The influence of the ACTN3 R577X polymorphism in the responsiveness to post-activation jump performance enhancement in untrained young men

A influência do polimorfismo R577X do gene ACTN3 na responsividade à melhora de desempenho de salto pós-ativação em indivíduos jovens destreinados

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Abstract – We aimed to investigate the influence of alpha-actinin-3 (ACTN3) R577X polymorphism on responsiveness to post-activation performance enhancement (PAPE) of countermovement jumps (CMJ) in untrained subjects. Sixteen untrained men were allocated into two groups according to their ACTN3 gene alleles: homozygous for the X allele (XX, n = 9) or homozygous for the R allele (RR, n = 7). CMJ height, mean power output and vertical force were determined twice (CMJ1 and CMJ2) in two conditions: control (CON) and potentiated (PAPE). In the CON condition, CMJ were performed before and after a 15-min rest. In the PAPE condition, CMJ were performed 15 min before and 4 min following five squats with a 5-repetition maximum (SRM) load. Different conditions were applied on separate days in a randomized order. Statistical analysis involved three-way ANOVAs to compare the differences between conditions (CON and PAPE), time (CMJ1 and CMJ2), and groups (XX and RR). Significance level considered was p < 0.05. Effect sizes were calculated as Cohen’s d. The effect sizes for changes in CMJ height for CON and following pre-activation for PAPE were 0.04 and 0.08, respectively. No significant differences were found for CMJ height between XX and RR at baseline (1.07 ± 2.54 cm e -0.82 ± 2.56 cm, respectively). No differences were found (p>0.05) in responsiveness to PAPE between the groups (XX = -0.20 ± 1.6 cm and RR = -0.81 ± 2.7 cm). We conclude that ACTN3 gene polymorphisms do not influence responsiveness to PAPE.

Keywords: Athletic performance; Exercise; Genetic polymorphism.

Resumo – Tivemos como objetivo investigar a influência do polimorfismo do gene ACTN3 na responsividade à potencialização do desempenho de salto com contra movimento (CMJ) pós-ativação (PAPE). Dezesseis homens destreinados foram divididos em dois grupos: homozigotos para o alelo X (XX, n = 9) ou R (RR, n = 7). A altura de CMJ, a potência média e a força vertical aplicada durante o salto pelos participantes foram determinadas duas vezes (CMJ1 e CMJ2) em duas condições: controle (CON) e potencializado (PAPE). Na condição CON, os CMJ foram realizados antes e depois de um período de 15 minutos de repouso. Na condição PAPE, os CMJ foram realizados 15 minutos antes e 4 minutos após a realização de cinco agachamentos com carga de cinco repetições máximas (SRM). As diferentes condições foram realizadas em dias separados e em ordem randomizada. Anova fatorial de três caminhos foi utilizada para comparar diferenças entre condições, tempos e grupos. O tamanho do efeito foi calculado pelo d de Cohen. Os tamanhos do efeito para alterações na altura de CMJ para os grupos CON e PAPE foram 0.04 e 0.08, respectivamente. Não houve diferenças significativas entre os grupos XX e RR na altura de salto em condição basal (1.07 ± 2.54 cm e -0.82 ± 2.56 cm, respectivamente). Não houve diferenças significativas na responsividade à PAPE entre os grupos (XX = -0.20 ± 1.6 cm e RR = -0.81 ± 2.7 cm). O polimorfismo do gene ACTN3 parece não ser influenciador isoladamente a responsividade à PAPE.

Palavras-chave: Desempenho atlético; Exercício físico; Polimorfismo genético.
INTRODUCTION

Post-activation performance enhancement (PAPE) is a phenomenon that temporarily increases power generation capacity, and it is often induced by performing pre-activation exercise at maximal or near maximal intensity\(^1\). Although PAPE is best observed when analyzing changes in force, especially rate of force development\(^1-5\), previous studies have shown improvements in jumping performance (e.g., jump height) following pre-activation activities (e.g., backsquat and halfsquat)\(^2-5\). Thus, PAPE can be an interesting strategy to improve an athlete’s performance during events that require substantial muscular strength and power\(^6\).

