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Acute Effect of Oral N-Acetylcysteine Supplementation on Fatigue Effect and Isometric Force Production in Physically Active People

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Abstract: The main objective of this study was to assess the ergogenic effect of N-acetylcysteine (NAC) on myotendinous isometric force production in physically active people after being subjected to a fatigue protocol. Twenty-three physically active people were randomly divided into the following two groups: NAC (n = 12; age = 26.8 ± 4.5 years, height = 173.1 ± 7.2 cm, and weight = 75.5 ± 7.5 kg), who received 2400 mg oral NAC, and control (n = 11; age = 23.4 ± 5.8 years, height = 175.9 ± 4.5 cm, and weight = 72.3 ± 9.9 kg), who received a placebo, for eight days. The isometric force production was assessed pre- and post-NAC supplementation during a maximal voluntary contraction test (MVC) and also during a fatigue protocol composed of seven sets of ten maximal isometric contraction repetitions of 5 s, with 5 s of rest between repetitions and 20 s between sets. No differences were observed between the groups in the force production values at any moment, and no side effects were found after NAC supplementation. After supplementation, a significant decrease in force was observed in both groups, but this significant loss of force started one set later in the NAC group compared to the control group (4th set vs. 5th set), which could be an ergogenic effect of the treatment. Therefore, oral daily supplementation with 2400 mg of NAC for eight days, could delay the decrease in force production during an isometric exercise protocol and without adverse side effects.

Keywords: N-acetylcysteine; muscle fatigue; muscle; strength; ergogenic effects; side effects

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1. Introduction

Neuromuscular fatigue has been defined as the exercise-induced reduction in the ability of skeletal muscle to produce force, regardless of task completion [1]. Factors commonly associated with muscle fatigue can be grouped into the following two categories: (a) central factors involving the central nervous system and neural pathways; and (b) peripheral factors occurring within the muscle, beyond the neuromuscular junction [2,3]. Muscle fatigue is a phenomenon commonly experienced during sport and limits performance in strenuous or prolonged activities. It also increases and restricts daily life under various pathological conditions, such as neurological, muscular, and cardiovascular disorders, as well as aging and frailty. Reducing the effect of fatigue or delaying the onset of fatigue at the same work intensity is one of the main objectives of training programs and ergogenic aids, although approaches vary depending on the stimulus of the exercise [4,5].

Changes in factors such as loss of strength, changes in the rate of muscle activation, and even stiffness resulting from strenuous exercise have been assessed mainly by artificial stimulation, i.e., mechanical and evoked muscle responses associated with electromyography or examinations of skeletal muscle and/or motor nerve structures at rest [6]. As well as contractile mechanisms, the decrease in voluntary and/or electrically induced force with fatigue also depends on the elongation capacity of elastic components, both in series and in parallel [7]. Fatigue may increase the compliance of the muscle–tendon complex due to repetitive cycles of long-duration contractions [8–10] that lengthen the force transmission time to the bone due to the inability to store and release elastic energy [9,11]. In addition, intense exercise is associated with the production of reactive oxygen species (ROS), the accumulation of which in muscle cells impairs muscle contraction and reduces the force production [12]. In intermittent sports or where the accumulation of competition occurs in a short space of time, this can be one of the factors that limits performance, so many recovery strategies have focused on fatigue control [13,14].

N-acetylcysteine (NAC) has been commonly used as an ergogenic aid for respiratory-related health problems, although there are already a number of research studies on its ergogenic effect in sport [15]. The main hypothesized mechanisms of action of NAC include improvement in potassium homeostasis, preservation of skeletal muscle Na+/K+ pump activity and inhibition of calcium ATPase oxidation in the sarcoplasmic reticulum [16]; so in sports where there is a high accumulation of exercise-induced ROS, their effect and performance decrement could be attenuated with an acute dose of NAC before or after exercise [17,18]. A recent review on the use of NAC in sport indicates that its antioxidant effect may be beneficial during the recovery process by scavenging ROS from muscle contraction and by providing cysteine for glutathione synthesis [4]. The studies analyzed in the review used a dose ranging from 1.2 to 20 g per day, for a loading period of 5 to 9 days. Following this dose recommendation, Corn and Barstow [5] found that oral NAC extended the time to fatigue at 80% of peak power, but other studies did not find a performance improvement under fatigue conditions [19,20].

