

applied sciences

Special Issue Reprint

Advances in Sport Physiology, Nutrition, and Metabolism

Edited by
Aleksandra Bojarczuk and Monika Michałowska-Sawczyn

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Guest Editors

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Monika Michałowska-Sawczyn



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This is a reprint of the Special Issue, published open access by the journal *Applied Sciences* (ISSN 2076-3417), freely accessible at: https://www.mdpi.com/journal/applsci/special_issues/067VH1X1O1.

For citation purposes, cite each article independently as indicated on the article page online and as indicated below:

Lastname, A.A.; Lastname, B.B. Article Title. <i>Journal Name</i> Year , Volume Number, Page Range.
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ISBN 978-3-7258-9045-3 (Hbk)

ISBN 978-3-7258-9046-0 (PDF)

<https://doi.org/10.3390/books978-3-7258-9046-0>

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About the Editors

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Advances in Sport Physiology, Nutrition, and Metabolism

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1. Introduction

The fields of sport physiology, nutrition, and metabolism are advancing at an unprecedented pace, driven by growing interest in understanding the biological mechanisms that govern human responses to physical exercise and training [1,2]. Human physical performance and physiological adaptation to exercise are no longer interpreted through a single physiological lens [3,4]. Rather, they represent complex phenomena resulting from the interaction of numerous intrinsic and extrinsic determinants [5,6].

Consequently, research in sport and exercise science aims to understand physiological responses to exercise [7], metabolic adaptations to training [8], and the role of nutritional strategies in supporting performance and recovery in physically active and athletic populations [9].

Despite the rapid expansion of research in sports science [10], important questions remain regarding the physiological and metabolic responses to different exercise stimuli, the mechanisms underlying training-induced adaptations [11], and the role of nutritional strategies in supporting recovery [12] and metabolic regulation [13]. In addition, evidence regarding exercise-based interventions in specific populations and sport-specific nutritional considerations remains comparatively limited [14,15]. Addressing these questions is essential for advancing both the biological understanding of exercise responses and their practical application in physically active and athletic populations.

Under this context, the Special Issue “Advances in Sport Physiology, Nutrition, and Metabolism” brings together seven original research articles and one review. These contributions address physiological responses to exercise, metabolic adaptations to training, and nutritional strategies, reflecting the breadth and contemporary focus of research in sport physiology, nutrition, and metabolism. The main findings of the included contributions are summarized in the following section.

2. Overview of Published Articles

The articles published in this Special Issue address several important questions in sport physiology, training adaptation, and nutrition.

Two studies in this Special Issue (contributions 2 and 6) examined post-activation performance enhancement (PAPE) using isometric conditioning activity (ICA) protocols and addressed methodological uncertainties in their experimental design. The article by Latocha et al. (contribution 2) investigated the role of inter-set rest intervals during ICA in 15 resistance-trained males, a factor that remains insufficiently examined due to substantial variability in protocol parameters such as contraction duration, joint angle, and contraction distribution in previous studies. By comparing rest intervals of 1, 2, and 3 min while keeping the total contraction time constant, they aimed to isolate the influence of recovery duration between ICA sets on subsequent countermovement jump (CMJ) performance. The

findings indicate that neither ICA nor the rest interval length significantly influenced CMJ height or relative peak power, suggesting limited effectiveness of the tested ICA protocols in resistance-trained individuals.

In contrast, Jarosz et al. (contribution 6) focused on another key parameter of ICA protocols, the total contraction volume, in a cohort of 14 highly trained male volleyball players. Specifically, the authors compared the effects of a single short ICA set (9 s) with a higher-volume protocol consisting of three sets (27 s in total) on CMJ performance. The results show that the higher-volume protocol significantly improved jump height and relative peak power within the first minutes following the conditioning activity.

Together, two studies (contributions 2 and 6) address an important methodological gap in the literature regarding the design of isometric conditioning activity (ICA) protocols to induce PAPE. While contribution 2 examined whether varying inter-set rest intervals (1–3 min) influence performance outcomes when total contraction time is held constant, contribution 6 directly compared different ICA volumes (9 s vs. 27 s total contraction duration). By isolating these key protocol variables across distinct trained populations, the studies help clarify how recovery structure and contraction volume independently affect the effectiveness of ICA-based warm-up strategies.

Extending the investigation of acute exercise responses (contributions 2 and 6), Skotniczny et al. (contribution 4) examined the immediate effects of maximal aerobic effort on the mechanical properties of the muscle–tendon unit. The authors investigated acute, tissue- and side-specific changes in viscoelastic properties following a progressive treadmill test to determine maximal oxygen uptake (VO_2max) in 21 high-level male soccer players. Measurements of tone, stiffness, elasticity, relaxation time, and creep were obtained before and immediately after the test using handheld myotonometry. Although muscle and tendon stiffness are known to influence athletic performance, fatigue, and injury risk, the immediate viscoelastic responses of muscle–tendon tissues to maximal aerobic exercise remain poorly understood and have rarely been systematically investigated in elite soccer players. The results reveal heterogeneous, side-dependent alterations in viscoelastic parameters across the muscles and tendons examined, with notable changes in the rectus femoris (RF) and Achilles tendon. Moreover, baseline viscoelastic properties of the RF were moderately associated with VO_2max , suggesting that tissue mechanical characteristics may serve as potential indicators of aerobic readiness.

Beyond studies examining acute responses to exercise (contributions 2, 4, and 6), three contributions in this Special Issue investigated the effects of structured training interventions implemented over longer periods (contributions 1, 3, and 8). These studies address distinct gaps in the literature concerning the design and physiological consequences of exercise training programs. Specifically, they examine (i) the effects of an integrated multicomponent training approach in elite rugby sevens athletes, (ii) the impact of plyometric training on anaerobic performance and biomechanical muscle properties in football players, and (iii) the effects of low-volume resistance training on biochemical markers related to muscle health, metabolic profile, and oxidative stress in older adults with cognitive impairment.

Marius et al. (contribution 1) evaluated the effects of a 12-week integrated training program in elite rugby sevens athletes, including both female and male players ($n = 24$; 12 women and 12 men). The intervention consisted of 12 training micro-cycles combining strength, speed, aerobic capacity, and rugby-specific exercises within an integrated training framework. Pre- and post-intervention assessments included sprint performance, jumping ability, resting metabolic rate, and cardiopulmonary exercise testing. The results show significant improvements in several performance variables, including sprint and jump performance, together with favorable changes in selected physiological responses to exercise. Sex-specific differences in adaptations were observed: females showed greater

improvements in aerobic capacity and jumping performance, while males demonstrated larger gains in explosive power and longer sprint distances. These findings highlight the potential value of integrated training approaches that simultaneously target multiple components of physical performance in rugby players.

A complementary perspective on training adaptations was provided by Matuszczyk et al. (contribution 8), who investigated the effects of a 10-week plyometric training program in football players. Twenty male athletes were randomly assigned to an experimental group ($n = 10$), which performed additional plyometric training twice per week, or a control group ($n = 10$) that maintained regular football training. The study assessed sprint performance (5 m and 30 m), reactive strength index, and biomechanical properties of the RF and vastus lateralis muscles. The experimental group demonstrated significant improvements in sprint performance and reactive strength index (RSI) following the intervention. However, no significant group-level changes were observed in muscle tension, stiffness, or elasticity. Instead, correlation analyses indicated that baseline elasticity of the vastus lateralis, and changes in the elasticity of the rectus femoris were associated with improvements in 5 m sprint performance. These findings suggest that plyometric training can enhance short-distance sprint performance and RSI, while individual biomechanical characteristics of muscle tissue may modulate training adaptations.

While the previous studies focused on athletic populations (contributions 1 and 8), Lopes et al. (contribution 3) explored the effects of exercise in a clinical population of older adults with cognitive impairment. In this study, 28 institutionalized older adults were assigned to a low-volume resistance training group ($n = 15$; 6 women and 9 men) or a control group ($n = 13$; 9 women and 4 men). The intervention consisted of an eight-week resistance training program performed twice per week using a single-set protocol. The authors examined biochemical markers related to muscle damage, liver function, lipid profile, glycemic control, and oxidative stress. Although no significant between-group differences were observed in any biomarker at baseline or post-test, the intervention group showed increases in serum lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase activities, all of which remained within physiological ranges, suggesting an acute-phase response rather than tissue damage. Additionally, within the intervention group, high-density lipoprotein cholesterol increased, and the atherogenic index decreased. These results suggest that low-volume resistance training may induce modest changes in selected biochemical markers and represent a feasible exercise strategy for older adults with cognitive impairment.

