing force generation [19]. Further, when beneficial effects of NAC on force generation/fatigue are seen, they occur more markedly at high (physiological) temperatures [6], as seen with other antioxidants as well [7, 23, 38]. Studies

in our laboratory, however, have not shown any effect of NAC on force in rested muscle nor on fatigue during repeated contractions in isolated type II limb muscles (flexor digitorum brevis and extensor digitorum longus (EDL)) [5, 29, 46]. Owing to the large number of factors that may have contributed to the variable results using NAC to study muscle function in earlier studies, we performed a controlled investigation of the effect of NAC on muscle force generation in the rested state and during fatigue. Specifically, we focused on temperature dependence, metabolism, and contractile properties of the isolated mouse EDL muscle, which was chosen since we previously established that NAC acts as an antioxidant in this preparation [29].

Methods

Materials and animals Antibodies against phosphorylated (S20) (ab2480) and total (ab11802) myosin light chain (MLC) were purchased from Abcam. All other chemicals were from Sigma or Boehringer.

Female C57Bl/6BKl mice weighing 18–22 g (approximately 7–12 weeks of age) were housed at room temperature with a 12:12-h light–dark cycle. Food and water were provided ad libitum. Animals were killed by rapid cervical dislocation, and thereafter, EDL muscles were isolated. The Stockholm North local ethical committee approved all animal care procedures.

Mounting, solutions, and stimulations Stainless steel hooks were tied with silk sutures to the muscle tendons. In experiments with isometric contractions, muscles were transferred to a stimulation chamber (World Precision Instruments) and mounted between a force transducer and an adjustable holder. The chamber temperature (25–40 °C) was maintained with a water-jacketed circulation bath. The muscle was bathed in a Tyrode solution consisting of (in millimolar) the following: NaCl 121, KCl 5, $CaCl_2$ 1.8, NaH_2PO_4 0.4, $MgCl_2$ 0.5, $NaHCO_3$ 24, EDTA 0.1, and glucose 5.5; the solution was continuously gassed with 95 % O_2–5 % CO_2, yielding a pH of 7.4. In most experiments, NAC was added to this solution to yield a final concentration of 20 mM (10 mM NaCl was added to the contralateral muscle to control for osmotic pressure); other additions to the solution are described in the text. Muscles were stimulated with plate electrodes lying parallel to the muscle using current pulses (0.5 ms duration, ~150 % of the current was required for maximum force response). Optimal length for tetanic force was set with 100-ms trains at 70 Hz.

Paired EDL muscles were preincubated for 60 min and then stimulated to perform 100 tetanic contractions (tetanic duration 300 ms, one train per 3 s) at frequencies of 70 Hz (25 °C), 100 Hz (30 °C), 150 Hz (35 °C), or 150 Hz (40 °C). These frequencies yielded a mean initial force that was ~85 % of the maximum (means at the four temperatures ranged from 81 to 88 %) and almost fused tetani that allowed for accurate and reproducible estimates of contractile properties. These frequencies were chosen on the basis of force frequency curves determined at the various temperatures (data not shown). In one series of experiments (35 °C), muscles were frozen immediately after five tetanic contractions for analysis of MLC phosphorylation and metabolites.

In another series of experiments, shortening velocity at zero load (V_0) was measured by the slack test at 25 °C [16]. After 60 min in NAC or control, muscles were stimulated to perform maximal tetanic contractions (120 Hz, 350-ms trains) at 1-min intervals. Muscles contracted isometrically during the initial 200 ms and were then allowed to shorten, and the subsequent isometric force redevelopment was measured (see Fig. 5). The shortening steps ranged from 0.5 to 2 mm, and muscles were stretched for 2 s prior to each contraction so that isometric force redevelopment always occurred at the optimal length. Three minutes after completion of the slack tests, muscles were stimulated to perform seven contractions (120 Hz, 350 ms trains, one train per 3 s). During the last three contractions, slack tests were again performed (1-, 2-, and 1-mm shortening steps, respectively).