Both in team sports (soccer, basketball, handball, etc.) and in individual sports, the competitive calendar is increasingly extensive, which means that long periods of time are accumulated at high physical intensity. This accumulation of competitions does not allow athletes to recover properly, so it is necessary to develop optimal recovery strategies. The use of NAC as a sports supplement has shown contradictions regarding its benefits for sports performance and oral intake should be further explored. Therefore, the main objective of this study was to assess the ergogenic effect of N-acetylcysteine on myotendinous isometric force production in physically active people after being subjected to a fatigue protocol.

2. Materials and Methods

2.1. Participants

Twenty-three physically active men (i.e., take part in physical exercise for a minimum of 2.5 h per week [21]) were invited to participate in the study. The participants were randomly divided into the following two groups using online software (http://www.randomization.com, accessed on 18 March 2022): control group (n = 11; age = 23.4 ± 5.8 years, height = 175.9 ± 4.5 cm, and weight = 72.3 ± 9.9 kg) and NAC group (n = 12; age = 26.8 ± 4.5 years, height = 173.1 ± 7.2 cm, and weight = 75.5 ± 7.5 kg). The sample size was previously calculated based on a study by Corn and Barstow [5], who analyzed the time to fatigue in cycling with and without NAC supplementation. The minimum number of subjects required to achieve a power of 0.9 and a bilateral alpha level of 0.05 was 10 participants. The experimental group received NAC supplementation, while the control group took a placebo of cellulose. All participants were informed of the testing procedures and possible risks involved, and all provided written consent. Ethical approval was granted from the Local Institutional Review Board (Hospital Clinico San Carlos; 22/513-E) in accordance with the latest version of the Declaration of Helsinki.

2.2. Procedure

The participants were familiarized with the measurement protocol before the start of the study. During the familiarization session, participants did four repetitions of five seconds of maximal voluntary isometric contraction (MVC) separated by three minutes of rest [22]. The study protocol consisted of two measurement periods, pre- and post-supplementation, each with two days of assessment. On the first day of both measurement periods, participants were assessed for their maximal isometric force production and carried out the fatigue protocol. On the second day of both evaluation periods, pre- and post-supplementation, participants only repeated the maximal isometric force test, to check the effect of fatigue 24 h later. The complete protocol is shown in Figure 1.

