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Research Article



Post-activation Potentiation: Effect of Recovery Duration and Gender on Countermovement Jump, Agility, and Linear Speed in Team-Sport Athletes

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Abstract

Background: Studies assessing post-activation potentiation (PAP) responses comparing male and female athletes are conflicting. **Objectives:** This study investigated whether differences exist in the duration for optimal post-preload stimulus measures on performance in male and female team sport athletes.

Methods: Twenty-four participants (12 males and 12 females) participated in the study. Two familiarization sessions were conducted with each participant. Then, three experimental conditions were implemented, incorporating a standardized warm-up, followed by back squats (conditioning exercises) and varying passive recovery times of $4 \min{(PAP_4)}$, $8 \min{(PAP_8)}$, or $12 \min{(PAP_{12})}$. Following the recovery, players performed three physical performance measures related to team sports: A countermovement jump, a modified agility t-test, and a 20-m linear sprint. The significance level was set at P < 0.05.

Results: All performance measures were significantly greater in PAP₁₂ than in PAP₄ and PAP₈ conditions in both males (1.50 to 2.95%) and females (1.09 to 5.79%) (P < 0.05). The PAP₁₂ condition also had significantly lower values for HR (3.18 to 5.15 beats.min⁻¹; P < 0.0005) and ratings of perceived exertion (RPE) (0.63 to 1.02; P < 0.05) than PAP₈ and PAP₄. Males performed better on all the performance tests (19.33 to 26.34%) compared to their female counterparts (P < 0.0005).

Conclusions: A pre-load stimulus consisting of one set of 5 repetitions of back squat at 85% one-repetition maximum can elicit a PAP response. A 12-min passive rest after the pre-load stimulus was most beneficial in improving physical performance measures in both male and female team-sport athletes.

Keywords: Post-activation Potentiation, Team-Sports, Gender, Passive Recovery

1. Background

Intermittent team sports, such as handball and volleyball, are characterized by high-speed actions, changes of direction, and explosive jumps, with success dependent on their interaction (1). In-game performance in team-sport athletes is directly influenced by the pre-game warmup (2, 3). To enhance and optimize performance, strength, and conditioning, practitioners have proposed warmup protocols that utilize specific components of raise, activate, mobilize, and potentiate

(RAMP) to induce readiness in both biomechanical and physiological variables related to sporting demands (4). The "potentiate" stage of a warm-up often induces a post-activation potentiation (PAP) response in human muscle to improve muscular performance characteristics. The term PAP constitutes an enhanced muscular response following voluntary muscular contraction (5), mainly due to delayed excitatory response on both myogenic and neurogenic systems (6). It has been shown to maximize power responses in team-sport athletes (7). The myogenic

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adaptation to PAP using high-intensity and heavy-strength exercises is well documented in athletes (8). Nevertheless, physiological and methodological factors can influence the efficacy of a given pre-load stimulus. Team-sport athletes require a combination of type I and II muscle fibers to fulfill competition demands that involve aerobic conditioning, speed, and agility and to generate strength and power (9).

Recent findings suggest that a low number of repetitions (\leq 6) of high-intensity and heavy (\geq 70% one-repetition maximum) strength exercises are effective as a "pre-load stimulus" to increase performance in jumps, throws, and sprints in athletes (10). It is well documented that the fiber type composition directly changes an athlete's response to PAP. Individuals with lower type II fiber sizes have also been shown to have a lower capacity to generate a PAP response (11). In addition, the rest duration between the pre-load stimulus and performance task is another important methodological consideration (7), with optimal rest durations ranging from 15 s up to 24 min in the literature (12-15). Our group recently demonstrated that incorporating high-intensity and heavy-strength exercises into the warm-up routine of university-level male handball players significantly improved their sport-specific performance measures. These improvements were observed when a 12-min passive rest period was given, as opposed to rest periods of 4 or 8 minutes (16). In female volleyball players, single leg jump distance significantly improved after 2, 6, and 10-min rest when they performed 5 repetition maximal back squats compared to no rest. No differences were observed in different recovery times (17). These findings contradict those established in a cohort of male handball players and might be associated with differences in population characteristics and study methodology.