Analytical Force signals were sampled online and stored in a personal computer for subsequent analysis. Times for force development and relaxation were estimated as time to 50 % of peak tension (1/2 TTP) and time to 50 % relaxation (1/2 RT). In the slack tests, the force redevelopment after the shortening step was fitted by a single exponential function. The time to take up the slack was determined as the time at zero force in each fitted curve. The release amplitudes were plotted against the time needed to take up the slack, and a straight line was fitted to the data points. V_0 was obtained by dividing the slope of this fitted line by the optimal length of the muscle.

Muscles were freeze dried and cleaned of nonmuscle constituents, powdered, thoroughly mixed, and separated into aliquots. One aliquot of muscle powder was extracted in ice-cold 0.5 M perchloric acid (160 μl/mg dry weight (wt)) and centrifuged, and the supernatant was neutralized with 2.2 M $KHCO_3$ and centrifuged again. The final supernatant was analyzed for ATP, phosphocreatine (PCr), creatine, glucose 6-P, lactate, inorganic phosphate (P_i), and malate using enzymatic techniques (changes in NAD[P]H) adapted for fluorometry [17].

A second aliquot was homogenized (100 μl/mg dry wt) with a ground glass homogenizer in ice-cold buffer consisting of (in millimolar) the following: HEPES 20, NaCl 150, EDTA 5, glycerol 20 % (*v*/*v*), KF 25, Na_2VO_4 1, protease inhibitor (Roche) 1 tablet/50 ml, and Triton X-100 0.5 %, pH 7.6. Homogenates were agitated for 30 min at 4 °C and centrifuged at 700×*g* for 10 min at 4 °C. The supernatant was diluted with Laemmli buffer +5 % β-mercaptoethanol (1:1) and heated at

95 °C for 5 min. Twenty micrograms of protein were separated by SDS-PAGE on 4–12 % Bis-Tris Gels (Invitrogen) at 150 V (~80 min) and transferred onto polyvinylidene fluoride membranes (Immobilon-FL, Millipore) (100 V, 3 h). Membranes were blocked for 1 h at room temperature in 3 % (*w*/*v*) bovine serum albumin in Tris-buffered saline containing 0.05 % Tween 20 (TBS-T). Thereafter, membranes were incubated with primary antibody diluted in blocking buffer (phospho-MLC and total MLC 1:2,500) overnight at 4 °C, washed with TBS-T, and incubated at room temperature with IRDye 680-conjugated goat anti-mouse IgG and IRDye 800 goat anti-rabbit IgG (1:15,000 LI-COR) diluted in TBS-T and LI-COR blocking buffer (1:1, *v*/*v*) for 1 h. The membranes were then washed with TBS-T. Immunoreactive bands were visualized using infrared fluorescence (IR ODYSSEY scanner, LI-COR Biosciences). Band densities were analyzed with ImageJ (NIH, USA; http//rsb.info.nih.gov/ij/).

Statistical Values are presented as means ± SE, unless stated otherwise. Statistical significance was set at $P<0.05$ and was determined with Student's paired *t* test.

Results

Following 1 h of incubation, NAC did not significantly affect submaximal peak force (~85 % of the maximum) at any temperature (Table 1). A series of fatigue experiments at 25–40 °C were then performed. Under control conditions, force initially went down rapidly at all temperatures, but this decrease was counteracted by NAC (Fig. 1). In general, the most noteworthy effect of NAC on force was observed after contraction 5 at all temperatures with a potentiating effect vs. control of ~25 % at 25 °C, ~20 % at 30 °C, ~35 % at 35 °C, and ~30 % at 40 °C (Fig. 1). Lower concentrations of NAC were also studied at 35 °C. No significant effect on force at any time was observed with 1 mM NAC; for instance, after five contractions, force had decreased to 92.5±0.9 % of initial in control muscles and to 90.8±0.6 % of initial in NAC-exposed muscles ($P>0.05$ NAC vs. control, $n=7$). However, 10 mM NAC resulted in a significant, albeit small, improvement in force after five contractions; force decreased to 90.0±0.7 % of initial in control muscles and to 94.1±0.5 % of initial in NAC-exposed muscles ($P<0.05$ NAC vs. control, $n=7$). Owing to the markedly more robust effect of NAC at 20 mM, all subsequent experiments were performed at 20 mM NAC.