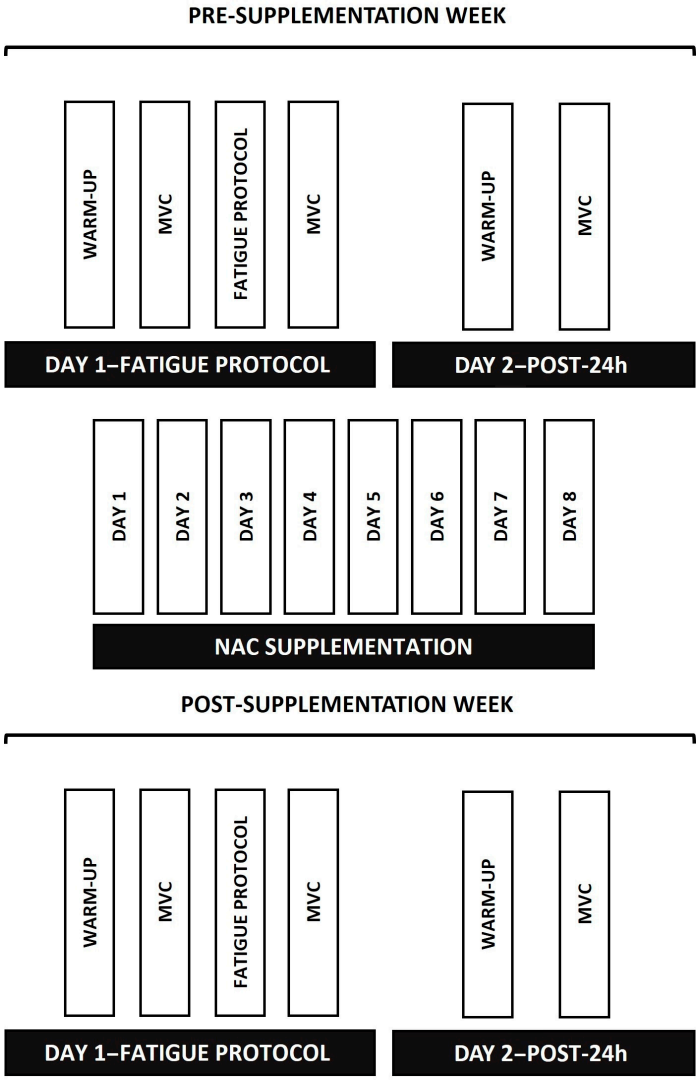

Figure 1. Study protocol. MVC = maximal voluntary isometric contraction test; NAC = N-acetylcysteine.

2.3. Strength Test

A Biodex Multi-Joint System 3 isokinetic dynamometer (Biodex Medical System, New York, NY, USA) was used to assess the maximal isometric strength of the knee extensor musculature of the dominant lower limb and to develop the fatigue protocol. A maximal strength test was conducted before and after the fatigue protocol, and it was also repeated on the remaining assessment days to analyze the effect of the fatigue protocol on MVC 24 h later. Before the tests, participants underwent a 5 min warm-up on the Wattbike cycle ergometer (Wattbike Ltd., Nottingham, UK) at an intensity of 100 W and a cadence of 80–90 rpm.

2.4. Maximal Voluntary Contraction Test

The MVC was calculated as the maximal force produced during an isometric contraction. The measurement protocol consisted of performing 2 maximal isometric contractions lasting 5 s each, with a 60 s rest between them. Isometric contractions were performed at 20° of knee flexion. MVC was defined as the average of the force values recorded by the isokinetic machine in the middle seconds of the test (2nd to 4th seconds), discarding the first and last seconds. Participants were positioned on the dynamometer chair aligning the knee flexion/extension axes with those of the dynamometer, seated with an angle of 110° between the backrest and the seat. The participant's position on the dynamometer was noted for replication in subsequent measurements. The upper thigh was secured to the chair and the most distal part of the leg to the dynamometer's padded lever arm. The non-evaluated limb remained free, and the trunk was stabilized against the backrest with two diagonal straps and another horizontal strap securing the hip to the seat.

2.5. Fatigue Protocol—Isometric Test

The protocol consisted of a total of 70 maximal isometric contractions, divided into 7 sets of 10 contractions each, to assess knee extensor muscle fatigue. Isometric contractions were maximal and lasted 5 s, with 5 s of rest between each. A total rest of 20 s was provided between each set (blocks of 10 contractions). The knee position was at 90° of flexion. Participants were positioned on the dynamometer chair in a similar fashion to the one used for the MVC test. The evolution of strength was assessed by recording the average strength of the force values measured by the isokinetic machine in the middle seconds of each isometric contraction (2 to 4 s), discarding the first and last seconds. The average force value was calculated for each of the series, and all series were compared with each other. During the fatigue protocol, the possible loss of strength and fatigue evolution were monitored, analyzing each contraction individually in terms of force data examinations.