Several physiological, biomechanical and metabolic mechanisms can explain PAPE. Previous studies have indicated the phosphorylation of the myosin regulatory light chain (RLC) as a mechanism that might determine PAPE magnitude. The greater approximation and interaction of contractile proteins and increased \(\text{Ca}^{2+}\) sensitivity\(^7\) can facilitate the formation of cross-bridges. As a result, tension development enhances maximizing rapid strength and power production capacity\(^8\). Moreover, the magnitude of myosin RLC phosphorylation seems to be related to muscle fiber type, with greater RLC phosphorylation occurring in type II muscle fibers\(^9\). Indeed, individuals and muscle groups with greater type II muscle fiber distribution are more responsive to PAPE than those with greater distribution of type I muscle fibers\(^6,9\).

The occurrence of genetic variations from polymorphisms affects both muscle fiber type distribution and exercise performance\(^10-13\). Among several existing polymorphisms, the R577X polymorphism of the alpha-actinin-3 (ACTN3) gene stands out for affecting the expression of muscle fiber types\(^10,14-15\). Individuals who are heterozygous (RX) or homozygous (RR) for the R577R allele have been reported to have a higher proportion of fast-twitch fibers\(^10,14\), while those who are homozygous for the R577X allele (XX) have a higher proportion of slow-twitch fibers\(^10,15\). Accordingly, Vincent et al.\(^14\) found that healthy RR young men had a greater number of type IIx fibers in the vastus lateralis than their XX counterparts. A study by Ahmetov et al.\(^15\) corroborated such a notion by showing that XX individuals exhibit a greater proportion of slow-twitch fibers in relation to RR individuals.

Since PAPE is more pronounced in individuals with greater expression of type II muscle fibers, while individuals with the ACTN3 RR polymorphism have greater expression of such fiber type, our hypothesis is that RR individuals would be more responsive to PAPE than XX individuals. Since no studies have investigated such relationship, our objective was to investigate the influence of the ACTN3 R577X polymorphism on responsiveness to PAPE in untrained subjects.

METHOD

Participants

Sixteen untrained young men who had not engaged in any type of strength training in the previous 6 months and were homozygous to the R577X and R577R ACTN3 gene polymorphisms participated in this study. None of the subjects had previous bone, joint, or muscle injuries in their lower limbs. Their
mean age, body mass, and height were 22.5 ± 1.9 years, 74.4 ± 12.4 kg and 175 ± 6 cm, respectively. Participants were allocated into two groups according to their ACTN3 polymorphisms: XX and RR. All participants provided written informed consent to participate in the study, which had been previously approved by the Institutional Ethics Committee. This study was conducted in conformity with the policy statement regarding the use of human subjects by the Declaration of Helsinki.

**Experimental design**

Each participant visited the laboratory in four occasions. In the first visit, the participants read and signed an informed consent term and had blood samples drawn to determine ACTN3 gene polymorphisms. Participants with the RR and XX polymorphisms of the ACTN3 gene were selected and recruited to attend further visits to the laboratory. The second visit was designed to assess anthropometric data and familiarize the participants with CMJ tests and equipment, in addition to determining five repetitions maximum loads (5-RM) in the squat exercise. To avoid any influence of muscle damage induced during 5-RM determinations, the participants returned for the first visit of the experimental protocol at least three days following the second visit.

The experimental protocol was performed in two sessions separated by intervals of at least three days. In the control (CON) session, the participants performed two sets of three CMJs (CMJ1 and CMJ2) interspersed by 15 minutes of rest. In the PAPE session, the participants performed the same three CMJs (CMJ1) followed by 15 minutes of rest. Subsequently, a pre-activation protocol (5RM squats) was performed followed by three CMJs (CMJ 2) four minutes later. The order at which each participant performed the PAPE and CON sessions was randomly determined. A light warm-up consisting of ten submaximal squats with no external load was performed 15 minutes before the beginning of the procedures described for the CON and EXP sessions. Figure 1 illustrates the experimental design.