Whereas the potentiating effect of NAC on force was present throughout fatiguing stimulation at 25 °C (Fig. 1), the effects disappeared more rapidly at higher temperatures.

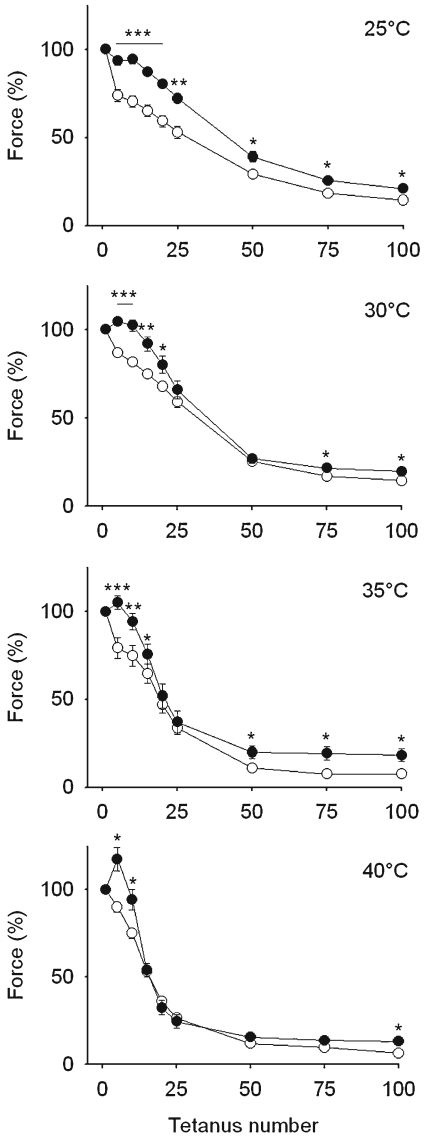

Fig. 1 Effects of NAC on force of EDL muscle during repeated tetanic contractions. Values are means ± SE for $n=7$ to 9 muscles. *Unfilled circles* denote control; *filled circles* denote NAC at the given temperatures. Force is expressed as a percentage of initial force (see Table 1 for absolute initial forces). *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. respective control value

Table 1 NAC does not affect submaximal isometric force of EDL muscle

Temperature (°C)	Force (mN)	
	Control	NAC
25	216±19	160±25
30	204±18	197±18
35	195±27	173±17
40	146±10	165±18

Values are mean ± SE for seven to nine muscles. Muscles were incubated at the given temperatures in the absence (control) or presence of NAC for 60 min and then stimulated with a 300-ms train at 70 Hz (25 °C), 100 Hz (30 °C), 150 Hz (35 °C), or 150 Hz (40 °C), which yields ~85 % of maximal isometric force

At 30 °C, the significant effect of NAC was lost by contraction 25, at 35 °C by contraction 20, and at 40 °C by contraction 15. Thus, the NAC effect on force potentiation was extended with decreasing temperature. Most subsequent experiments were performed at 35 °C, since this is the normal temperature of limb muscles in the living mouse [45].

NAC is a general antioxidant that will buffer oxidizing species in all cell compartments. Recently, others demonstrated that reactive oxygen species (ROS) derived from mitochondria were responsible for the depression of force in mechanically skinned rat EDL muscle fibers at physiological temperature (37 °C) [38]. Therefore, we used SS31, a cell-permeable antioxidant targeted to mitochondria [36, 47]. In our hands, SS31 has biological effects in isolated mouse cardiomyocytes [1]. However, SS31 did not affect force production during fatiguing stimulation at 35 °C (Fig. 2a). Next, we assessed whether NAC was working on another ROS generator that has been implicated in muscle function: NADPH oxidase [28]. We used concentrations of apocynin and diphenyleneiodonium (DPI) shown to inhibit NADPH oxidases [8, 18, 44]. However, apocynin had no noteworthy effect on force (Fig. 2b). Following 1 h of exposure to 1 mM DPI, initial force was decreased by more than 70 % (control=220±30 mN, DPI=63±5; $P<0.05$; $n=4$). At 10 μM, DPI had no effect on initial force and tended to decrease force throughout the fatigue run ($n=2$, data not shown).