2.6. Supplementation

Participants in the NAC group received their daily dose already prepared along with intake instructions supervised by an endocrinologist specializing in sports supplementation (C.L.). The supplementation guidelines were as follows [4]: 2.4 g of NAC per day, divided into 2 daily doses of 1200 mg each (~32 $mg \cdot kg^{-1}$). The individual dose of 1200 mg of NAC was consumed in a capsule with 100 mL of water. The first dose was taken in the early morning with breakfast and the second dose 2 h before dinner; The last dose was taken 2 h before the start of the post-evaluation. The control group took a placebo of 1200 mg of cellulose per dose (two doses in total) dissolved in 100 mL of water. An alphanumeric code was assigned to each trial to blind participants and investigators to the group assigned (NAC or control). This code was only accessible to investigators after the analysis of the variables.

Prior to the start of the study, all participants were informed about the most common side effects of NAC, such as diarrhea, vomiting, and headache [4]. Additionally, a daily record of any incidents related to the intake during the eight days of supplementation was kept.

2.7. Side Effects Evaluation

During the supplementation period, all participants responded to an online survey about discomforts typically associated with NAC ingestion. This survey included 14 items on a yes/no scale. This questionnaire was based on previous publications about side effects derived from the ingestion of NAC [4,20]. It recorded upset stomach, nausea, gas, cough, indigestion, vomiting, metallic taste, rash, itching, local erythema (redness), redness of eyes, face or hands, conjunctivitis, lightheadedness, and sleepiness. The participants had the option to indicate other side effects that were not included in the list at the end of the questionnaire.

2.8. Statistical Analysis

The quantitative data results are shown as the mean ± standard deviation. The normality of the variables was checked with the Shapiro–Wilk test. Once the normality of the variables was assumed ($p > 0.05$), a *t*-test for independent samples was used to ensure baseline comparability between groups for descriptive variables (age, height, and weight), which showed no statistically significant differences between groups at baseline ($p > 0.05$ for all variables). A two-way ANOVA was used to analyze the effect of fatigue on MVC force and on force production during the fatigue protocol. The difference between the average basal condition force (first set of isometric contractions in the fatigue protocol) and each of the other moments in the fatigue protocol was calculated. In addition, to determine from which set of isometric contractions the decrease in force production no longer changed, the difference between the lowest value of the force production and the other repetitions was also calculated. A paired *t*-test was employed to analyze these differences. The effect size was calculated (Cohen's d) for each variable. The qualitative data results (side effects) are presented as percentages. The differences in side effects were analyzed using the McNemar test. The significance criteria were set at $p < 0.05$.

3. Results

3.1. Maximal Isometric Contraction

Table 1 shows the results of the MVC force values at each measurement time. Maximal strength significantly decreased by 70.8 ± 17.4% in the NAC group ($p < 0.001$) after the fatigue protocol and by 61.4 ± 20.9% in the control group ($p < 0.001$). These differences were similar to those found at the pre-intervention time points in both groups (before supplementation). There were no significant differences between pre- and post-24 h, neither before or after supplementation in any of the two groups ($p = 0.092$). There were also no significant differences between groups at any time point of measurement ($p = 0.228$) and the decrease in strength was similar in both groups ($p = 0.085$).

Table 1. Maximal voluntary contraction evolution (mean ± standard deviation).

	PRE (N·m)	POST (N·m)	POST-24 h (N·m)		F	*p*-Value	ηp^2
Pre-supplementation intervention							
NAC	61.03 ± 16.87	28.96 ± 14.24 *	57.54 ± 24.82				
CONTROL	56.88 ± 24.82	37.35 ± 14.95 *	62.68 ± 17.37	Group Effect	0.110	0.745	0.006
Post-supplementation intervention				Time Effect	28.391	<0.001	0.916
NAC	67.85 ± 23.26	42.17 ± 19.32 *	73.69 ± 24.39	Time × Group	0.955	0.479	0.269
CONTROL	65.86 ± 12.83	46.59 ± 13.85 *	69.01 ± 15.22				

NAC, N-acetylcysteine group; Control, control group; PRE, pre-fatigue protocol; Post, post-fatigue protocol; Post-24 h, 24 h after fatigue protocol; ηp^2, partial eta squared; Group, main effect of the group in the ANOVA results; Time, main effect of time in the ANOVA results; Group × Time, main effect of group × time in the ANOVA results. * $p < 0.05$, compared with pre-fatigue protocol at each period (pre-supplementation and post-supplementation).