![Figure 1](image-url)
Genetic testing

To determine the participants’ ACTN3 genotype, 4 ml of blood were extracted from the antecubital vein using EDTA tubes and stored at -80°C. Genomic DNA was extracted from 500 μL of whole blood according to a salting out method, while the quality and integrity of the sample were tested through spectrophotometry (Nanodrop, ThermoScientific – GE). DNA samples were stored at -20°C for no longer than three days until further analyses. ACTN3 gene polymorphism was determined using a real-time polymerase chain reaction (PCR) procedure. Approximately 100 ng of genomic DNA was amplified with the primers Vic-CTGACGAGACGCA and Fam-AGGCTGACTGAGACG (Applied Biosystems, USA) for the R and X alleles, respectively. Allele discrimination was performed in a genomic sequence detection system (Sequence Detection System 7000, Applied Biosystems, USA) using a commercial genotyping assay (TaqMan PCR Master Mix, Applied Biosystems, USA). PCR conditions were an initial denaturation at 95°C for 10 minutes followed by 40 cycles at 94°C for 15 seconds and a final cycle of 60 seconds at 60°C. Following PCR, the equipment determined the R577 polymorphism of the ACTN3 gene.

Procedures

Countermovement Jump (CMJ)

Jump performance was assessed as vertical force (VF), mean power output (MPO), and height calculated based on the flight time recorded during CMJs, with VF as the main biomechanical marker of CMJ performance, while MPO and height are considered as secondary outcomes of CMJ performance. Flight time was assessed by filming the participants’ feet during CMJ on a smartphone with a capture frequency of 240 frames per second (iPhone SE, Apple, USA). The footage was analyzed in a validated\textsuperscript{16,17} app (MyJump2, Carlos Balsalobre, Spain) that allows examiners to determine the moment when the feet of the participants leave the ground (i.e., takeoff) and the moment when the feet regain contact with the ground (i.e., landing). Flight time was calculated as the interval between takeoff and landing and used to calculate jump height according to the following equation:

\[ h = t^2 \times 1.22625 \] (1)

where \( h \) is jump height and \( t \) is flight time\textsuperscript{16}. CMJ height is presented as absolute values (cm) and normalized by the length of the participant’s lower limbs (cm/cm).

VF during CMJ was calculated based on CMJ height, body mass, and the push-off distance, according to the following equation:

\[ VF = BM \times g \times \left( \frac{h}{Pd} + 1 \right) \] (2)

where \( MF \) is VF (N), \( BM \) is body mass (kg), \( g \) is the acceleration of gravity (m/s\(^2\)), \( h \) is jump height, and \( Pd \) is push-off distance\textsuperscript{18}. VF values were normalized based on the participant’s body masses (N/kg).

MPO during CMJ was calculated based on push-off velocity and VF through the following equation:
\[ MPO = VF \times \sqrt{\frac{g \times h}{2}} \]  

(3)

where \( MPO \) is mean power output (W), \( VF \) is vertical force (N), \( g \) is the acceleration of gravity (m/s\(^2\)), and \( h \) is jump height\(^{18}\). MPO values were normalized based on the participant’s body masses (W/kg).

Each CMJ started with the participants in a standing position. Participants were instructed to rapidly squat until their knees reached 90° of flexion and immediately jump as high as they could. To eliminate the influence of arm swing during CMJs, the participants were instructed to keep their arms still with their hands on their hips during all jumps. Each jump was preceded by a 30-second rest.

Each participant performed six CMJ (three in CMJ1 and three in CMJ2) per condition (PAPE and CON). The mean values for CMJ height, MPO and MF were calculated and used for analyses. This study used a mean coefficient of variation for flight time using such procedure of 3.9 ± 5.3%.