To examine the basis for the potentiating effect of NAC on force, we determined the degree of myosin light chain phosphorylation, since this results in force potentiation during submaximal isometric contraction [34] and myosin light chain is redox sensitive [9, 31]. NAC did not affect MLC phosphorylation in the basal state (Fig. 3). MLC phosphorylation increased (~50 %) similarly during five tetanic contractions in control and NAC-exposed muscles. Thus, MLC is not involved in the potentiating effect of NAC.

Next, we examined whether NAC exposure induced metabolic changes that could account for the enhanced force after five contractions. In the basal state, there were no noteworthy effects of NAC on metabolites, with the exception of a small but significant increase in glucose 6-P (Table 2). Following the five contractions, the increase in P_i was similar in control and NAC-exposed muscles. Similarly, the changes in PCr and lactate after contractions did not differ between groups. Interestingly, the increase in glucose 6-P after five tetani was ~2-fold greater in NAC-exposed muscles, whereas the increase in malate was reduced by ~75 % in muscles treated with NAC vs. control.

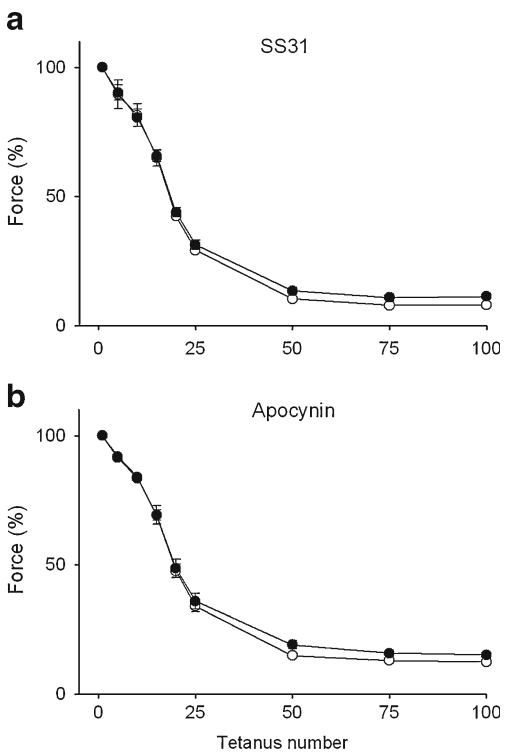

Fig. 2 SS31 and apocynin do not prevent the decline of force of EDL muscle during repeated tetanic contractions. Values are means ± SE for $n=5$ to 6 muscles. *Unfilled circles* denote control; *filled circles* denote 3 μM SS31 (**a**) or 1 mM apocynin (**b**) at 35 °C. Force is expressed as a percentage of initial force. Absolute values for initial force (in millinewton) are as follows: control=261±13, SS31=229±8 (**a**) and control=209±13, apocynin=230±38 (**b**)