The number of training years and strength levels of individuals also play a role in the effectiveness of the PAP response to a given pre-load stimulus, while gender differences are less straightforward (10). A study conducted by Tsolakis et al. (18) reported that an isometric PAP protocol significantly decreased leg power output compared to a plyometric PAP protocol in male fencers but not in females. Interestingly, the authors observed that male fencers were almost twice as strong as female fencers and believed that the total strength capacity heavily influences these observed differences. However, in highly trained weightlifters, although men performed significantly better than their female counterparts during jump testing following maximal isometric and dynamic PAP protocols (19), the greatest PAP responses were observed in the highest strength-trained males and females (9). Also, PAP responses observed in the literature are conflicting when comparing males and females, with few studies assessing both simultaneously. Other aspects also influence responses, such as age differences and physiological profiles associated with a sport.

Previous studies assessing PAP responses have shown a lack of consideration/large differences in athlete backgrounds (differences in age, physiological profiles, training years, and strength levels). The current study controlled these factors as much as possible and is one of the few studies investigating differences in gender and PAP effects on performance related to team sports.

2. Objectives

The purpose of this study was to expand on recent work from our group and investigate whether gender influences the optimal recovery durations following a pre-load stimulus, using high-intensity and heavy strength exercises on performance measures related to team-sport athletes.

3. Methods

3.1. Hypothesis

We hypothesized that performance in team-sport athletes improves most significantly after a PAP protocol following a 12-min passive rest period in both male and female athletes.

3.2. Selection and Description of Participants

Using statistical power software (G*Power v3.1.10, Germany), the sample size required for this study was estimated to be 11. This estimation was based on detecting a meaningful difference of 5% in at least one of the performance variables, a statistical power of 0.8, and an alpha level of 0.05 (16) between conditions.

Twelve males (mean \pm SD: Age 20.7 \pm 1.9 years, height 1.78 \pm 0.05 m, and body mass 70.5 \pm 6.4 kg) and twelve females (mean \pm SD: Age 21.1 \pm 2.0 years, height 1.66 \pm 0.07 m and body mass 57.3 \pm 5.8 kg) university team-sport players volunteered to take part in this study. All participants were athletes from the University Pendidikan Sultan Idris volleyball or handball team. Players trained a minimum of three times per week at any time of the day and were involved in one game per week. Players were only selected if they had at least 3 years of resistance training experience and 3 years of experience in squat exercises. Before participating in this study, no players had a history of recent musculoskeletal injuries. No one was taking any dietary supplements or pharmaceutical drugs during the study. All were free from illness during

the study period. Everyone gave their written informed consent. The study was approved by the Human Ethics Committee of the Sports Science Department, Sultan Idris Education University, Malaysia, and conformed to the Helsinki Declaration. All the tests were performed between October and December.

3.3. Design

The experimental study was counterbalanced and randomized in design. Each participant performed two familiarization sessions before participating in the main experiment to minimize learning effects. They performed a three-repetition maximum back squat test to determine a one-repetition maximum following the guidelines of the National Strength and Conditioning Association (20). They were instructed to attempt three repetitions to 90° of knee flexion of the chosen set load. After three successful repetitions, the weight was increased by \sim 15 kg until the weight could no longer be lifted through the full range of motion. The three-repetition maximum squat test required 3 - 4 attempts to complete during the first familiarization session. A 5-min passive recovery was given between each set of three repetitions (21). A one-repetition maximum estimation was then determined using the table from Haff et al. (22) based on the data collected during the three-repetition maximum squat test. Once completed, the participants underwent full familiarization with the physical performance tests used in the study.