Together, these studies (contributions 1, 3, and 8) demonstrate that structured training programs, including integrated conditioning, plyometric training, and resistance exercise, can induce measurable physiological adaptations across diverse populations. Such programs may also contribute to improvements in physical performance or health-related outcomes. In addition to studies examining acute responses and structured training interventions, other contributions in this Special Issue explore the role of nutritional strategies in supporting exercise performance and recovery. Under this context, Drviš et al. (contribution 7) examined nutritional recommendations for breath-hold divers, a population exposed to unique physiological stressors, including prolonged apnea, hypoxia, and hypercapnia. The authors highlight that, despite the extreme physiological demands of breath-hold diving, sport-specific nutritional frameworks remain largely underdeveloped. To address this gap, the review synthesizes the available literature. It proposes tailored nutritional strategies for different breath-hold diving disciplines, highlighting Mediterranean and alkaline dietary patterns as potentially useful approaches to support recovery, performance, and long-term health in freedivers.

Beyond physiological and nutritional considerations, the accurate monitoring of performance and injury risk remains a central challenge in applied sport science. Addressing this aspect, Michailidis et al. (contribution 5) investigated the relationship between asymmetries detected through a commonly used field-based test—the single-leg countermovement jump—and those identified using laboratory-based isokinetic strength assessments in 63 elite male youth football players. Given that inter-limb asymmetries have been associated with both injury risk and reduced performance, the study explored whether practical jump-based screening tools could serve as proxies for more complex strength measurements. The findings indicate only limited relationships between the two assessment methods, suggesting that simple field tests cannot fully replace laboratory-based evaluations and that a combination of approaches may be necessary for comprehensive monitoring of lower-limb asymmetry in football players.

3. Conclusions

The studies included in this Special Issue highlight several interconnected directions in contemporary sport physiology, training adaptation, and applied sport science research. Two primary research axes emerge across the contributions. The first concerns acute physiological responses to exercise and conditioning stimuli (contributions 2, 4, and 6), including the methodological optimization of isometric conditioning activity protocols to induce post-activation performance enhancement and the investigation of the immediate mechanical responses of muscle–tendon tissues to maximal aerobic effort. The second axis focuses on longer-term training interventions and their physiological consequences across both athletic and clinical populations (contributions 1, 3, and 8).

In addition to these primary research axes, this Special Issue also includes two complementary topics. One contribution (7) addresses sport-specific nutritional considerations in breath-hold divers, highlighting the limited availability of dedicated dietary frameworks for this unique discipline. Another study (5) focuses on performance monitoring in football players, examining the relationship between asymmetries detected using field-based jump tests and those identified through laboratory-based isokinetic strength assessments.

Research examining acute responses to exercise indicates that specific protocol characteristics influence the outcomes of conditioning strategies. In particular, studies investigating isometric conditioning protocols show that manipulating parameters such as rest intervals or contraction volume does not necessarily yield consistent improvements in countermovement jump performance. However, certain configurations—such as higher total contraction volume—may produce short-term increases in jump height and relative peak power in trained individuals. At the same time, investigations of maximal aerobic exercise demonstrate that exhaustive treadmill running can induce heterogeneous, tissue-specific alterations in the viscoelastic properties of muscles and tendons in high-level soccer players, suggesting that acute exercise responses may vary across different muscle–tendon structures.

Studies addressing longer-term training interventions further show that structured exercise programs can lead to measurable physiological and performance-related changes across different populations. An integrated multicomponent training approach was shown to improve several performance and physiological parameters in elite rugby sevens athletes, including sprint ability, jumping performance, and indicators of cardiovascular efficiency. Similarly, a 10-week plyometric training program improved sprint performance and RSI in football players, although no significant group-level changes were observed in biomechanical muscle properties. In a clinical context, low-volume resistance training performed over eight weeks produced modest but potentially beneficial changes in selected metabolic markers in older adults with cognitive impairment, while enzyme changes remained

within physiological ranges and were suggestive of an acute-phase response rather than tissue damage.

Complementary contributions also emphasize the importance of supporting factors in applied sport science research. A review focusing on breath-hold diving highlights the current lack of dedicated nutritional recommendations for freedivers and proposes dietary strategies based on Mediterranean and alkaline dietary patterns. In addition, research on lower-limb asymmetry in youth football players shows that asymmetry indices derived from jump tests cannot replace those obtained through isokinetic strength assessments, indicating that a combination of testing approaches may be necessary for a more comprehensive evaluation of lower-limb asymmetry in football players.

Building on the directions highlighted by the studies in this Special Issue, subsequent work should further refine conditioning protocols and clarify the mechanisms underlying performance enhancement and fatigue responses, particularly in the context of acute exercise (contributions 2, 4, and 6). This encompasses systematic exploration of key methodological parameters of isometric conditioning activity—such as contraction intensity, joint angle, contraction duration, and recovery structure—and the integration of objective indicators of neuromuscular function and fatigue. Additionally, research into the viscoelastic responses of muscle–tendon structures to intense exercise should assess how training and recovery shape longer-term adaptations and determine whether targeted interventions affect performance readiness or injury risk.

Regarding longer-term training interventions (contributions 1, 3, and 8), future research should examine the effectiveness of structured training programs across diverse populations and contexts. Longitudinal studies are needed to better understand training adaptations over extended periods, including the role of integrated training strategies, plyometric interventions, and resistance training protocols. Particular attention should be paid to individual variability in training responses, the influence of athlete characteristics, and the optimization of exercise interventions in both athletic and clinical populations.

Complementary areas (contributions 5 and 7) underscore the significance of broader performance support systems. Ongoing inquiry into sport-specific nutritional strategies for disciplines with unique physiological demands (e.g., breath-hold diving) remains important, as does the advancement of diagnostic methods for monitoring performance and injury risk. Integrating field- and laboratory-based assessments to evaluate lower-limb asymmetry and function will strengthen these efforts.

Looking forward, multidisciplinary approaches that bridge physiology, nutrition, biomechanics, and performance analysis are likely to yield the most comprehensive insights. While the studies in this Special Issue address a range of topics, most focus on individual domains.

Unifying these perspectives within integrated research frameworks will better capture the multifactorial nature of athletic performance and adaptation [16], paving the way for a deeper understanding of exercise mechanisms, training effects, and recovery processes.

Conflicts of Interest: The author declares no conflicts of interest.

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Article

Integrated Training Program for Rugby Sevens: A Multivariate Approach of Motor, Functional, and Metabolic Components

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Abstract

Purpose: This study assessed the adaptations resulting from implementing an experimental, integrated training program tailored to sex-specific traits. The aim was to enhance motor abilities, aerobic capacity, and metabolic variables in female and male rugby sevens athletes. **Methods:** Employing a combined observational and experimental design, initial and post-intervention assessments were conducted over three months (March–June 2023) with 24 elite professional players, divided equally by sex (12 females, 12 males). The protocol consisted of 12 micro-cycles, each lasting 7 days and comprising 12 training sessions. The evaluations included sprint and jumping tests, as well as functional assessments such as resting metabolic rate and cardiopulmonary exercise testing. **Results:** Using one-way repeated measures ANOVA, significant improvements were noted across all performance parameters ($p < 0.001$), with effect sizes ranging from small to very large. Sex-specific differences were evident, with females demonstrating consistent improvements in aerobic capacity and jumping ability, while males excelled in explosive power and longer sprints. Despite initial performance disparities, both sexes improved in short-distance sprints (10 m and 40 m). Cardiovascular efficiency improved as indicated by reduced maximum heart rates and lower respiratory quotients. **Conclusions:** Males showed superior progress in strength and explosive power tests, reflecting distinct physiological traits. These findings underscore the need for individualized and sex-specific training programs to optimize performance in high-intensity sports, such as rugby sevens.

Keywords: rugby sevens; integrated training; sex differences; metabolic adaptations; motor performance

1. Introduction

Modern sevens rugby involves a high level of physical, technical, tactical and psychological demands, requiring athletes to undergo multidimensional preparation to meet the requirements of high-level competition. Modern sevens rugby imposes complex physiological demands characterized by intermittent high-intensity efforts, repeated physical contacts and multiplanar neuromuscular demands [1,2]. In rugby, motor actions involve the simultaneous activation of neuromuscular, cardiovascular, and metabolic systems in a functional synergy that cannot be replicated through segmented training [3,4]. This specificity requires reconsidering traditional training paradigms that treat performance

components in isolation. Contemporary research predominantly addresses the development of motor capacities through segmented methodologies; however, this methodological fragmentation overlooks the principle of functional interdependence in biological systems and fails to generate optimal adaptations for the specific requirements of rugby according to sex [4–7].