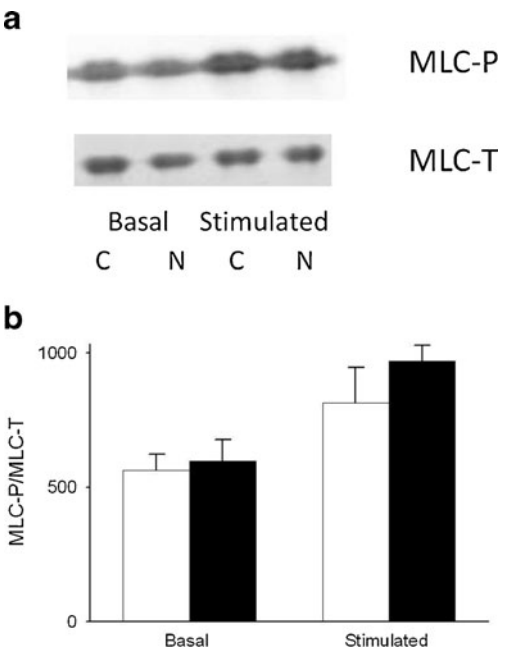

Fig. 3 NAC does not alter the degree of myosin light chain phosphorylation of EDL muscle. Muscles were stimulated to perform five tetanic contractions (150 Hz, 300 ms trains at 35 °C). **a** Representative western blots for phosphorylated myosin light chain (*MLC-P*) and total myosin light chain (*MLC-T*). *C* denotes control; *N* denotes NAC. **b** MLC-P/MLC-T means ± SE for $n=5$ (basal) or $n=6$ (stimulated) muscles. *Unfilled bars* denote control; *filled bars* denote NAC. Values are expressed relative to basal control

Table 2 Effects of NAC on metabolite levels in EDL muscle

	Basal		Stimulated	
	Control	NAC	Control	NAC
Glucose 6-P	0.51±0.07	0.70±0.10*	2.38±0.07	5.22±1.04*
Lactate	1.3±0.2	0.9±0.2	19.4±3.1	16.8±3.9
Malate	0.07±0.02	0.05±0.01	0.49±0.06	0.16±0.04***
ATP	25.0±0.3	25.5±0.3	22.6±0.6	23.5±1.2
Phosphocreatine	64.3±2.6	63.0±1.2	39.8±2.6	37.4±4.4
P_i	14.6±0.5	13.8±0.4	33.9±3.7	30.9±4.5
Total creatine	84.7±3.4	90.0±1.6	88.8±3.0	89.9±3.1

Values are mean ± SE for seven muscles and are given in micromoles per gram dry weight. Muscles were stimulated to perform five tetanic contractions (150 Hz, 300 ms trains at 35 °C)

*P<0.05; ***P<0.001 (vs. corresponding control)

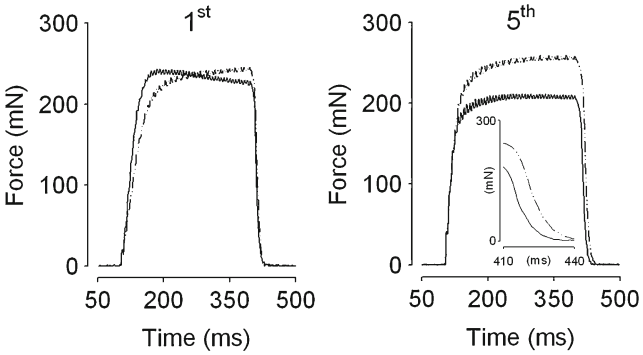

Fig. 4 Representative force recordings for two EDL muscles from one mouse during the first and fifth isometric tetanic contractions (150 Hz, 300 ms trains at 35 °C). *Solid line* denotes control; *dashed line* denotes NAC. Note the increase in force and slowing of relaxation in the presence of NAC during the fifth contraction. *Inset* is a magnification to clarify the slowing of relaxation

We also assessed whether NAC affected contraction and relaxation times in the unfatigued state and during repeated contractions (Table 3). In the unfatigued state, NAC decreased the speed of contraction (measured as 1/2 TTP) at 30–40 °C (Table 3 and Fig. 4). And the higher the temperature, the greater is the slowing induced by NAC. However, during repeated contractions, 1/2 TTP became significantly faster at all temperatures and the effect of NAC was virtually lost, although there was still a small but significant effect at 40 °C. There was no relationship between the force-enhancing effects of NAC and slowing of 1/2 TTP.

In the unfatigued state, NAC did not affect the speed of relaxation (measured as 1/2 RT), with the exception of a small degree of slowing at 35 °C. Additional contractions slowed 1/2 RT at all temperatures. This slowing was significantly larger with NAC after five contractions at all temperatures. This is illustrated in typical force recordings from the first and fifth contractions at 35 °C (Fig. 4). This effect was lost by 25 contractions at all temperatures, with the exception of a sustained slowing at 25 °C (recall that force was enhanced by NAC at this point as well). By 100 contractions, however, NAC again resulted in the slowing of 1/2 RT at all temperatures, which, again, was associated with a small but significant force enhancement. Noteworthy is that at 10 mM NAC, where the effect of NAC on force was minor (see above), relaxation was marginally more slowed after five contractions in six of seven NAC-exposed muscles and the difference did not reach statistical significance (NAC=24.3±0.7 ms, control=22.7±0.5 ms; n=7). Thus, tetanic force was robustly enhanced only when NAC clearly slowed relaxation (cf. Fig. 1 and Table 3).