### 5-RM squat

The 5-RM load for the squat exercise was assessed to determine the pre-activation protocol load. 5-RM was defined by determining the greatest load that subjects could lift five times with proper squat movement until 90° of knee flexion. A minimum number of 10 unloaded squat movements were performed 3 minutes before 5-RM tests as a warm-up protocol. A 3-minute recovery period between the warm-up and the first attempt was provided to reduce any effects of fatigue. For the first attempt to determine 5-RM squat load, examiners considered an individualized load estimate for the participants to be able to perform five maximal repetitions. When the subjects failed to complete 5 repetitions or were able to perform more than that, the load was changed accordingly, and after 5 minutes of rest interval the participants performed another attempt. A limit of five attempts per session was respected. This study considers 5-RM expressed normalized to individual body mass.

### Statistical analyses

Data were analyzed on a statistical software package (GraphPad Prism Version 8.3.0; GraphPad Software Inc., San Diego, CA) and a Shapiro-Wilk’s test confirmed Gaussian distribution. The assumptions of data homogeneity and sphericity were confirmed by the Levene and Mauchly tests, respectively. Data variance was verified through three-way ANOVAs considering three factors: group (XX and RR) vs time (CMJ1 and CMJ2) vs condition (CON and PAPE). If significant group vs. time vs. condition interactions were identified, pairwise analyses would be performed with Tukey’s post hoc, which did not occur. The level of significance was set at p < 0.05 for all tests. Data are expressed as means ± SD. Effect sizes (ES) were calculated as Cohen’s d. Effect sizes were considered small, medium, and large when Cohen’s d were < 0.5, 0.5 < d < 0.8, and d > 0.8, respectively.
RESULTS

Baseline measurements

No significant differences between the groups were found for baseline values or any of the assessed anthropometric characteristics (age, body mass, and height) or 5-RM squats load (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>XX (n=9)</th>
<th>RR (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.3 ± 1.4</td>
<td>22.7 ± 2.5</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>71.3 ± 11.07</td>
<td>78.5 ± 13.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.7 ± 6.7</td>
<td>174.7 ± 5.8</td>
</tr>
<tr>
<td>5-RM Load (kg)</td>
<td>83.3 ± 15.7</td>
<td>83.7 ± 17.1</td>
</tr>
<tr>
<td>Normalized 5-RM (kg/kg)</td>
<td>1.17 ± 0.16</td>
<td>1.08 ± 0.23</td>
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Table 1. Anthropometric data and 5-RM load for squat exercise.

Values are means ± SD; XX: homozygous for the R577X allele group; RR: homozygous for the R577R allele group; 5-RM load: repetitions maximum loads in squat exercise.

Changes in CMJ height

No significant (p < 0.05) effects of polymorphism (Absolute: F = 4.2; Normalized: F = 3.6), pre-activation (Absolute: F = 3.9; Normalized: F = 3.8), session (Absolute: F = 0.2; Normalized: F = 0.2) or polymorphism vs pre-activation vs session interactions (Absolute: F = 0.8; Normalized: F = 0.5) were observed for CMJ height. Absolute and normalized CMJ were not significantly (p < 0.05) different between polymorphism at CMJ1 of the CON and PAPE sessions. Absolute and normalized CMJ heights were not significantly (p < 0.05) different at CMJ2 in relation to CMJ1 in the CON and PAPE sessions (Figure 2A, B).

Changes in MPO

No significant (p < 0.05) effects of polymorphism (F = 6.7), pre-activation (F = 0.2), session (F = 0.6) or polymorphism vs pre-activation vs session interactions (F = 0.2) were observed for MPO during CMJ. MPO was not significantly (p < 0.05) different between polymorphism at CMJ1 of the CON and PAPE sessions. MPO was also not significantly (p < 0.05) different at CMJ2 in relation to CMJ1 in the CON or PAPE sessions (Figure 2C).

Changes in VF

No significant (p < 0.05) effects of polymorphism (F = 6.4), pre-activation (F = 0.3), session (F = 0.6) or polymorphism vs pre-activation vs session interactions (F = 0.2) were observed for VF during CMJ. VF was not significantly (p < 0.05) different between polymorphism at CMJ1 of the CON and PAPE sessions. VF was also not significantly (p < 0.05) different at CMJ2 in relation to CMJ1 in the CON and PAPE sessions (Figure 2D).