In this context, integrated training strategies are gaining increasing relevance, as they enable the synergistic development of multiple motor and physiological qualities necessary for optimal performance. An integrated approach involves the intelligent combination of physical training within a periodized planning framework, adapted to the specificity of rugby [2,8,9]. Recent studies show that training programs that simultaneously address multiple aspects of physical performance—such as speed, strength, endurance, and agility—yield significant gains in players’ physical efficiency compared with training focused on a single parameter [8–11]. Moreover, these multivariate approaches enable training to be personalized for each athlete, taking into account their unique characteristics, strengths, and areas needing development. Thus, by adapting the program to each athlete’s specific needs, the body’s physiological adaptations are optimized, and superior sports performance is achieved [10–16]. Unlike most studies that analyze either motor or physiological parameters in isolation, our study proposes an integrated approach, simultaneously evaluating motor and functional parameters in a rugby-specific context. Additionally, our study is distinguished by its analysis of sex differences in training response, offering a comparative perspective between female and male athletes, an aspect frequently neglected in high-performance sports research [11,17–19].

Studies indicate that faster rugby players are more successful at breaking through defenses, evading tackles, and scoring tries than slower teammates. Their speed allows them to engage the defensive line more quickly, prompting opponents to make inefficient defensive choices and creating opportunities for missed tackles. From a defensive angle, faster players also demonstrate higher tackle efficiency [20,21]. Rugby players require physical actions like high-intensity running and directional changes during games, making physical fitness crucial for performance [22]. The significance of developing motor qualities has been emphasized, especially among coaches of adolescent players [23]. In contact sports such as rugby, optimizing physical fitness is essential for actions such as tackles and scrums [24,25]. Throughout the competitive season, training for strength, speed, and explosive power is vital, although elite athletes often see limited improvement in these areas [26]. This has spurred ongoing efforts to find innovative training methods [27–29].

Recent advancements in rugby research have illuminated substantial deficiencies, particularly in examining isolated performance components, while largely overlooking the intricate interactions among motor skills, functional capabilities, and metabolic processes. Most studies tend to adopt a univariate perspective, which inadequately captures the multifaceted nature of athletic performance. This limitation is further exacerbated by the current methods for quantifying training load, which predominantly rely on a narrow range of physiological markers, often constrained by field-based measurement limitations. As a result, there remains a significant gap in understanding the synergistic mechanisms underpinning integrated physiological adaptations and their practical implications for rugby training methodologies. Currently, the literature lacks a holistic perspective that accounts for the interplay among various performance determinants. This fragmentation underscores an urgent need for a comprehensive multivariate framework to effectively integrate these diverse physiological elements. Such an approach would not only enhance the relevance of academic research to the sport’s physiological demands but also improve training practices by aligning them more closely with the complex realities of rugby performance.

Our study presents a personalized and integrated training program, assessed through multivariate analyses that include standardized tests, and advanced evaluations, such as indirect calorimetry and cardio-pulmonary exercise testing (CPET). This integrated model aims to enhance sports training theory and optimize rugby performance.

This study aims to evaluate adaptations following the implementation of an experimental integrated training program tailored to sex-specific characteristics, targeting improvements in motor components, aerobic capacity, and metabolic variables in female and male rugby athletes. We hypothesized that the implementation of a 12-week integrated training program would result in significant improvements in the physical fitness of rugby players and that the magnitude of these adaptations would differ by sex, particularly in motor abilities, aerobic capacity, and metabolic variables.

2. Materials and Methods

2.1. Participants

The study included 24 elite professional senior rugby sevens players, comprising 12 females (50%) and 12 males (50%). Female players had a mean height of 162.8 cm and a mean body mass of 59.4 kg at baseline, while male players had a mean height of 182.0 cm and a mean body mass of 87.1 kg. The study sample consisted of 24 elite professional senior rugby sevens (7 s) players, including 12 females (50%) and 12 males (50%). All participants were active members of the national high-performance rugby sevens program and competed regularly at national and international levels throughout the competitive season. The athletes had a minimum of five years of competitive rugby experience and were accustomed to the high training volumes and intensities specific to this discipline.

At the time of the study, all athletes were clinically fit, free from injury, and medically cleared for high-intensity exercise. They followed a centralized training program conducted under the supervision of coaches and strength and conditioning specialists. Both female and male groups were integrated within the same training framework, with sex-specific adaptations applied when necessary.

Inclusion criteria: active athletes, absence of injury in the last 6 months (including during the study), minimum age 18 years, minimum 4 years of experience as active players, minimum two national team selections, participation in the entire experimental program, and complete completion of evaluations from both initial (It) and final (Ft) sessions. G*Power 3.1.9.4., analysis for the study with 12 subjects per group ($n = 24$ total) and a statistical power ($1 - \beta$) of 0.95 indicates adequate statistical power (82.5%). Athletes were excluded from the study if they had experienced musculoskeletal injuries within the previous six months, undergone surgical interventions, or presented medical conditions (cardiovascular, respiratory, metabolic, or neurological) incompatible with high-intensity exercise; if they used substances or medications that could influence physiological responses; failed to fully participate in the training program or in the initial and final assessments; were involved in other concurrent training interventions; experienced acute illness episodes during the testing period; or did not provide informed consent. This G*Power analysis indicated that a minimum of 11 participants in the study group was necessary to achieve the desired level of statistical power.

2.2. Research Design

The study used observational and experimental methodologies, including initial baseline and post-intervention evaluations, and was conducted over a 3-month period from March to June 2023, during which 12 training micro-cycles were performed, each micro-cycle having 7 days, with 12 training sessions per micro-cycle. The study included

an initial evaluation (It), implementation of the 12-week experimental program, and final evaluation (Ft).

The training periodization was carried out according to the three specific periods in sports preparation: preparatory, pre-competitive, and competitive. The experimental program included integrated training in the context of rugby, as well as training for strength optimization, conducted both in the strength room (indoor) and on the rugby field (outdoor). The program was individualized for each player, with a muscle activation program adapted to the sport's particularities and their fitness level. Integrated exercises were implemented in the first part of the 12 training sessions, each lasting 30 min. During the preparatory period, the micro-cycle intervention consisted of 6 integrated training sessions, structured as follows: 2 anatomical adaptation sessions with integrated field exercises; 2 sessions targeting aerobic capacity in game contexts; and 2 sessions to educate movement speed, performed with the ball. During the pre-competitive period, the training sessions—the 6 training sessions—were structured as follows: 2 sessions for developing speed and agility; 2 sessions for improving specific aerobic explosive power through workshop work with the ball and through aerobic power exercises 3 vs. 2 players; 2 sessions focused on developing explosive strength with integrated means (on the rugby field outdoor).

The integrated training program was organized at the level of each training session into three distinct sections: warm-up, main training phase, and cool-down. The structure of each session aimed to progressively prepare the athletes for the mechanical, metabolic, and neuromuscular demands specific to rugby, while also facilitating recovery and injury prevention.

The warm-up lasted approximately 15–20 min and included general aerobic activation (light running), joint mobility exercises, and dynamic stretching targeting the ankle, knee, hip, and spinal joints. This was followed by neuromuscular activation and coordination drills (skipping, low-intensity jumps), as well as progressive accelerations and changes of direction. During this phase, exercises such as single-leg balance followed by a lateral sprint and drop jump combined with a sprint and change of direction were integrated to prepare the athletes for explosive actions and rugby-specific demands.

The main training phase, lasting approximately 60–75 min, combined strength, speed, aerobic capacity, and rugby-specific exercises in an integrated manner (Table 1). Examples of exercises included barbell squats followed by lateral movement and controlled contact, horizontal sled pushes followed by a sprint, Nordic hamstring exercises followed by a rapid stand-up and a 1 vs. 1 duel, zig-zag sprints with passing and a final sprint. In addition, small-sided games (3 vs. 3) with intensity-based rules were incorporated, integrating intermittent aerobic and anaerobic efforts, decision making, and physical contact. Through this structure, mechanical, metabolic, and neuromuscular demands reflected the real requirements of competition, promoting effective functional adaptations and direct transfer to game performance.

The cool-down, lasting approximately 10–15 min, consisted of active recovery exercises (light running or cycling), mobility and flexibility exercises, as well as breathing and relaxation techniques. During this phase, low-intensity running combined with joint mobility and controlled breathing was used to reduce residual fatigue, optimize recovery, and maintain long-term training availability.

The training program was structured throughout the competitive season, respecting the alternation between preparatory, competitive, and recovery phases, with particular emphasis on training load control and the progressive adaptation of the athletes. The program combined strength training, rugby-specific exercises, and aerobic capacity development, and was tailored to positional demands and the players' training level.

Table 1. Training load was periodized and recovery strategies were integrated; however, nutrition and objective recovery markers were not systematically controlled.