Finally, the effect of NAC (20 mM) on V_0 and the rate of force redevelopment after shortening were examined. In the unfatigued state, NAC did not significantly affect the speed of shortening (control=3.76±0.19 lengths/s vs. NAC=3.56±0.05 lengths/s; n=7, P>0.05) (Fig. 5). These values are comparable to V_{max} values reported for mouse EDL bundles studied under similar conditions [4]. In early fatigue (tetani 5–7), where NAC had its largest force-enhancing effect, V_0 was not changed in

Table 3 Effects of NAC on contractile properties of EDL muscle

Temperature (°C)	Contraction 1		Contraction 5		Contraction 25		Contraction 100	
	Control	NAC	Control	NAC	Control	NAC	Control	NAC
Time to 50 % of peak tension (1/2 TTP, ms)								
25	35±2	37±1	25±1	27±1	24±2	24±1	24±2	21±1
30	26±1	32±2**	20±1	23±1	21±1	21±2	16±1	17±1
35	24±1	34±2**	17±1	19±1	19±2	17±1	12±2	15±1*
40	21±1	52±6**	14±1	18±2*	11±1	13±1*	10±1	13±1**
Time to 50 % of decrease of tension (1/2 RT, ms)								
25	27±1	29±2	29±2	36±2*	45±2	59±3**	41±2	55±3**
30	22±1	22±2	27±2	31±2**	44±3	47±3	33±2	38±2*
35	15±1	17±1*	19±1	24±1***	29±1	30±1	21±1	27±1**
40	15±1	15±1	19±1	20±1*	25±1	24±1	16±1	21±2**

Values are means ± SE for seven to nine muscles

*P<0.05; **P<0.01; ***P<0.001 (vs. corresponding control)

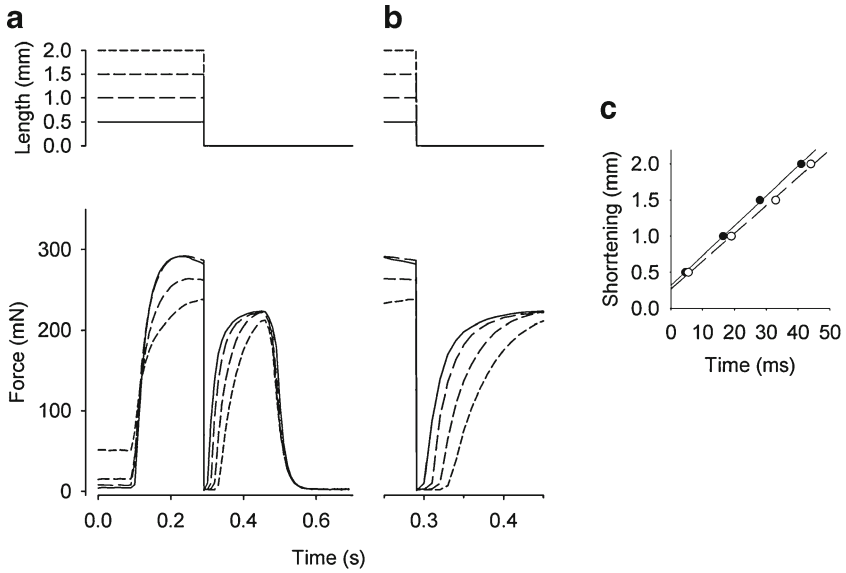

Fig. 5 Representative force recordings from a slack test experiment performed in control solution in the unfatigued state. **a** Length changes (*top*) and the corresponding force traces (*bottom*). Tetanic stimulation (120 Hz) started at 0.1 s and ended at 0.45 s. Length=0 mm represents the optimal length. **b** Records from the time of the shortening in **a** on an expanded time scale. **c** Measurements of the time until force starts to redevelop after different shortening amplitudes. *Filled circles* (*full line*) show data from the experiment depicted in **a** and **b** and *unfilled circles* (*dashed line*) show data from the contralateral muscle exposed to NAC. Note the good fit of a straight line to the data points in both conditions and the similar slopes, i.e., similar V_0