Responsiveness to PAPE

Changes in absolute CMJ height following pre-activation reached -0.20 ± 1.6 (ES = 0.03) and -0.81 ± 2.7 (ES = 0.19) for XX and RR individuals, respectively. Changes in normalized CMJ height following pre-activation ranged 0.00 ± 0.02 (ES = 0) and -0.01 ± 0.03 (ES = 0) for XX and RR individuals, respectively.
Changes in MPO during CMJ following pre-activation pointed to $-0.13 \pm 1.28$ (ES = 0.03) and $-0.56 \pm 2.04$ (ES = 0.24) for XX and RR individuals, respectively. Changes in VF during CMJ following pre-activation were $-0.05 \pm 0.52$ (ES = 0.04) and $-0.21 \pm 0.87$ (ES = 0.12) for XX and RR individuals, respectively. Figure 3 shows individual changes in absolute CMJ height (A), normalized CMJ height (B), MPO during CMJ (C), and VF during CMJ (D) discriminated by ACTN3 gene polymorphism. Figure 4 shows mean changes in the same variables considering the XX and RR polymorphisms, as well as the whole sample as one single group (i.e., regardless of ACTN3 gene polymorphism).

**DISCUSSION**

The main purpose of this study was to investigate the influence of the ACTN3 gene polymorphism in responsiveness to PAPE. We tested the hypothesis that RR individuals would show greater responsiveness than XX individuals. The results obtained showed that ACTN3 gene polymorphism may have negligible influence on responsiveness to PAPE, demonstrated by the lack of significant group vs. time vs. condition interaction effects for VF ($F = 1.88$) – a direct estimate of CMJ performance – and indirect markers of CMJ performance, such as height ($F = 0.80$), normalized height ($F = 0.54$), and MPO ($F = 1.56$). Additionally, pre-activation had no effect on any of the variables assessed for either groups (Figure 2).

Evidence has shown that several factors can influence the responsiveness to post-activation performance enhancement, including individual characteristics (e.g., fiber type distribution, training status, training background, and gender) and pre-activation protocol configuration (e.g., exercise type, resting periods, intensity, and set distribution)\textsuperscript{19-21}. Hence, the absence of differences between PAPE responses among groups as well as the lack of performance enhancement for all subjects grouped may be related to both individual- and protocol-related characteristics, with minor contribution of ACTN3 gene polymorphism.

Other studies have pointed to a relationship among ACTN3 polymorphism, muscle fiber type distribution, and responsiveness to PAPE\textsuperscript{9,14,15}. Vincent et al.\textsuperscript{14} demonstrated that individuals with the RR genotype presented a higher percentage of type IIx muscle fibers ($14 \pm 2\%$) than individuals with the XX genotype ($9 \pm 1\%$). Similarly, Ahmetov et al.\textsuperscript{15} found a significantly greater proportion of slow-twitch fibers in XX individuals comparing with RR individuals. Additionally, Hamada et al.\textsuperscript{9} showed that greater twitch potentiation ($+127\%$ vs $+40\%$ increase in peak twitch torque at rest) is observed in individuals containing more fast-twitch fibers than those with greater proportions of slow-twitch muscle fibers. Furthermore, Delmonico et al.\textsuperscript{22} showed a significantly ($p = 0.01$) greater increase in relative knee extensor peak power (PP) following strength training (ST) in RR female individuals in relation to their XX counterparts, suggesting that RR individuals might show greater increases in power following ST than XX individuals. Although force production and power generation capacity are different manifestations, both heavily depend on type II muscle fibers. When taken together, these data suggest that subjects with the RR polymorphism of the ACTN3 gene may present a greater percentage of type II muscle fibers, thus possibly exhibiting greater PAPE. However, our results did not support this notion. Instead, we observed that ACTN3 polymorphisms alone did not influence the individuals’ responsiveness to PAPE since no significant group effect (i.e., RR vs XX) was found. Therefore, phenotypical factors (e.g., training...
status or background), rather than ACTN3 gene polymorphisms and fiber type distribution, may have a greater influence on the magnitude of PAPE response.