Integrated Exercise	Integrated Components	Brief Description	Main Objective
Squat + lateral movement + controlled contact	Strength + stability + rugby-specific	3–5 barbell squats followed by a 5–7 m lateral movement and controlled contact using a pad or partner	Increase contact tolerance and knee/hip joint stability
Drop jump + sprint + change of direction	Explosive power + speed + decision making	Plyometric jump followed by a 10–20 m sprint and a change of direction in response to a visual or auditory cue	Improve acceleration and reactive ability in game situations
Nordic hamstring + rapid stand-up + 1 vs. 1 duel	Eccentric strength + injury prevention + game-specific	2–4 Nordic hamstring repetitions followed by an explosive stand-up and a short 1 vs. 1 duel	Reduce the risk of posterior thigh muscle injuries
Small-sided game (3 vs. 3) with intensity rules	Aerobic capacity + tactics + contact	30–60 s bouts with short recovery periods, including mandatory tackling/ruck involvement	Enhance intermittent high-intensity performance specific to rugby
Single-leg balance + lateral sprint	Proprioception + agility + neuromuscular control	5–10 s single-leg balance followed by a 5–10 m lateral sprint and controlled deceleration	Prevent ankle sprains and improve movement control
Horizontal push (sled) + sprint	Rugby-specific strength + speed + anaerobic capacity	10–15 s horizontal pushing followed by a 10 m sprint	Improve scrum-related explosive power production and transition speed
Zig-zag sprint + passing + final sprint	Speed + coordination + metabolic stress	Rapid changes of direction with passing while running, followed by a final sprint	Maintain technical accuracy under fatigue
Low-intensity running + mobility + breathing	Recovery + injury prevention + autonomic regulation	Active recovery combined with joint mobility exercises and controlled breathing	Optimize recovery and reduce accumulated fatigue

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the National University of Physical Education and Sport in Bucharest, with no. 103/2023.09.26.

The integration of training modalities was designed so that the mechanical, metabolic, and neuromuscular demands reflected the real requirements of the game, thereby reducing training fragmentation and promoting effective functional adaptations. Emphasis was placed on the development of foundational strength, neuromuscular stability, movement control, and the progressive management of external load, which are considered essential for performance enhancement.

External variables were partially controlled in accordance with the specific demands of rugby. Training load was structured through periodized micro-cycles and competitive phases, respecting the principles of progression, work–recovery alternation, and sport specificity. Exercise volume and intensity were adjusted to the athletes’ training level, and recovery strategies were systematically integrated into the training process.

Nutritional intake and objective recovery markers (e.g., sleep quality or biological indicators of fatigue) were not standardized or individually monitored throughout the intervention, which should be considered when interpreting the study findings.

2.3. Evaluation Tools

The study included motor and functional tests selected to relate to the objective. The motor tests were structured into two categories: running tests and jumping tests, as well as specific physical performances related to speed and explosive strength, which were evaluated.

- Running tests: 10 m, 40 m, 100 m speed test performed with the Smart Speed Timing Gate system [30]. Motor evaluation was carried out with a free start from the standing position, in a straight line, measuring times for each running distance.
- Vertical jump test with prior flexion, performed on the Optojump platform (Microgate, 39100 Bolzano, Italy, 2020) [31]. A minimum of 3 jumps were performed to calculate the average for Counter Movement Jump (CMJ).
- Squat Jump test (SJ) without preloading phase, used to evaluate pure jumping capacity and explosive power specific for Squat jump (SJ).
- Lower-limb explosive performance was assessed using the Optojump Next system (Microgate, 39100 Bolzano, Italy, 2020). For the countermovement jump (CMJ), participants started from an upright position with hands on hips, performed a rapid countermovement, and immediately executed a maximal vertical jump. For the Squat Jump (SJ), participants began from a static semi-squat position ($\sim 90^\circ$ knee flexion), held for 2–3 s to eliminate the stretch–shortening cycle. Then they performed a maximal vertical jump without prior countermovement.
- Three trials were performed for each test, with 30–60 s of rest between attempts. The highest jump height (cm), calculated from flight time by the Optojump software 2020, was retained for analysis.

The functional tests were standardized and performed under the same conditions for all subjects. Functional tests evaluated aerobic capacity, resting metabolic rate, and physiological reactions to effort:

- Resting metabolic rate (RMR): evaluated through indirect calorimetry using the COSMED system, considered the gold standard for measuring energy consumption at rest [32,33].
- Cardio-pulmonary exercise test (CPET): for evaluating exercise capacity, performed with the Quark CPET system using the Bruce protocol on a treadmill. This graded test enabled the estimation of $\text{VO}_{2\text{max}}$ and the monitoring of cardiovascular and respiratory responses during progressive effort.

The evaluation was carried out after the first accommodation micro-cycle. Each evaluation was performed on different days, in the first part of the day, before the first training session. The order of test performance was as follows: running tests, jumping tests, and functional tests, RMR and CPET.

2.4. Statistical Analysis

A detailed statistical analysis was performed to evaluate training adaptations and sex differences in performance. Descriptive statistics included means, standard deviations (SD), and variances. Normality was verified using Shapiro–Wilk tests ($p > 0.05$). Repeated Measures ANOVA examined within-subject changes, providing F-statistics and p -values while adjusting for individual baselines. Confidence intervals (95%) indicated plausible population values, with intervals excluding zero suggesting significant effects. We calcu-

lated the Partial Eta Squared (η^2), with effect sizes classified as * ≥ 0.01 (small effect), ** ≥ 0.06 (moderate effect), and *** ≥ 0.14 (large effect). Post hoc pairwise comparisons with the Bonferroni correction were used to calculate mean differences and confidence intervals. Independent samples *t*-tests were used to compare sex differences between groups at both time points (initial and final tests), reporting mean differences, standard errors (SE), and *p*-values. Results were considered statistically significant at $p < 0.01$ **, $p < 0.05$ *.

3. Results

In Table 2, we present the primary descriptive statistics for the study sample. Table 3 presents the results of a one-way repeated-measures ANOVA, accompanied by pairwise comparisons, to evaluate performance changes between the initial and final assessments for both female and male groups, thereby highlighting their respective progress. In Table 4, we report the mean differences computed using an independent Student's *t*-test, enabling a comparative analysis between the female and male groups within the study.

In Table 2, the motor components assessment data demonstrate consistent improvements across all measured parameters following the intervention period. The aerobic capacity and metabolic data reveal substantial changes following intervention. The speed assessment data show consistent improvements in sprint performance for both sexes across all distances, with normal distributions maintained for parametric analysis (Shapiro–Wilk $p > 0.17$). Notable improvements were observed in short-distance sprints, with females reducing 10 m times by 5.7% (from 2.01 ± 0.07 to 1.90 ± 0.09 s) and males by 6.6% (from 1.70 ± 0.09 to 1.59 ± 0.13 s). For the 40 m sprint, females improved by 4.9% (from 6.01 ± 0.34 to 5.71 ± 0.32 s) and males by 6.8% (from 5.27 ± 0.25 to 4.91 ± 0.21 s). The 100 m sprint saw smaller gains, with females improving by 2.8% (from 15.50 ± 0.88 to 15.07 ± 0.73 s) and males by 3.5% (from 12.36 ± 0.43 to 11.92 ± 0.35 s). Improvements diminished with increased sprint distance, indicating adaptations were greater in acceleration and short bursts than in speed endurance. Males showed greater absolute improvements across all distances.

The explosive power variables showed normal distributions (Shapiro–Wilk tests: $p > 0.05$), validating parametric statistical methods. In the Countermovement Jump (CMJ), females improved from 28.86 ± 2.36 cm to 32.10 ± 2.66 cm (11.2% increase), while males increased from 41.72 ± 7.13 cm to 44.63 ± 6.41 cm (6.9% increase). For Squat Jump Height (SJ), females improved 12.6% (28.93 ± 2.42 cm to 32.56 ± 2.75 cm), and males improved 9.3% (38.55 ± 7.41 cm to 42.13 ± 6.38 cm). Both groups maintained normal distributions ($p > 0.07$).

In aerobic capacity, both groups exhibited significant improvements, with females' Maximal Oxygen Uptake ($\text{VO}_{2\text{max}}$) increasing by 80.1% and males by 136.4%. Absolute Oxygen Consumption (VO_2/L) increased by 23.3% in females and by 6.1% in males, with stable distribution characteristics. Both groups significantly reduced their Heart Rate Maximum (HRmax), indicating improved cardiovascular regulation, with normal distributions maintained.

The metabolic assessment indicated modest improvements in energy expenditure, with females increasing daily energy expenditure by 2.6% and males by 4.4%. Significant shifts in the Respiratory Quotient (RQ) toward greater fat oxidation were observed, indicating enhanced fat utilization and improved metabolic flexibility, with both sexes converging toward similar final RQ values. These results suggest enhanced metabolic efficiency, characterized by increased energy output and a shift toward fat-based fuel utilization patterns.

Table 2. Descriptive statistics for the parameters of the study components.