control solution (−3.4±2.8 % of the original, $P>0.05$), whereas it was slightly decreased in the presence of NAC (−5.7±2.1 % of the original, $P<0.05$). Force redevelopment after the shortening step was well described by a single exponential function in all conditions. In the unfatigued state, force redevelopment had similar half-times ($t_{1/2}$) in control solution (20.7±0.8 ms) and in the presence of NAC (23.2±1.6 ms, $P>0.05$). The rate of force redevelopment was significantly increased in early fatigue ($P<0.01$), but there was still no difference between the two conditions (15.7±0.6 ms in control vs. 16.6±0.6 ms with NAC, $P>0.05$).

Discussion

The major findings of the present study are that (1) NAC transiently enhances force generation during repeated tetanic contractions at 25–40 °C, (2) the loss of the NAC effect is accelerated at higher temperatures, (3) force enhancement is not associated with MLC phosphorylation or changes in P_i concentration but is associated with slowing of relaxation, and (4) NAC markedly increases glucose 6-P, but attenuates malate accumulation during repeated contractions.

We used a NAC concentration of 20 mM in the majority of the present experiments, because this concentration has previously been shown to alter isolated muscle contractility and metabolism under various experimental conditions [6, 29, 43, 46]. NAC is a general antioxidant and can scavenge various oxidants in all cell compartments [3]. We attempted to identify the site at which NAC exerted its force-potentiating effect during repeated contractions by using the mitochondrially targeted antioxidant SS31 and inhibitors of NADPH oxidases. However, these treatments had no positive effect on force generation. Previously, we demonstrated that NAC functions as an antioxidant in the isolated mouse EDL muscle during repeated contractions (based on changes in glutathione status and fluorescence of an intracellular ROS indicator) [29]. This suggests that NAC is enhancing force by scavenging ROS in a manner that cannot be achieved by using SS31 or inhibitors of NADPH oxidases.

The effects of the cellular redox state on muscle force generation are complex. Oxidants can increase or decrease force in isolated muscle, and the effects will depend on the nature of the oxidant, duration of exposure, and concentration [2, 19, 24, 31]. The mechanism whereby NAC increases submaximal force during the early phase of repeated contractions in the present study is not clear. It is not due to alterations in MLC phosphorylation or accumulation of metabolites (P_i and lactate). Rather, the increase of force with NAC was associated with a slowing of relaxation, which would favor enhanced tension during the not fully fused contractions used in the present study. Slowing of relaxation could be due to slowed Ca^{2+} sequestration by the sarcoplasmic reticulum (SR) or altered cross-bridge kinetics. In frog muscle fibers, the fatigue-induced slowing of relaxation can be ascribed to the combined effect of less effective removal of Ca^{2+} from the cytosol by the SR and altered cross-bridge kinetics [41]. However, in mouse fast-twitch muscle fibers, the fatigue-

induced slowing of relaxation was found to be caused solely by altered cross-bridge kinetics [39, 41]. That being the case, increased force in the presence of NAC may not be due to an increase in $[Ca^{2+}]_i$, but rather to slowed cross-bridge kinetics, as has been suggested earlier [24, 31]. In support of this, we observed a small decrease in V_0 in early fatigue in the presence of NAC but not in the control. Both the relaxation speed and V_0 depend on cross-bridge detachment, although the exact mechanisms of detachment differ in the two conditions [10]. On the other hand, the rate of force development at the onset of contractions and the rate of force redevelopment after the shortening step increased in early fatigue, and both these parameters depend on the rate of cross-bridge attachment [10]. There are several redox-sensitive sites on myofibrillar proteins where NAC could be exerting its effects. These include troponin, tropomyosin, actin, and myosin heavy chains [9, 22, 24, 31]. In summary, a possible explanation for the early force-enhancing effect of NAC is slowed cross-bridge detachment, owing to changes in the redox state of myofilament proteins. Thus, the slowing of relaxation, as seen with NAC, would tend to enhance fusion and hence force generation during submaximal isometric contraction.