Figure 2. Counter movement jump (CMJ) height (A & B), mean power output (C), and vertical force (D) assessed in pre- (CMJ1) and post-rest/pre-activation (CMJ2) for the XX and RR groups at the control (CON) and post-activation performance enhancement (PAPE) sessions. Lines represent mean values and circles/squares represent individual values.

Figure 3. Individual changes in countermovement jump (CMJ) height (A & B), mean power output (C), and vertical force (VF) in the PAPE session discriminating ACTN3 gene polymorphism.
In fact, training background and/or chronic adaptations to a specific exercise training modality have both been suggested as important factors influencing responsiveness to PAPE\textsuperscript{19,23}. According to Boulosa et al.\textsuperscript{19}, the magnitude of an individual’s response to PAPE response may depend on the compatibility between the type of training background (e.g., endurance or power training) and the characteristics of the pre-activation protocol, which can stimulate predominantly slow- or fast-twitch muscle fibers. The pre-activation protocol adopted in this study was designed to stimulate type II (i.e., fast-twitch) muscle fibers due to the heavy loads and lower number of repetitions (5-RM)\textsuperscript{23}. Hence, individual training background might have influenced responsiveness to PAPE, suggesting that the phenotype may have a greater impact than the genotype on responsiveness to potentiation. This study included only untrained participants, which was established as an inclusion criterion (i.e., no experience with strength training for at least six months preceding experiment). However, some individuals may have experienced different exercise training modalities in their life span (e.g., resistance, endurance, or collective sports training) which could have promoted long-term adaptations that might have influenced responsiveness to PAPE. Additionally, it is a limitation of our study not to have monitored physical activity levels (e.g., inactivity) in the months preceding the experiment, since neuromuscular adaptations are not exclusive to exercise training, but also to daily life physical activities\textsuperscript{24}. Thus, the pre-activation protocol was likely to be appropriate to a specific subgroup of participants with a determined training background, while it would not be the best approach to induce PAPE in the remaining subjects with a different training background. Notwithstanding, further research is necessary to elucidate the relevance of training background in the prescription of pre-activation activity.

This study found no significant effect of the pre-activation protocol on CMJ height for both groups of untrained individuals (Figure 4). However, the pre-activation protocol adopted herein has already been used in previous
and shown to be effective in improving CMJ height in trained subjects. Mitchell & Sale\textsuperscript{23} showed improved jumping performance (2.9%, ES = 0.20) in 11 rugby union players subjected to pre-activation protocol. Young et al.\textsuperscript{25} observed improved jumping performance (2.8%, CC = 0.73, p = 0.02) in 10 trained men (at least 1 year experience in half-squats) following a similar pre-activation protocol (1 x 5 half-squat at 5-RM with 4 min resting period). Moreover, Crewther et al.\textsuperscript{2} also found improved CMJ height at 4 (3.8 ± 1.9%, ES = 0.31), 8 (3.5 ± 1.5%, ES = 0.32), and 12 (3.0 ± 1.4%, ES = 0.27) minutes following one set of three back squats with a 3-RM load in 9 sub-elite male rugby players.

Force (VF) and power output (MPO) were also not affected by the pre-activation protocol in this study (Figures 2-4). These findings do not corroborate with previous studies that found increased force and power output following similar pre-activation protocols in different populations\textsuperscript{26-27}. Kilduff et al.\textsuperscript{26} found significantly higher CMJ height (34.3 ± 1.2 cm vs. 36.0 ± 1.2 cm; p < 0.01), peak rate of force development (12358 ± 673 N.s\textsuperscript{-1} vs. 16290 ± 810 N.s\textsuperscript{-1}; p < 0.05), and power output (p = 0.029) at 8 min recovery (p<0.001) following conditioning contractions (3 sets of 3 repetitions of back squats at 87% 1-RM) in relation to the baseline in rugby players. In a similar study, Kilduff et al.\textsuperscript{27} found increases in CMJ height (6.8 ± 7.2% and 8.0 ± 8.0%) and power output at 8 min and 12 min (4,568 ± 509 W vs. 4,862 ± 485 W; and 4,568 ± 509 W vs. 4,911 ± 444 W, respectively) following one set of 3RM squat in rugby players. Together, these results indicate that the compatibility between training status and the pre-activation protocol configuration can significantly influence jumping performance and responsiveness to PAPE.