3.4. Testing Protocol

The participants were asked to lead a "normal life" between sessions. No caffeinated beverages and other training or heavy exertion were allowed 48 hours before experiments. On arrival, compliance with the protocols' sleeping, food intake, and exercise restrictions was assessed verbally. Then, they put on a heart rate monitor (Polar S710; Polar Electro Oy, Kempele, Finland) and completed a 5-min general warmup (self-paced jog), followed by 2 sets of dynamic stretching of the lower musculature (5 repetitions of bodyweight squats and 5 repetitions of lunge walks on each leg) over a distance of 10 m. Once completed, they were asked to perform one set of 5 repetitions of back-squat at 85% one-repetition maximum (16). This load has previously been shown to stimulate PAP effectively (8, 16). After undergoing the stimulus, they were then instructed to rest passively for a total duration of 4 min (PAP₄), 8 min (PAP₈), or 12 min (PAP₁₂). Following the recovery, they were instructed to perform a counter movement jump (CMJ), a 20-m linear sprint, and an agility t-test in sequence. The tests

chosen were based on the specific characteristics and competencies required to excel in team sports. A 2-min rest between trials and tests was included to minimize the effects of fatigue. Both heart rate and ratings of perceived exertion (RPE; scale 6 - 20) were taken throughout the experimental conditions. These were counterbalanced in order of administration to minimize potential learning effects (23), with at least 72 h to provide sufficient recovery between trials (Figure 1).

3.5. Physical Performance Tests

3.5.1. Countermovement Jump

A CMJ test was performed to measure the explosive power of the leg musculature. This was assessed on a force platform (Quattro jump: Kistler, Winterthur, Switzerland). The test required athletes to place their hands on their hips and was repeated three times. A 30-s rest was provided in between each jump. The estimate of the height change in the athlete's center of mass, considering the total duration the athlete spends in the air with no ground contact, was assessed via jumping height. The best CMJ height (cm) was then used for subsequent analysis.

3.5.2. Agility t-Test

Agility facets, including balance control, acceleration, and deceleration, were determined using a modified agility *t*-test to predict team-sport performance (Figure 2). The agility *t*-test was administered using the modified protocol as previously used by Sassi et al. (24) and Ishak et al. (16). Agility times were recorded using timing gates (Microgate, Bolzano, Italy). The position of the timing gate was standardized as instructed by the guidelines the manufacturer set. All athletes performed 3 trials, with the fastest time used for subsequent analysis.

3.5.3. Linear 20-m Sprint Test

The maximum running speed and acceleration were determined during the 20-m linear sprint test, a relevant performance parameter in team-based sports (25). The athlete was required to run a single maximal sprint over a 20-m distance. Sprint times were recorded using timing gates. Each sprint's starting position was standardized, placing the dominant foot at the front. All athletes performed the linear sprint 3 times and were given a 1-min passive recovery period between each set to ensure reliable results. The best 20-m linear sprint time was used for further analysis.

3.6. Statistical Analyses

All data were analyzed using Statistical Package for the Social Sciences (SPSS IBM, Chicago, IL, USA) version

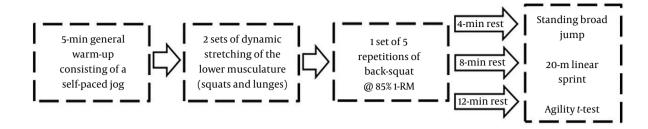


Figure 1. Schematic overview of the protocol for the 3 experimental conditions consisting of 4 min (PAP₄), 8 min (PAP₈), or 12 min (PAP₁₂) passive rest after standardized warmup and PAP stimulation. Once rested, the participants performed the 3 performance tests. PAP, post-activation potentiation.

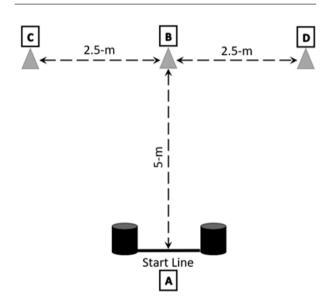


Figure 2. Illustration of the modified agility *t*-test assessment. Grey triangles display the cones around which the athletes must run and shuffle. The black cylinder displays the placement of timing gates (Microgate, Bolzano, Italy), set 1-m apart, 1 m in height, and 1 m from the pre-marked start and finish line.