Components	Motor Test	Group	Time	Mean	SD	Variance	Shapiro–Wilk <i>p</i>
Speed	10 m	Female	It	2.008	0.066	0.004	0.287
			Ft	1.895	0.089	0.008	0.353
		Male	It	1.703	0.087	0.008	0.176
			Ft	1.591	0.130	0.017	0.170
	40 m	Female	It	6.010	0.340	0.116	0.218
			Ft	5.713	0.317	0.101	0.453
		Male	It	5.265	0.245	0.060	0.060
			Ft	4.910	0.213	0.045	0.342
	100 m	Female	It	15.500	0.879	0.773	0.433
			Ft	15.065	0.728	0.531	0.805
		Male	It	12.356	0.428	0.184	0.864
			Ft	11.919	0.352	0.124	0.982
Explosive power	CMJ (cm)	Female	It	28.862	2.359	5.565	0.326
			Ft	32.100	2.657	7.061	0.997
		Male	It	41.724	7.127	50.801	0.124
			Ft	44.626	6.408	41.068	0.201
	SJ (cm)	Female	It	28.925	2.415	5.837	0.142
			Ft	32.562	2.747	7.548	0.618
		Male	It	38.545	7.405	54.836	0.257
			Ft	42.125	6.382	40.733	0.170
Aerobic capacity	VO ₂ max	Female	It	34.256	4.907	24.081	0.404
			Ft	39.531	3.580	12.822	0.285
		Male	It	40.125	4.558	20.782	0.797
			Ft	45.587	5.520	30.479	0.393
	VO ₂ /L	Female	It	2.093	0.323	0.105	0.809
			Ft	2.581	0.398	0.159	0.152
		Male	It	3.562	0.495	0.245	0.468
			Ft	3.781	0.545	0.298	0.176
	HRmax	Female	It	186.312	9.890	97.829	0.612
			Ft	176.187	5.647	31.896	0.164
		Male	It	192.500	8.302	68.933	0.959
			Ft	182.625	8.578	73.583	0.709
Metabolic changes	Kcal/day	Female	It	1860.125	316.897	1004.117	0.905
			Ft	1908.250	326.482	1065.000	0.112
		Male	It	2530.625	398.028	1584.917	0.585
			Ft	2641.437	300.138	900.196	0.990
	RQ	Female	It	0.826	0.039	0.002	0.213
			Ft	0.732	0.036	0.001	0.313
		Male	It	0.822	0.086	0.007	0.281
			Ft	0.733	0.038	0.002	0.257

SD—Standard deviation, Shapiro–Wilk *p*—level of significance of normality test.

Table 3. ANOVA—one-way repeated measures and Pairwise Comparisons between initial and final test of female and male groups.

Component	Motor Test	Group	Within-Subjects Effects			Pairwise Comparisons			
			F	<i>p</i>	ηp^2	$\Delta X(Ft - It)$	<i>p</i> ^a	95% CI	
								Lower	Upper
Speed	10 m	Female	42.207	<0.001	0.738	−0.114 *	<0.001 **	−0.151	−0.076
		Male	29.405	<0.001	0.662	−0.112 *	<0.001 **	−0.157	−0.068
	40 m	Female	60.307	<0.001	0.801	−0.297 *	<0.001 **	−0.378	−0.215
		Male	46.447	<0.001	0.756	−0.355 *	<0.001 **	−0.466	−0.244
	100 m	Female	36.198	<0.001	0.707	−0.436 *	<0.001 **	−0.590	−0.281
		Male	110.188	<0.001	0.880	−0.437 *	<0.001 **	−0.526	−0.349
Explosive power	CMJ (cm)	Female	155.496	<0.001	0.912	3.237 *	<0.001 **	2.684	3.791
		Male	115.338	<0.001	0.885	2.902 *	<0.001 **	2.326	3.478
	SJ (cm)	Female	81.179	<0.001	0.844	3.637 *	<0.001 **	2.777	4.498
		Male	43.943	<0.001	0.746	3.581 *	<0.001 **	2.429	4.732
Aerobic capacity	VO ₂ max	Female	36.159	<0.001	0.707	5.275 *	<0.001 **	3.405	7.145
		Male	152.640	<0.001	0.990	5.463 *	<0.001 **	2.785	8.140
	VO ₂ /L	Female	53.634	<0.001	0.658	0.488 *	<0.001 **	0.294	0.681
		Male	23.003	<0.001	0.167	0.219	<0.001 **	−0.050	0.488
	HRmax	Female	25.906	<0.001	0.633	−10.125 *	<0.001 **	−14.365	−5.885
		Male	26.069	<0.001	0.635	−9.875 *	<0.001 **	−13.997	−5.753
Metabolic changes	Kcal/day	Female	1432.479	<0.001	0.990	48.125	<0.001 **	−221.177	317.427
		Male	1090.189	<0.001	0.986	110.813	<0.001 **	−61.381	283.006
	RQ	Female	9921.368	<0.001	0.998	−0.094 *	<0.001 **	−0.117	−0.071
		Male	2604.500	<0.001	0.994	−0.089 *	<0.001 **	−0.119	−0.059

It—initial test; Ft—final test, ΔX —difference of means, CI—confidence interval; *p*—statistical significance; ηp^2 —Partial Eta Squared. The mean difference is significant at the 0.05 level. a—Adjustment for multiple comparisons: Bonferroni; *p* < 0.01 **; *p* < 0.05 *.

The one-way repeated measures ANOVA revealed statistically significant improvements across all measured parameters (*p* < 0.001), with Partial Eta Squared ranging from small to very large, indicating substantial training-induced adaptations. Notably, there were sex-specific differences in adaptation patterns and the magnitudes of the effects (Table 3).

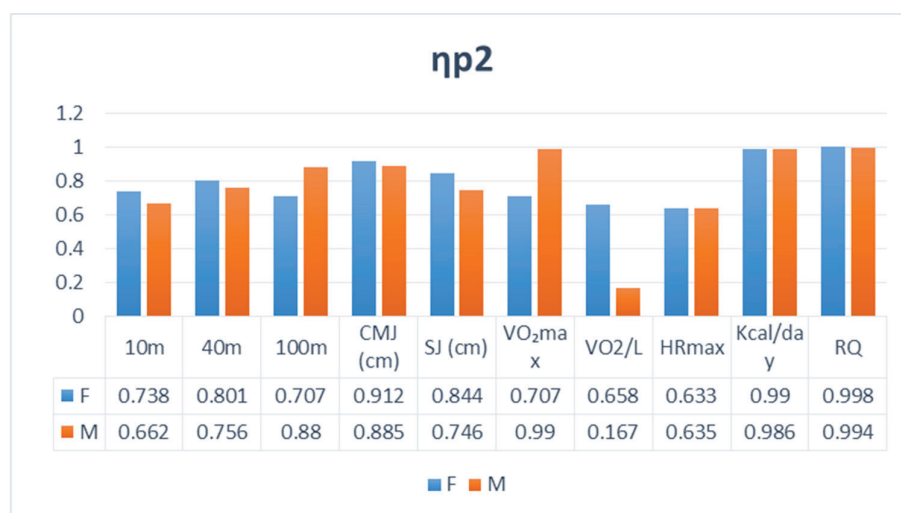
Speed performance across all distances showed significant improvements, with effect sizes ranging from large to very large. The 10 m sprint improved similarly for both sexes (females: −0.114 s, males: −0.112 s; $\eta p^2 = 0.738$ –0.662), while the 40 m sprint highlighted the largest sex differences (females: −0.297 s, males: −0.355 s; $\eta p^2 = 0.801$ –0.756). The 100 m sprint's improvements were nearly identical (females: −0.436 s, males: −0.437 s), with males showing the largest effect size ($\eta p^2 = 0.880$) (Table 3, Figure 1).

Explosive power metrics also improved significantly ($\eta p^2 = 0.707$ –0.924), with CMJ performance increasing in both groups (females: +3.237 cm, males: +2.902 cm; $\eta p^2 = 0.912$ –0.885) (Figure 1).

Table 4. Mean differences of the Independent Student *t*-test for the male and female groups of the study.

Component	Motor Test	Initial Test (It)			Final Test (Ft)		
		Mean Difference	SDE	<i>p</i>	Mean Difference	SD	<i>p</i>
Speed	10 m	−0.305	0.027	<0.001 **	−0.303	0.039	<0.001 **
	40 m	−0.744	0.104	<0.001 **	−0.802	0.095	<0.001 **
	100 m	−3.143	0.244	<0.001 **	−3.145	0.202	<0.001 **
Explosive power	CMJ (cm)	12.8	1.876	<0.001 **	12.526	1.734	<0.001 **
	SJ (cm)	9.620	1.947	<0.001 **	9.563	1.737	<0.001 **
Aerobic capacity	VO ₂ max	5.868	1.674	0.001 *	6.056	1.645	0.001 *
	VO ₂ /L	−1.468	0.147	<0.001 **	−1.200	0.168	<0.001 **
	HRmax	−6.187	3.228	0.061	−6.437	2.567	0.017 *
Metabolic changes	Kcal/day	670.500	127.193	<0.001 **	733.187	110.8	<0.001 **
	QR	−0.003	0.023	0.003 *	0.001	0.0137	0.926

It—initial test; Ft—final test, CI—confidence interval; *p*—statistical significance; *p* < 0.01 **; *p* < 0.05 *.