3.2. Effects of Fatigue

In the pre-intervention moment, no significant time × group effects (F = 2.453; $p = 0.108$) were found in the isometric force production during the fatigue protocol. In addition, no differences were found between the NAC and control groups in any set of isometric contractions ($p = 0.467$; 95% confidence interval (CI) of the mean difference (MD) = −62.17 to 29.50 N·m), although a significant decrease in force production compared to basal level (time effect) was described for both groups (F = 53.962; $p < 0.001$). The force production of the control group decreased 34.1 ± 10.7% at the end of the fatigue protocol and a decrease of 38.1 ± 14.1% was observed in the NAC group. After the supplementation intervention, there was no significant time × group effect (F = 2.1; $p = 0.151$). In addition, no differences between the NAC and control groups were found in any set of isometric contractions ($p = 0.467$; 95% CI of MD = −62.17 to 29.50 N·m), although a significant decrease in the force production compared to the basal level (i.e., time effect) was described for both groups

(F = 24.6; $p < 0.001$). The force production of the control group decreased 26.9 ± 10.3% at the end of the fatigue protocol and a decrease of 32.1 ± 16.6% was observed in the NAC group. The decrease in the isometric force production was the same for both groups, the control group and NAC group, at the pre-intervention moment ($p > 0.054$) and post-intervention moment ($p > 0.396$). Finally, after the supplementation intervention, the control group reached a maximum decrease in the isometric force production at set 4 (no differences were found in force production from set 4 to the end of the fatigue protocol ($p = 0.167$), but the NAC group reached the maximum decrease in the isometric force production from set 5 to the end of the fatigue protocol ($p = 0.095$). The results of the isometric force production during the fatigue protocol are shown in Figure 2.