28. Using the Shapiro-Wilk test, the normality of data distribution was examined, confirming the homogeneity (P > 0.05). The differences between conditions were evaluated using a repeated-measures analysis of variance for all performance variables. A three-way general linear model with repeated measures was used to assess HR and RPE measures (condition (3 levels) x time (3 levels) x gender (2 levels)). A general linear model with two-way repeated measures was used to assess performance variables (condition (3 levels) x gender (2 levels)). To correct violations of sphericity, the degrees of freedom were corrected, using Huynh-Feldt ($\varepsilon > 0.75$) or Greenhouse-Geisser (ε < 0.75) values for ε , as appropriate. Graphical comparisons between means and Bonferroni pairwise comparisons were made where main effects

were present. Effect sizes were calculated from the ratio of the mean difference to the pooled standard deviation. The magnitude of the ES was classified as trivial (≤ 0.2), small (> 0.2 - 0.6), moderate (> 0.6 - 1.2), large (> 1.2 - 2.0), and very large (> 2.0). The results are presented as the mean \pm standard deviation throughout the text. Ninety-five percent confidence intervals are presented where appropriate. Following convention, the alpha significance level was set at 5%, where P values < 0.05 were referred to as "significant." Values of "0.000" are shown here as P < 0.0005.

4. Results

4.1. Comparison of Males and Females

Mean \pm SD values and other results are shown in Table 1 and Figure 3. There were significant differences between males and females in CMJ, agility, and linear sprint results (P < 0.0005). Men jumped significantly higher (11.00 to 12.14 cm; 23.20%), performed the modified agility *t*-test faster (1.50 to 1.59 s; average: 19.33%), and ran the 20-m linear sprint faster (average: 26.34%) than females in all conditions (P < 0.0005, Figure 3). Males had a significantly lower HR (2.67 beats.min⁻¹; P < 0.0005) and RPE (0.33; P = 0.41) than their female counterparts throughout the testing protocol.

4.2. Comparison Between Post-activation Potentiation Conditions

4.2.1. Counter Movement Jump

The CMJ height in PAP₁₂ showed a significant improvement of 2.95% in males (1.35 cm; 95% CI: 0.76 -1.95 cm, P < 0.0005, ES = 0.61) and 2.35% in females (0.90 cm; 95% CI: 0.17 - 1.63 cm, P = 0.005, ES = 0.32) compared to PAP₈. The CMJ height was also significantly better in PAP₁₂ by 2.25% in males (1.45 cm; 95% CI: 0.69 - 2.22 cm, P < 0.0005, ES = 0.63) and 5.78% in females (2.14 cm, 95% CI: 1.02 - 3.26 cm, P < 0.0005, ES = 0.65) compared to PAP₄. In

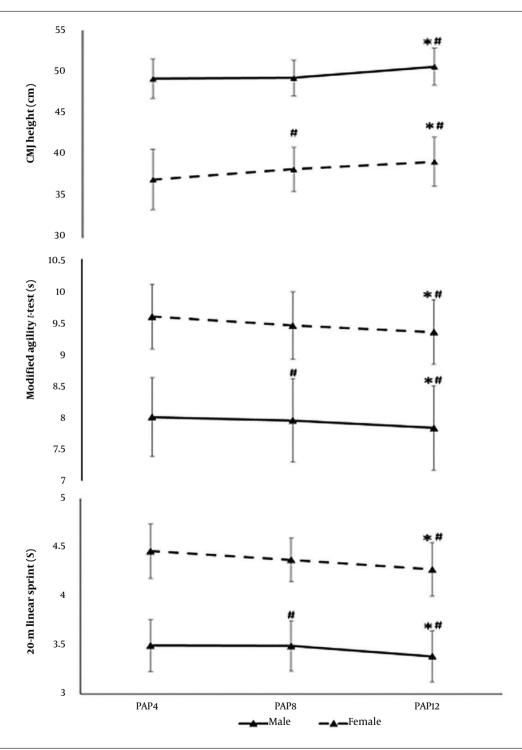


Figure 3. Mean ± SD for countermovement jump height (cm), modified agility t-test (s), and 20-m linear sprint times (s) for all conditions. *: Denotes a statistical significance compared to PAP₈. #: Denotes a statistical significance compared to PAP₄.