**Figure 1.** Partial Eta Squared (ηp^2) values.

In aerobic capacity, VO₂max showed significant differences (females: $F = 36.159$, males: $F = 152.640$; $\eta p^2 = 0.707$ – 0.990), with females improving in absolute VO₂ (+0.488 L/min, $\eta p^2 = 0.658$) while males showed non-significant changes (Graph 1). Maximum heart rate (HRmax) reductions were significant in both groups (females: -10.125 bpm, males: -9.875 bpm; $\eta p^2 = 0.633$ – 0.635).

Metabolic adaptations showed significant changes in caloric expenditure but questionable practical significance, while respiratory quotient improvements were highly significant ($\eta p^2 = 0.998$ – 0.994). The findings indicate meaningful training adaptations that support practical effectiveness in enhancing athletic performance. Females excelled in aerobic and jumping parameters, while males showed advantages in explosive power and sprinting, suggesting that sex-specific training strategies could improve outcomes.

Independent samples *t*-tests conducted between groups on the initial and final study tests revealed significant baseline performance differences by sex across most parameters, with different adaptation patterns observed after the intervention period.

Baseline sex differences in speed progress were highly significant across all sprint distances ($p < 0.001$). Males showed better initial performance advantages of 0.305 s (10 m), 0.744 s (40 m), and 3.144 s (100 m). After the intervention, these sex gaps remained almost the same (0.304 s, 0.803 s, and 3.146 s, respectively), indicating similar improvement patterns with males maintaining their performance advantage (Table 4).

Independent samples *t*-tests revealed significant baseline performance differences between sexes in initial and final tests, particularly in speed and explosive power. Males outperformed females in sprint distances (10 m: 0.305 s, 40 m: 0.744 s, 100 m: 3.144 s) with minimal change post-intervention. Males consistently held advantages in explosive power measures, such as CMJ (12.86 cm) and SJ (9.62 cm), ($p < 0.001$), highlighting superior training adaptation in males.

In aerobic capacity, initial VO_2 Max showed a male advantage of 5.86 mL/kg/min ($p = 0.001$), which persisted post-intervention (6.05 mL/kg/min, $p = 0.004$). Absolute VO_2 slightly decreased from 1.47 to 1.20 L/min, indicating stable oxygen consumption improvements. Cardiovascular adaptations varied, with non-significant baseline differences evolving into significant post-intervention gaps ($p = 0.017$). Males also exhibited substantial metabolic advantages both initially (670.5 kcal) and post-intervention (733.2 kcal), with a 9.4% increase in the sex gap.

The results indicated that males retain performance advantages in speed and explosive power while also displaying better aerobic adaptation. Females showed similar relative improvements across most parameters, indicating that they respond to training similarly, despite having lower absolute performance levels.

4. Discussions

4.1. Specific Discussion of the Study

The study aimed to investigate the impact of an integrated training program, conducted over 12 weeks, on the development of speed, strength, explosive power, aerobic capacity, and metabolic adaptations in senior rugby players, with a focus on sex. The obtained results highlight statistically significant improvements in both groups (female and male) for all evaluated parameters, but with notable differences in magnitude and specificity between sexes. The results of the study demonstrate that the integrated approach produces superior adaptations compared to unidimensional training methods. The simultaneous improvements in aerobic and anaerobic capacity, explosive strength, and agility suggest that synergistic interactions between different physiological systems can be optimized through careful programming. This finding is consistent with previous studies emphasizing the importance of competitive training in team sports, in which players must simultaneously demonstrate multiple physical qualities [34–37].

The superior progress recorded by the male group, compared to the female group, in strength and explosive power tests (CMJ—countermovement jump, SJ—squat jump, and SJ_Explosive power—power developed in jumping) is attributed to physiological differences such as higher testosterone levels, greater muscle mass, and more developed fast-twitch muscle fibers, as well as a more intense adaptive response of the body to strength training, giving males a consistent and reproducible advantage in developing explosive capacities. At the same time, the data reveals that both groups recorded consistent progress in speed over short distances (10 m and 40 m), but with maintenance of the initial performance difference, males maintaining an absolute advantage. Recent studies reveal that most of the time, through any applied methods, males have greater progress [38–42].

Regarding aerobic capacity, both females and males showed significant increases in $\text{VO}_{2\text{max}}$, but the adaptive response was more pronounced in males, who maintained a higher absolute performance level. The decrease in HRmax in both groups confirms

improved cardiovascular efficiency, as a more efficient cardiovascular system requires fewer beats per minute to sustain the same physical effort, reflecting a positive adaptation of the heart to training. However, sex differences became significant post-intervention, indicating a different physiological response to effort: males typically exhibit a greater capacity to reduce heart rate due to a larger stroke volume (the heart pumps more blood per beat), superior cardiac mass, and improved cardiorespiratory efficiency, while females tend to maintain slightly higher heart rates for the same level of effort, reflecting differences in heart size, blood volume pumped, and hormonal response to intense exercise [43–45].

At the metabolic level, both groups exhibited moderate increases in daily energy consumption and a significant reduction in RQ (Respiratory Quotient), indicating a shift toward more efficient use of lipids as an energy source. In this regard, metabolic adaptations manifested similarly between groups, indicating a convergence of physiological mechanisms for energy efficiency [46–48].

The results of this study contribute to a deeper understanding of how a structured, complex integrated training program differentially influences motor and physiological parameters by sex. The results align with previous studies that have shown that males tend to exhibit a consistent advantage in strength and speed parameters, while females demonstrate greater consistency in aerobic adaptations and cardiovascular regulation. Additionally, our data complements the specialized literature regarding how metabolic adaptations (increased energy consumption and efficiency of fat oxidation) occur regardless of sex, emphasizing the relevance of complex interventions in sports preparation [46–51].

Our study emphasizes that implementing a diverse training program that combines the development of speed, strength, explosive power, and aerobic capacity significantly enhances overall physical performance in rugby sevens. Prior research supports this integrated approach, demonstrating that rugby sevens simultaneously requires multiple energy systems and various physical qualities [52–55]. Additionally, sex differences in training adaptation highlight the need for personalized programs that leverage each group's potential and address specific characteristics of sports development and performance. Studies have documented notable sex differences in responses to strength and explosive power training, as well as in energy metabolism [56,57]. Hunter [58] also demonstrates that women have greater resistance to muscle fatigue but develop lower absolute strength than men, which requires differentiated training strategies. A number of studies have argued for sex-specific training programs in team sports, emphasizing that applying the same protocols to both sexes may be suboptimal [59,60]. Implementing such a program requires multidisciplinary skills and a deep understanding of the interactions among physiological systems, underscoring the importance of collaboration among sports science specialists. Bompa and Buzzichelli [61], advocate a holistic approach to training periodization that integrates knowledge from physiology, biomechanics, and sports nutrition. A number of studies have demonstrated that training programs that account for the complex interactions among physiological systems elicit superior adaptations compared to isolated approaches [62–65].

4.2. The Limits of Study, Practical Implications and the Future Direction of the Study

The research has several limitations, including a small sample size of 24 athletes, which affects the generalizability of the results to all rugby players. The 12-week intervention highlights short-term adaptations but does not address long-term sustainability. The study exclusively involved professional athletes, limiting its relevance to amateurs or those in training. Additionally, it did not monitor factors such as nutrition, sleep quality, or psychological stress, which could affect performance. Lastly, while sex differences were analyzed, the study did not fully consider the physiological adaptations of rugby players.

The practical implications of this study significantly enhance the sports training process in rugby sevens by establishing a comprehensive research model that integrates multiple performance dimensions and facilitates effective theory-practice transfer. Results demonstrate that integrated training programs produce substantial improvements across key performance indicators: speed, strength, explosive power, aerobic capacity, and metabolic efficiency. Sex-specific analysis reveals that personalized training approaches optimize physiological adaptations, with male athletes demonstrating superior gains in strength and explosive power due to hormonal and neuromuscular advantages, while female athletes exhibit greater consistency in aerobic adaptations, reflecting different cardiovascular response patterns. The implementation of advanced evaluation technologies—including cardiopulmonary exercise testing and indirect calorimetry—provides coaches with precise, objective data to individualize training loads, monitor real-time progress, and prevent overtraining, effectively combating performance plateaus and supporting sustainable long-term athletic development.