The efficacy with which a pre-conditioning activity can acutely enhance neuromuscular performance and induce PAPE depends on the balance between optimal recovery of fatigue induced by pre-activation and the prevalence of some of the mechanisms that underpin performance enhancements (e.g. increased neural drive/muscle activation, muscle temperature, muscle water content, changes in muscle architecture etc)\textsuperscript{8,20,21}. Numerous factors affect such balance, including, but not limited to, training status, recovery period, and intensity of the pre-conditioning exercise\textsuperscript{8,20}. In our study, fatigue induced by performing a high-intensity conditioning activity (e.g. 5-RM squat) seems not to have been sufficiently recovered in the 4-min interval respected between the pre-conditioning activity and CMJ2, which might be related to the training status of the investigated population (i.e., active, but untrained).

Previous studies indicated that a single set of resistance exercise performed at moderate intensities (60–84% 1-RM, ES=1.06) associated with a moderate recovery interval duration (7-10min, ES: 0.70, p<0.05) are ideal for eliciting PAPE in untrained subjects comparing with high intensities (>85% 1-RM, ES=0.31) and short-duration recovery intervals (3-7min, ES:0.54)\textsuperscript{20,28}. In this study, the participants were subjected to a single-set pre-activation protocol at an intensity corresponding to ~87% of individual 1-RM\textsuperscript{29}, with a 4-min recovery interval before CMJ assessment. Therefore, it is plausible to infer that the intensity of the pre-activation protocol and the duration of the recovery interval in our study may have negatively influenced by the PAPE response, which might have contributed to the absence of significantly higher CMJ height, VF, MPO. Nevertheless, it may be inappropriate to generalize an optimal resting period\textsuperscript{30}, since other studies have shown a large inter-individual variability of
the optimal recovery interval duration between pre-activation exercise and power assessment, which was associated with numerous factors (e.g., strength level, training status, and myotypology). Hence, further investigation should be carried out to elucidate the optimal recovery interval duration to induce PAPE for each population.

Although relevant, our findings should be interpreted with caution. Our sample size consisted of sixteen subjects, nine of which were homozygous for the X allele, while the other seven are homozygous for the R allele. Even though our sample size is sufficient to show that the pre-activation protocol did not result in significant enhancements in CMJ performance for the entire sample, each group has a small number of participants, which might have resulted in a type II error, representing a limitation. Further studies should address larger sample sizes to be more effective in determining the impact of ACTN3 gene polymorphism on responsiveness to PAPE.

CONCLUSION

Our study indicates that only one polymorphism of the ACTN3 gene is not enough to define the responsiveness to PAPE. Hence, coaches and practitioners should consider the contribution of other factors (e.g., training status or training background) that appear to better determine which athletes may benefit from a greater PAPE response. Moreover, our results suggest that a high-intensity pre-activation protocol (i.e., 5-RM backsquat) with a short-duration recovery period between potentiation and power assessment is ineffective at inducing PAPE in untrained subjects.

COMPLIANCE WITH ETHICAL STANDARDS

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Ethical approval

Ethical approval was obtained from the Ethics Committee on Human Research of the Institute of Biosciences of São Paulo State University – Rio Claro, and the protocol (no.86496518.4.0000.5465) was written in accordance with standards set by the Declaration of Helsinki. The participants provided their written informed consent to participate in this study.

Conflict of interest statement

The authors have no conflict of interests to declare.

Author contributions

Conceived and designed the experiments: GM and LL. Performed the experiments: GM, RB and VA. Analyzed the data: GM, RB and VA. Contributed
with reagents/materials/analysis tools: CJ, CA, BD and CG. Wrote the paper: GM, LL, and RV. All authors contributed to manuscript revision and approved the submitted version.

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