Table 1. Mean ± SD of Physical Performance Variables and Subjective Measures in 3 Post-activation Potentiation Conditions. In bold is Indicated the Statistical Significance (P < 0.05)

Variables	Male			Female			P-Value			
	PAP4	PAP8	PAP ₁₂	PAP4	PAP8	PAP ₁₂	Condition	Sex	Time	Interaction
CMJ (cm)	49.2 ± 2.4	49.3 ± 2.2	50.6 ± 2.2	37.1± 3.6	38.3 ± 2.7	39.2 ± 3.0	< 0.0005	< 0.0005		0.801
Agility(s)	8.00 ± 0.62	7.95 ± 0.66	7.83 ± 0.67	9.59 ± 0.51	9.45 ± 0.53	9.34 ± 0.51	< 0.0005	< 0.0005		0.967
Linear sprint (s)	3.50 ± 0.27	3.49 ± 0.26	3.39 ± 0.26	4.46 ± 0.28	4.37 ± 0.22	4.27 ± 0.28	< 0.0005	< 0.0005		0.855
RPE (6 - 20)	13.9 ± 2.9	13.5 ± 2.8	12.9 ± 2.8	14.2 ± 3.0	13.9 ± 3.0	13.2 ± 2.8	< 0.0005	0.041	< 0.0005	0.923
HR(bpm)	151.5 ± 21.4	149.8 ± 21.4	146.5 ± 21.8	154.4 ± 21.6	152.2 ± 21.2	149.2 ± 21.8	< 0.0005	< 0.0005	< 0.0005	0.999

Abbreviations: CMI, counter movement jump; RPE, perceived exertion; PAP, post-activation potentiation

females, the CMJ height was significantly higher in PAP₈ than in PAP₄ by 3.35% (1.24 cm, CI: 0.22 - 2.27 cm, P < 0.0005, ES = 0.39).

4.2.2. Agility

Agility times in PAP $_{12}$ showed a significant improvement of 1.50% in males (0.17 s, 95% CI: 0.09 - 0.25 s, P < 0.0005, ES = 0.27) and 2.56% in females (0.25 s, 95% CI: 0.14 - 0.35 s, P < 0.0005, ES = 0.48) compared to PAP $_{4}$. Agility was also significantly improved in PAP $_{12}$ compared to PAP $_{8}$ by 2.14% in males (0.12 s, 95% CI: 0.06 - 0.17 s, P = 0.002, ES = 0.18) and 1.09% in females (0.10 s, 95% CI: 0.02 - 0.19 s, P = 0.005, ES = 0.20). In females, agility in PAP $_{8}$ was significantly faster than in PAP $_{4}$ by 1.48% (0.14 s, 95% CI: 0.00 - 0.28 s, P < 0.0005, ES = 0.27).

4.2.3. Linear Sprint

Twenty-meter linear sprint times significantly improved in PAP $_{12}$ by 3.20% in males (0.11 s, 95% CI: 0.04, 0.17 s, P < 0.0005, ES = 0.41, small) and 2.24% in females (0.10 s, 95% CI: 0.03 - 0.17 s, P = 0.002, ES = 0.39) compared to PAP $_{8}$. Values for sprint times in PAP $_{12}$ significantly improved by 3.06% in males (0.11 s, 95% CI: 0.05 - 0.18 s, P = 0.002, ES = 0.43) and 4.19% in females (0.19 s, 95% CI: 0.10 - 0.27 s, P < 0.0005, ES = 0.68) compared to PAP $_{4}$. In females, sprint times were significantly faster in PAP $_{8}$ than in PAP $_{4}$ by 2.00% (0.09 s, 95% CI: 0.01 - 0.19 s, P = 0.003, ES = 0.36).