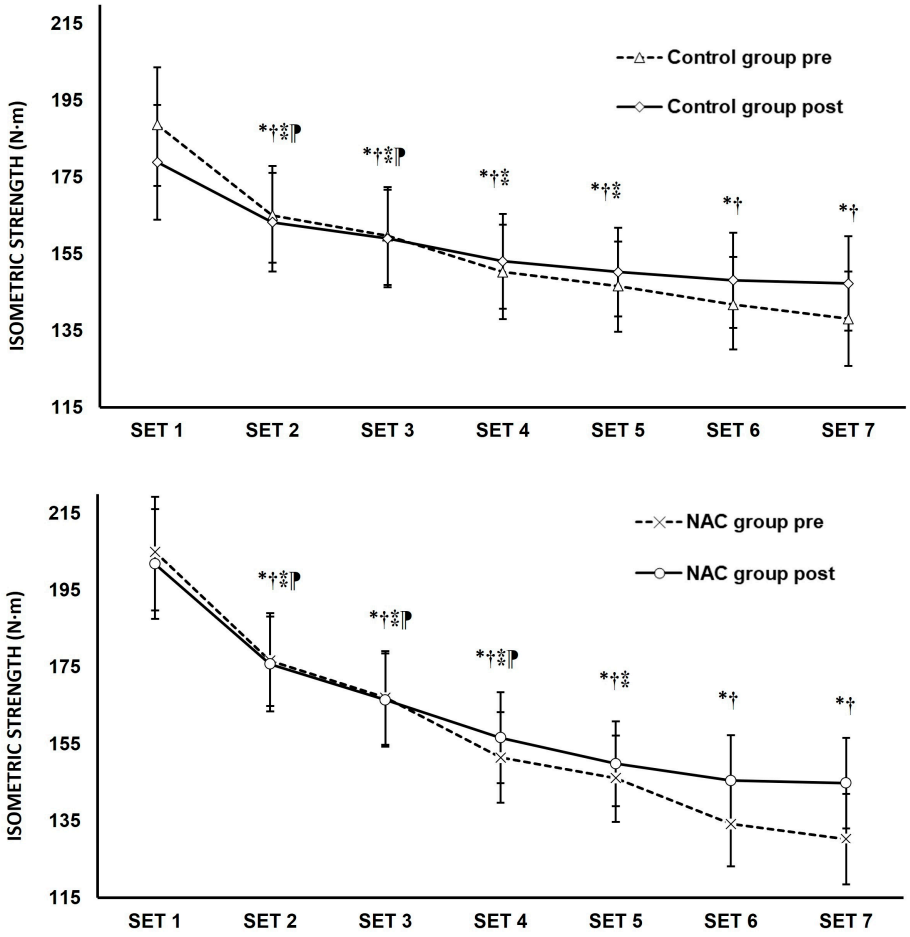

Figure 2. Isometric force production during the fatigue protocol (mean ± standard error). * Significant differences compared to set 1 at the pre-intervention moment; † significant differences compared to set 1 at the post-intervention moment; ‡ significant differences compared to set 7 at the pre-intervention moment; ℙ significant differences compared to set 7 at the post-intervention moment; significance criteria: $p < 0.05$.

In the intra-group comparison of strength loss (all comparisons were made against the first set of the fatigue protocol) between pre- and post-intervention; in the control group, the strength loss was significantly lower at the post-intervention moment from set 5 ($p < 0.017$). In the NAC group, the strength loss at the post-intervention moment was lower compared to the pre-intervention moment from set 4 ($p < 0.045$) (Figure 3).

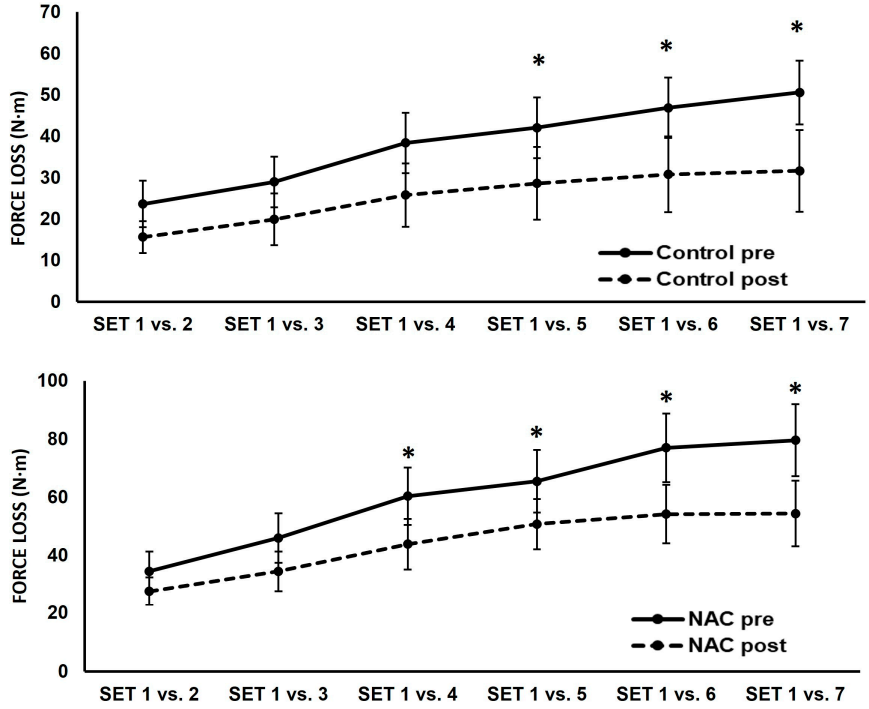

Figure 3. Isometric force loss during the fatigue protocol (mean ± standard error). Set 2 vs. 1 = isometric force production difference between set 2 and set 1 of the fatigue protocol; set 3 vs. 1 = isometric force production difference between set 3 and set 1 of the fatigue protocol; set 4 vs. 1 = isometric force production difference between set 4 and set 1 of the fatigue protocol; set 5 vs. 1 = isometric force production difference between set 5 and set 1 of the fatigue protocol; set 6 vs. 1 = isometric force production difference between set 6 and set 1 of the fatigue protocol; set 7 vs. 1 = isometric force production difference between set 7 and set 1 of the fatigue protocol; * significant differences compared to pre-intervention moment; significance criteria: $p < 0.05$.