Future research should expand along several critical directions: longitudinal multi-season studies examining career longevity and injury prevention; integration of wearable technology, GPS tracking, and AI-powered algorithms for real-time monitoring and predictive modeling; biomechanical analysis using 3D motion capture to assess technical efficiency and injury risk patterns; nutritional interventions and recovery protocols to enhance training adaptations; psychological and cognitive performance assessment under fatigue conditions. These future directions would substantially expand the scientific foundation for evidence-based training in rugby sevens and contribute to advancing high-performance sport science methodologies.

5. Conclusions

The study confirms the effectiveness of an integrated training program in enhancing the performance of rugby sevens players, with significant improvements in motor, functional, and metabolic parameters. Both male and female athletes made progress; however, their adaptations varied. Male showed greater improvements in strength, explosive power, and speed, while women displayed more consistent enhancements in aerobic and cardiovascular fitness. The reduction in maximum heart rate in both groups indicates increased cardiovascular efficiency, and the decrease in respiratory quotient suggests a better ability to utilize lipids for energy, which is essential for endurance in intermittent games. These findings underscore the importance of training programs that account for individual and sex-specific traits to optimize performance. The study also highlights the benefits of advanced evaluation methods, such as indirect calorimetry and cardiopulmonary exercise testing (CPET), for objectively tracking physiological changes in athletes. Overall, it supports integrated training as an effective strategy for enhancing the performance of elite rugby players and preventing stagnation. Future research should investigate the application of integrated training across various age groups and levels in rugby, as well as other factors that impact training and performance.

Author Contributions: Conceptualization, S.M. and A.A.D.; methodology, S.M. and A.A.D.; validation, S.M. and A.A.D.; formal analysis, S.M., D.B. and A.A.D.; investigation, S.M. and A.A.D.; data curation, S.M., D.B. and A.A.D.; writing—original draft preparation, S.M., D.B. and A.A.D.; writing—review and editing, S.M., D.B. and A.A.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the National University of Physical Education and Sport in Bucharest, with no. 103/2023.09.26. The participants provided written informed consent to participate in this study.

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

Data Availability Statement: The original contributions are included in the article.

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

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Article

Acute Effects of Isometric Conditioning Activity with Different Rest Intervals Between Sets on Countermovement Jump Performance in Resistance-Trained Participants

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Abstract

This study investigated the acute effects of different rest intervals between sets of isometric conditioning activity (ICA) on countermovement jump (CMJ) performance. Fifteen resistance-trained males completed four conditions: three ICA protocols with 1, 2, or 3 min rest intervals between sets and a control condition (CTRL). ICA was performed in the half-squat position at a 90° knee angle against an immovable barbell, with each protocol consisting of three sets of short maximal voluntary isometric contractions (3 × 3 s per set). CMJ performance was assessed before and at 3, 6, 9, and 12 min after ICA or CTRL. The results showed no significant effects of the ICA or rest interval on CMJ height or relative peak power. However, independent of rest duration, ICA induced transient alterations in jump mechanics, characterized by decreased stiffness at 9 min and reduced eccentric peak velocity at 6–12 min post-ICA. Similar fluctuations were observed in the CTRL condition, suggesting that these changes may reflect time-dependent variability rather than specific potentiation effects. In conclusion, the studied ICA protocols did not enhance CMJ height or power output, and the length of the rest interval did not influence the outcomes, indicating limited applicability of this approach in resistance-trained males.

Keywords: post-activation performance enhancement; PAPE; rest interval; jump height; stiffness; eccentric velocity

1. Introduction

Post-activation performance enhancement (PAPE) refers to a short-term improvement in muscular performance in tasks such as sprinting and jumping following a specific conditioning activity (CA) [1]. While PAPE is often discussed alongside post-activation potentiation (PAP), the two phenomena differ in their underlying mechanisms and time course [2]. PAP has traditionally been linked to myosin light chain phosphorylation and assessed through single muscle twitch responses [2]. In contrast, PAPE likely involves additional physiological mechanisms, including increased motor unit recruitment, neural drive, and enhanced excitation-contraction coupling, which contribute to its prolonged effects beyond those of PAP alone [2,3]. In practical terms, a typical PAPE protocol involves performing a high-intensity resistance exercise (such as a heavy squat or loaded jump) followed by a short rest interval and then a biomechanically similar dynamic task, such as an unloaded jump or a sprint. This sequencing is designed to transiently enhance the

neuromuscular readiness for the subsequent explosive task, thereby reflecting the PAPE effect [4]. Furthermore, optimizing neuromuscular readiness through PAPE-inducing protocols may have implications not only for enhancing performance in high-velocity tasks but also for improving joint stability during rapid transitions between concentric and eccentric phases, such as those occurring in jumping and landing tasks [5]. Isometric conditioning activity (ICA) has garnered growing interest in both scientific literature and athletic practice, owing to its potential to elicit performance benefits without inducing substantial mechanical or metabolic fatigue [6,7]. This makes it particularly appealing in contexts where rapid recovery and minimal muscle damage are desired, such as pre-competition warm-ups or in-season training [8,9]. The elicitation and magnitude of the PAPE effect induced by ICA are influenced by various factors, including joint angle [10], contraction intensity [11], the total duration and number of ICA sets [12], contraction distribution [13], and the athletes' strength level and training experience [10,14,15]. Although various parameters have been explored, limited research has specifically examined the role of rest interval duration between ICA sets, which may be an important determinant of PAPE outcomes [12]. Investigating this variable is crucial because the balance between potentiation and fatigue is time-dependent: too short rest may not allow sufficient recovery and thus diminish performance, whereas excessively long intervals may attenuate the potentiation effect [16,17]. Determining the optimal rest duration could therefore enhance the practical application of ICA in both training and competition settings, ensuring that athletes maximize performance benefits while minimizing unnecessary fatigue.

Another important consideration is the duration of rest between completing CA and performing the subsequent explosive task, as research on traditional dynamic CA protocols generally recommends 3 to 8 min to optimize the potentiation response [9,18]. Together, these perspectives suggest that both intra-CA recovery (between sets) and post-CA recovery (between CA and the following task) should be systematically examined to establish evidence-based guidelines for the effective use of ICA in training and competition. It is important to consider that ICA may elicit different physiological responses compared to traditional dynamic CA, due to factors such as minimal muscle fiber disruption, lower metabolic cost, and reduced accumulation of peripheral fatigue [6,19]. These characteristics may influence the onset, magnitude, and duration of the PAPE response in ways distinct from those observed with dynamic resistance exercises [6]. These factors suggest that findings derived from research on dynamic CA may not be entirely generalizable to ICA [12]. Therefore, it is crucial not to rely solely on the existing literature regarding dynamic CA but to conduct direct comparisons of the inter-set rest durations specific to ICA. While rest intervals for dynamic CA have been investigated, particularly in the context of high-intensity, multi-joint movements, the appropriate recovery time between ICA sets remains less clearly defined and may be highly dependent on factors such as contraction intensity, volume, and an individual's neuromuscular profile [12,20]. For example, Jarosz et al. [12] reported improvements in CMJ performance following three isometric sets (9 s each, half-squat position) with significant effects observed at 3 and 6 min post-ICA. Similarly, Spieszny et al. [14] found enhanced CMJ height after three sets of maximal isometric squats, with improvements occurring at both 4 and 8 min post-ICA. In contrast, Tsoukos et al. [10] demonstrated performance enhancements after protocols involving three sets of 3 s maximal isometric efforts with shorter 1 min rest periods between sets. In addition to rest interval duration, their protocols differed not only in rest duration but also in contraction length and joint position (90° and 140° of knee flexion). On the other hand Jarosz et al. [13] and Krzysztofik et al. [21] found no performance improvements when using the same 1 min rest period and number of sets (i.e., 3 sets) as in the study by Tsoukos et al. [10], but with different contraction structures. Specifically, Jarosz et al. [13] applied sets totaling 9 s each but distributed as either 9×1 s, 3×3 s, or 1×9 s isometric

contractions, whereas Krzysztofik et al. [21] used 3×3 s maximal isometric half-squats. Jarosz et al. [13] employed the half-squat at approximately 120° of knee flexion, while Spieszny et al. [14] used 90° . These discrepancies in contraction duration, joint angle, and rest interval complicate direct comparisons and underscore the need to systematically examine how these factors interact to affect the PAPE response. Taken together, the inconsistencies highlight a clear methodological gap—there is a lack of studies that directly isolate the effect of rest interval duration between ICA sets while keeping other parameters (such as contraction time, joint position, and contraction distribution) constant [13].

Given conflicting reports on optimal rest, this study tested whether 1, 2, or 3 min rest intervals between ICA sets, with total contraction time held constant at 27 s across three sets, differentially affect subsequent CMJ performance in resistance-trained participants under the CTRL condition. It was hypothesized that protocols with longer inter-set rest intervals would be more likely to elicit improvements in CMJ performance, although these effects might emerge later during the recovery period compared to shorter rest conditions.