4.2.4. Heart Rate and Ratings of Perceived Exertion

The PAP₁₂ condition had significantly lower values for HR (3.18 to 5.15 beats.min⁻¹; P < 0.0005) and RPE (0.63 to 1.02; P < 0.05) than PAP₈ and PAP₄. Only the HR was significantly lower in PAP₈ than in PAP₄ (1.97 beats.min⁻¹; P = 0.021). Also, HR (22.90 to 50.63 beats.min⁻¹; P < 0.0005) and RPE (3.82 to 6.43; P < 0.0005) significantly increased after the 20-m linear sprint compared to when CMJ and agility tests were done. In addition, both HR (22.90 beats.min⁻¹; P < 0.0005) and RPE (2.62; P = 0.41) significantly increased after the modified agility *t*-test compared to after CMJ. There were no significant differences in RPE between PAP₈ and PAP₄ (P = 0.055).

5. Discussion

This study investigated whether differences exist in the duration for optimal post-pre-load stimulus measures on performance measures in male and female team-sport athletes. Our main finding was that CMJ, agility, and 20-m linear sprint increased significantly after the PAP stimulus when a 12-min passive rest period was provided in both males and females. The 4-min passive rest following the PAP protocol yielded the worst physical performance results, while the 12-min passive rest yielded the best results in males and females. These findings align with previous work conducted by Kilduff et al. (7), who reported that the optimal recovery duration to maximize the PAP response on peak power output was between 8 and 12 minutes in professional rugby athletes.

Countermovement jumps increased by 2.95% in males and 2.35% in females following 12 minutes of passive rest compared to 8 minutes. Compared to 4 minutes of passive rest, this increased by 2.25% and 5.78%, respectively. Similar observations were made in linear sprints, where sprint times improved by 3.06 - 3.20% in males and 2.24 - 4.19% in females following a 12-min passive rest. Values for agility also displayed such patterns, with improvements ranging from 3.06 - 3.20% in males and 2.24 - 4.19% in females following the 12-min rest period. Previous studies have found that the optimal recovery time required to maximize the PAP effect can be as little as 15 s or as much as 24 min (12-15), although some studies found no significant improvements in physical responses (26). Several physiological and methodological factors influence the efficacy of a given pre-load stimulus on performance. Appropriate intensity and duration of the conditioning activity and the type and duration of recovery between the pre-load stimulus and subsequent exercise affect subsequent performance (10, 26). A PAP strategy consisting of a low number of repetitions (≤ 6) of high-intensity and heavy (> 70% 1RM) strength exercises is an effective pre-load stimulus to increase performance in jumps, throws, and sprints in athletes (10), as supported by our findings. A similar study conducted by Ishak et al. (16)

established that in male university-level handball athletes, the greatest benefits in handball-specific performance measures were observed after completing a 12-min passive rest period following a pre-load stimulus (1.55 to 3.65%). In Rugby players, a similar pre-load stimulus with 10-min of rest improved 20-m sprint performance by 3.3% compared to no pre-load stimulus (27). Not all investigations have found 5 repetitions of back-squat at 85% 1-repetition maximum to improve performance. A study by Jo (28) observed that using a pre-load stimulus in recreationally trained individuals failed to influence performance after a heavy-load exercise using different recovery durations. It was observed that the more an individual is trained, the less rest period is required to potentiate performance. Findings suggest that athletes require a minimum of 3 years of resistance training experience to respond optimally to conditioning activities This aligns with our inclusion criteria, where participants were required to have at least 3 years of experience in resistance training and back squatting. Therefore, our athletes were sufficiently "trained" to induce PAP and could respond to this pre-load stimulus favorably. It has been suggested that individuals who are "weaker and/or less trained" take longer to potentiate than "stronger and/or better trained" individuals (30).