3.3. Side Effects Evaluation

No significant differences were found in the reported side effects between the NAC and control groups. The results are shown in Table 2.

Table 2. Side effects of N-acetylcysteine (NAC) supplementation.

Variable	NAC Group		Control Group		*p*
	Yes (%)	No (%)	Yes (%)	No (%)	
Upset stomach	3.3	96.7	0.0	100	0.625
Nausea	0.0	100	0.9	99.1	1.000
Gas	15.4	84.6	1.8	98.2	0.125
Cough	1.1	98.9	0.0	100	1.000
Indigestion	4.4	95.6	0.0	100	1.000
Vomiting	0.0	100	0.0	100	1.000
Metallic taste	0.0	100	0.0	100	1.000
Rash	0.0	100	0.0	100	1.000
Itching	2.2	97.2	0.0	100	1.000
Local erythema (redness)	3.3	96.7	0.0	100	1.000
Redness of eyes, face, or hands	0.0	100	0.0	100	1.000
Conjunctivitis	3.3	96.7	0.0	100	1.000
Lightheadedness	0.0	100	0.9	99.1	1.000
Sleepiness	0.0	100	6.4	93.6	1.000
Others	0.0	100	0.0	100	1.000

4. Discussion

Antioxidant effects can be of great benefit for sports performance, as they can help to reduce ROS and increase exercise time to exhaustion [23]. Some studies have shown benefits of the intake of vitamin-C-rich foods [24], and the use of drugs such as NAC [25] also appears to be beneficial for sports performance. Most studies conducted with NAC use the intravenous administration method, since the bioavailability of orally administered NAC is lower [20,26], although this reduces its application to healthcare personnel, making it more difficult to access for athletes/sports teams with fewer resources. Therefore, the main objective of this study was to assess the ergogenic effect of the oral application of N-acetylcysteine on myotendinous strength production in physically active people after being subjected to a fatigue protocol. After supplementation, the main effect observed was that the isometric strength loss in the NAC group compared to the pre-intervention assessment was significantly lower in the last four exercise sets, and this significant reduction in strength loss started one set (4th set vs. 5th set) before compared to the control group. Therefore, oral NAC supplementation may be an effective tool to delay the fatigue onset and improve athletic performance in repeated effort sports.

The use of NAC as a sports supplement has been commonly employed in cyclic exercise such as running and cycling at submaximal and maximal intensities in order to increase time to exhaustion [25,27]. In a study by Cobley et al. [27], after 6 days of supplementation, two yo-yo tests were performed on consecutive days to observe whether athletes were able to maintain their performance despite the accumulation of fatigue, with an improvement in the results in the intervention group in the fatigue state. Similar to these results, another study showed that the time to exhaustion at submaximal intensities (80% VO2max) was significantly longer in the amateur cyclists taking NAC [5]. In this line of thought, our results show that in the NAC group there were smaller reductions in isometric force production in the 4th, 5th, 6th, and 7th sets of the post-intervention moment compared to the pre-intervention moment, so an 8-day NAC supplementation could help to increase fatigue tolerance. In addition, compared to the control group, the decrease in force production in the NAC group at the post-intervention time was significantly lower compared to the pre-intervention time from one set earlier than in the control group (4th set vs. 5th set), corresponding to force losses of more than 30% of their maximal force. Previous research has already described NAC as an ideal supplement for working at submaximal intensities, close to the lactate steady state, so the succession of maximal isometric contractions with short recovery times may have managed to replicate this lactate steady state. Another reason that could explain the differences between the NAC and control groups is that NAC supplementation has been shown to be effective at reducing perceived pain [4], which is effective for prolonging maximal exertion. Future research should record pain at the end of each set to try to relate the increase in pain to the loss in strength.