Earlier, it was shown that NAC effects on muscle function were temperature dependent [6] and others using another antioxidant (Tempol) arrived at similar conclusions [7], where antioxidants dramatically improved muscle function at 37 °C compared with 22 °C. During repeated tetani in the present study, NAC initially increased submaximal tetanic force more as temperature increased (cf. contraction 5 vs. 1 in NAC experiments, Fig. 2). The loss of the NAC effect, thereafter, also appeared to be dependent on temperature, with the NAC effect disappearing more rapidly when the temperature was higher. These results suggest a complex ROS–temperature interaction with respect to force.

An unexpected finding was the markedly increased accumulation of glucose 6-P and diminished accumulation of malate after only five contractions in the presence of NAC. The NAC-mediated increase in glucose 6-P is not likely to reflect a block in glycolysis as judged by the similar accumulation of lactate between conditions. Malate accumulation reflects acceleration of oxidative metabolism [11, 45] and occurs as a consequence of expansion of the tricarboxylic acid (TCA) intermediate pool (anaplerosis), which is dependent on adequate glycolysis [32, 33]. Thus, it is unlikely that NAC is attenuating malate accumulation by inhibiting a step prior to pyruvate formation. Rather, NAC is either inhibiting influx of TCA cycle precursors into mitochondria or mitochondrial function. Clearly, NAC is affecting several steps in carbohydrate metabolism. Previously, we showed that NAC inhibits contraction-mediated activation of AMP-dependent protein kinase and glucose transport in isolated mouse EDL muscle [29, 46], yet another aspect of carbohydrate metabolism. If and/or how the latter events are linked to those described in the current study is at present not known. We do not believe, however, that the current metabolic changes induced by NAC have any direct effects on the force results, but they could impact on muscle performance under other conditions where metabolic flux rates could be critical (for example lower intensity, more prolonged exercise).

What then are the physiological implications of the current findings? In unfatigued muscle at 25 °C, the rate of tension development is slow and not affected by NAC. This is consistent with low ROS production at low temperatures (see above). At higher temperatures, tension development speeds up but NAC slows this process, most markedly at 35–40 °C (Table 3, contraction 1). This suggests that ROS contributes to the rate of tension development at physiologic temperatures in unfatigued muscle. While NAC does not affect peak force during a prolonged isometric contraction, it would be expected to markedly lower the force × time integral during a short isometric contraction or power during a short dynamic contraction, which can have a duration of less than 100 ms in human muscle [42]. The implication is that during the first contraction of an intense exercise (e.g., the first step-out of starting blocks or power lift), performance would be markedly impaired by NAC. Thereafter, the effect of NAC on the rate of tension development will disappear. Instead, NAC will result in the slowing of relaxation in the subsequent contractions, which suggests that ROS speeds up relaxation during repeated contractions (even at low temperatures). During dynamic contractions (e.g., during sprinting), the slowing of relaxation induced by NAC should also result in poorer performance. Thus, the present results suggest that NAC will negatively affect performance during intense dynamic exercise.

Conclusions

These results demonstrate that NAC slows tension development in unfatigued mouse fast-twitch muscle in a temperature-dependent manner. Additionally, NAC transiently enhances isometric force generation during repeated contractions by a mechanism that is independent of changes in myosin light chain phosphorylation and inorganic phosphate. The concomitant slowing of relaxation, which seems to be caused by altered myofibrillar function and slowed cross-bridge detachment, suggests that NAC enhances isometric force by facilitating fusion. The initial slowing of tension development and subsequent slowing of relaxation suggest that NAC would impair exercise performance during intense dynamic exercise.

Acknowledgments This research was supported by grants from the Swedish National Center for Sports Research and the Swedish Research Council and by funds from the Karolinska Institutet. A. Hernández was supported by a grant from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (no. IF32AR057619).

Conflict of interest No conflict of interest, financial or the like, is declared by the authors.

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