Previous findings have shown that male individuals are stronger than their female counterparts in several measures of strength (31), have a better CMJ (32), perform an agility test faster (27), and are quicker over 20 m (27). In a cohort of fencers, leg power output was only affected in males, with no benefits observed in female fencers (18). Nevertheless, jump testing has shown improvements in both males and females, with the greatest improvements in the highest strength-trained individuals (7). Findings in the literature are conflicting, and different results are observed between males and females for different performance variables. Nevertheless, we agree with previously observed findings that have shown a pre-load stimulus to improve performance irrespective of gender and that the training experience of different individuals affects results (29). It may therefore be hypothesized that the volume and intensity of this pre-load stimulus may be a suitable option for both handball and volleyball players, regardless of gender. Team-sport athletes are stronger and more powerful than other sporting athletes, such as middle-distance runners (1), due to the high jumping demands in professional volleyball and handball players (3). The player position demands vary in handball and volleyball, with differences in strength and high-intensity actions present across different playing positions (2). When assessing volleyball players, the greatest total jump loads are observed in the setters and middle blockers positions, while the most high-intensity jumping actions (> 70% maximum jump height) are performed by opposites (3). Previous research has found that type II fibers are a potentially greater target for PAP interventions than type I fibers (11). This results in greater potential gains in more explosive athletes who are typically required to carry out more high-intensity power actions during the competition, such as handball and volleyball players. Regardless of player position, handball and volleyball athletes' general strength levels are sufficient to elicit a significant PAP response, as observed by our results.

Finally, building upon our recent study (16), we once again observed significant decreases in both heart rate and RPE after performing PAP₁₂ compared to both PAP₄ and PAP₈ exercises. Notably, these findings were consistent among both male and female team sports players. There is often a trade-off between potentiation and fatigue, and indicators of fatigue can often arise via increases in both perceived effort and heart rate responses for a given exercise task (5, 33). Based on the secondary outcome measures of this study, these data may further support the use of 12-min recovery once a pre-load stimulus has been completed to maximize physical capacity in both volleyball and handball athletes. Compared with longer rest durations, a 12-min recovery from a warm-up maintains other important physiological and neuromuscular factors, such as increased muscle temperature better (4). Since muscle performance following a pre-load stimulus depends on the balance between muscle fatigue and potentiation (5, 33), a pre-load stimulus should aim to maximize potentiation and minimize fatigue to generate the best PAP response (34).

Findings suggest that using a set of 5 repetitions of back-squat at 85% of 1-repetition maximum can elicit a PAP response in team-sport athletes and has profound implications for players, strength and conditioning coaches, and coaches involved in these disciplines. The key findings of this study suggest that by performing a pre-load stimulus, after which a 12-min passive recovery is provided, significant improvements in measures of CMJ, agility, and linear sprint are established. These results imply that PAP could be elicited in both male and female players in performance variables related to handball and volleyball and can be used before sports performance and/or training sessions. However, in agreement with current recommendations in the literature (33), whether using PAP after heavy strength repetitions improves field performance during handball and volleyball matches is currently unknown. Practitioners are thus required to ensure that the pre-load stimuli provided to athletes are individualized per the player's characteristics and that a sufficient recovery window is given.

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Footnotes

Authors' Contribution: Al and FYW conceived and designed the research and acquired the data. CEB, EV, RA, and CS designed the evaluation, performed parts of the statistical analysis, and helped draft the manuscript. SC and SP provided the analysis and interpretation of the data, drafted the manuscript, and helped with some of the design. All authors helped with the writing of the manuscript.

Conflict of Interests: The authors have no conflict of interest in this study.

Data Reproducibility: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: The study was approved by the Human Ethics Committee of the Sports Science Department, Sultan Idris Education University, Malaysia, and conformed to the ethical standards of the Declaration of Helsinki (ethics code: 2022-081-02).

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Informed Consent: Everyone gave their written informed consent.

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