This is one of the first studies to analyze the effect of NAC on the physical performance of physically active people, who had no previous experience with the measurement test. Despite familiarization, a better understanding of the test may have helped participants to reduce the effect of fatigue on post-test maximal voluntary contraction force loss [28]. Furthermore, there were no significant differences in the MVC force values between groups at any of the measurement times, so we can establish that the fatigue caused by the protocol did not have any effects 24 h after the test. Despite the possible benefit of NAC in delaying the onset of fatigue, we found no significant differences between groups for any of the strength values, and both groups improved their post-intervention performance. Another reason why no differences were found between the groups may be due to the fact that the last dose ingested by the participants in the NAC group was 2 h before the test, and according to different authors, the peak of the greatest effect of NAC is about 20–40 min after the last intake [26], so it would be necessary to check whether the benefits were greater for the same intake of NAC closer to the time of the evaluation. Finally, it has been reported that the bioavailability of NAC in the body is low (~6%) [29], and, moreover, there are

different responses for the same load, so in future interventions the response of athletes to the dose should be monitored in order to adjust the dose [20].

Side effect avoidance should be an objective when aiming to improve sports performance through the intake of ergogenic aids. In our study, no significant episodes of side effects were described in the NAC group, contradicting the findings of other researchers who administered NAC orally [4,30]. The main side effects of NAC supplementation are related to the appearance of stomach upset and increased gas, although much higher doses per kg of body weight may be necessary to observe these adverse effects. Ferreira et al. [30], who analyzed the side effects of different NAC doses, described adverse reactions with doses higher than 35 mg/kg of body weight. In this vein, no significant performance improvements were observed with approximately 15 mg/kg body weight or less, which is 50% lower than what our participants received on average. Therefore, it seems reasonable to indicate that with doses lower than 35 mg/kg (~32 $mg \cdot kg^{-1}$) of body weight, the side effects are mitigated, although the improvement in sports performance may be conditioned by the type of effort made [5].

Our study has several limitations. First, the last intake of NAC was 2 h before the evaluation, which may reduce its ergogenic effect. Furthermore, the same dose of NAC was given to all participants, so those with a higher body weight received lower levels in relation to their weight, which may have reduced the benefit of the supplementation. This aspect may have also contributed to the low presence of side effects. Future studies should examine the ergogenic effect of orally administered NAC at doses approaching 35 mg/kg body weight in isometric exercise. Furthermore, our study only assessed the effects of NAC supplementation on strength performance; in future studies, the assessment of muscle activity by EMG should be included to distinguish between central and peripheral fatigue. Finally, the study participants were physically active people (2.5 h of physical exercise per day), so further studies are needed to test the effect of NAC in people with higher physical performance and also with different types of exercise.

Practical Applications

The delaying effect of fatigue on force production may be a key factor in athletic performance. From the results of our study, we can point out that oral supplementation with 2400 mg of NAC for 8 days can delay the loss of strength. Therefore, in preparation for a competition in which the efforts are repeated, it may be recommended to take NAC during the 8 days prior to the event. As some studies point out, the peak effect of NAC occurs 20–40 min after the last intake [26], although in our research positive results were already observed when the last intake was at least 2 h before the tests. Finally, NAC appears to be a safe supplement that does not cause side effects in athletes at the doses given in our research.

5. Conclusions

In conclusion, our results suggest that a dose of 2400 mg daily for eight days can reduce the fatigue effects on force production; however, it was not enough to improve the maximal voluntary contraction force after the fatigue protocol. In addition, oral supplementation of 2400 mg NAC appears to cause no side effects, so it may be an appropriate dose to improve performance in repeated-gesture sports.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Clinical Hospital San Carlos (protocol codes: 22/513-E and 22 August 2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author in Zenodo at https://doi.org/10.5281/zenodo.13901238 (accessed on 8 October 2024).

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Conflicts of Interest: The authors declare no conflict of interest.

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