2. Materials and Methods

2.1. Experimental Approach to the Problem

The experiment employed a randomized crossover design, in which each participant completed four experimental sessions to evaluate the acute effects of maximal isometric squats (used as the ICA) on subsequent CMJ performance. Prior to testing, participants underwent a familiarization session followed by four experimental sessions. In each experimental session, participants performed one of three ICA protocols or a CTRL condition. The ICA protocols differed in the rest intervals between sets: 1 min (ISO-1), 2 min (ISO-2), and 3 min (ISO-3). CMJ performance was assessed approximately 3 min before the ICA and at 3, 6, 9, and 12 min after its completion [10,13]. In the CTRL condition, CMJ measurements were taken at the same time intervals but without performing the ICA (Figure 1). Consecutive sessions were spaced 3 to 7 days apart.

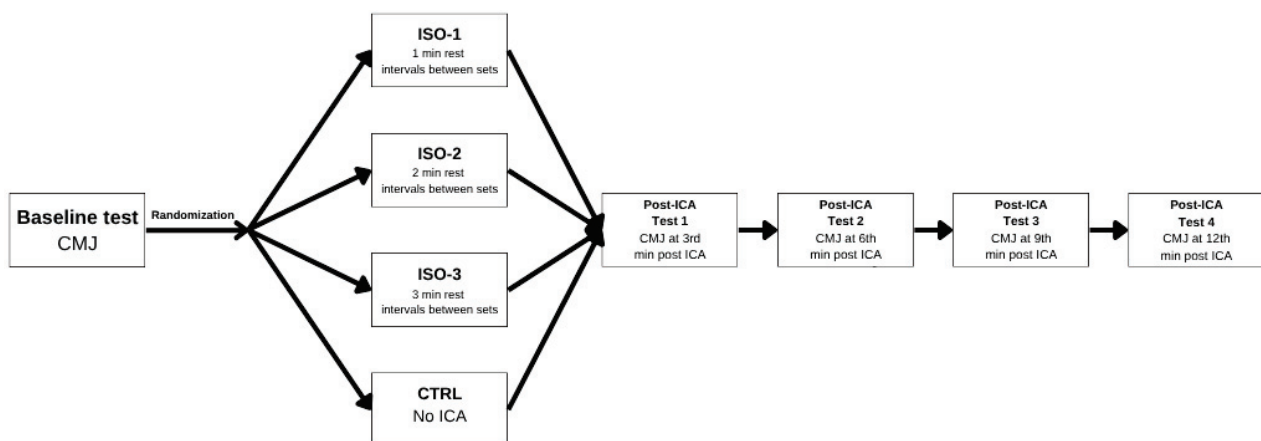


Figure 1. Study design flowchart. CMJ—countermovement jump; ICA—isoemetric conditioning activity; ISO-1—condition with 1 min rest intervals between sets; ISO-2—condition with 2 min rest intervals between sets; ISO-3—condition with 3 min rest intervals between sets; CTRL—control condition (without ICA).

2.2. Participants

The sample size estimation was performed using G*Power software (version 3.1.9.2, Düsseldorf, Germany). The calculation was based on the parameters for a “repeated measures ANOVA, within-subject factors” design, assuming one group of participants, four experimental conditions, and five measurement points. A statistical power of 0.8, a significance level of 0.05, and an anticipated medium effect size of approximately $d = 0.5$

were adopted, consistent with prior research investigating the acute effects of isometric activation exercises on jump performance [14,21]. The analysis indicated that a minimum of 10 participants was required.

The study involved 15 resistance-trained male participants classified based on training status and performance caliber (trained) according to McKay et al. [22] (Table 1). The inclusion criteria were as follows: (a) participation in resistance training at least three times per week for a minimum of two years; (b) no muscle injuries (resulting in an absence from training for more than four weeks) for at least six months prior to the start of the study. Participants were regularly engaged in traditional resistance training programs, including multi-joint free-weight exercises such as squats, deadlifts, and bench presses, performed at least three to four times per week. Participants were instructed to refrain from engaging in any resistance training for 48 h before the experimental session. Additionally, they were advised to maintain their usual dietary habits and avoid consuming any supplements or stimulants during the week preceding the experiment. At the beginning of the experimental session, body composition was assessed using multi-frequency bioelectrical impedance analysis conducted under laboratory conditions with the InBody 770 device (InBody, Seoul, Republic of Korea). Participants were informed about the potential risks and benefits associated with their involvement and were reminded of their right to withdraw from the study at any point without justification, prior to providing written informed consent. The experiment utilized a randomized crossover design. Although participants were aware of the immediate tasks being performed (e.g., isometric contractions or walking), they were blinded to the overall purpose and specific hypotheses of the study. Randomization was carried out using the randomization.org generator, which assigned each participant a unique number and session sequence. Written informed consent was obtained from all participants; however, detailed information concerning the study's aims and expected outcomes was intentionally withheld to minimize bias.

Table 1. Descriptive characteristics of the study participants.

Age [years]	20.5 ± 1
Body mass [kg]	79 ± 9
Resistance training experience [years]	3 ± 1
Body fat [%]	10.4 ± 3.6
Relative 1RM BS [kg/bm]	1.26 ± 0.23

1RM—one repetition maximum, BS—back squat, bm—body mass.

All experimental procedures were conducted at the Academy of Physical Education in Katowice, Poland. The study protocol was reviewed and approved by the Bioethics Committee for Scientific Research at the Academy of Physical Education in Katowice (approval no. 3/2021), adhering to the ethical principles of the 1983 Declaration of Helsinki.

2.3. Procedure

All testing sessions were performed at the same time of day (± 1 h) under identical laboratory conditions (temperature 21–22 °C, same equipment setup, and same supervising researchers) to ensure environmental and procedural consistency. Each session included an individualized, habitual warm-up routine for each participant. After completing the warm-up, participants performed a baseline CMJ assessment. Following a rest interval of approximately 3 min, they executed the isometric squats serving as the ICA according to the assigned condition, or no ICA in the CTRL trial, following a randomized order. During the ICA, an immovable barbell, securely fixed in place, was positioned across the participants' shoulders. The squat depth was standardized to a 90° knee flexion angle [21], as determined during a familiarization session conducted three days before the main

experimental trials. This measurement was performed by an experienced coach using a goniometer (EasyAngle, Meloq AB, Stockholm, Sweden). A qualified strength coach ensured consistent body positioning across all conditions, maintaining an upright trunk posture. At the verbal cue from the researcher, participants were instructed to “push the barbell vertically upward as hard and as fast as possible,” exerting maximal force by pressing their back against the barbell and driving their feet into the ground [12]. No restrictions were imposed on squat depth during the CMJ.

Participants completed, in randomized order, four conditions: CTRL (5 min of treadmill walking at $5 \text{ km} \cdot \text{h}^{-1}$ as an unrelated exercise condition to determine whether the conditioning activity was unique in its ability to elicit a performance response) and ISO-1/ISO-2/ISO-3 (each: three sets of ICA comprising $3 \times 3 \text{ s}$ maximal isometric contractions; 27 s total contraction time) with inter-set rest of 1, 2, or 3 min, respectively. The time required to complete each condition was monitored using a stopwatch.

2.4. Measurement of Countermovement Jump Performance

CMJ performance was assessed using a force platform (Force Decks, Vald Performance, Australia) operating at a sampling frequency of 1000 Hz [23], which has previously been recognized as a reliable instrument for assessing vertical jump kinematics [23]. Each participant performed three CMJs without arm swings, with 3 s rest intervals between attempts to ensure a stable standing position and correct posture before the next jump, while maintaining the continuity of a single testing series. During the measurement, participants began from a standing position with their hands on their hips, maintaining a straight back posture to minimize angular hip displacement. They were instructed to stand as still as possible for 1 s before initiating the countermovement. Then, they dropped into the countermovement position to a self-selected depth and were immediately followed by a maximal effort vertical jump. They were instructed to land in the same position as their take-off, centered on the force platform. All CMJ assessments were supervised by the same experienced evaluator (strength and conditioning coach with over five years of experience) to ensure consistent execution and cueing. The study's dependent variables included various parameters related to jump height. First, jump height (JH) was calculated based on the velocity of the center of mass at take-off, using the impulse-momentum equation [12]. Additional variables, including relative peak power output (PP), eccentric peak velocity (EPV), and stiffness (S), were considered as independent factors potentially influencing jump height.

3. Statistical Analyses

All statistical analyses were conducted using JASP software (version 0.18.3; JASP Team, University of Amsterdam, Amsterdam, The Netherlands) and are presented as mean values with corresponding standard deviations ($\pm \text{SD}$). Statistical significance was established at $p < 0.05$. Both absolute values and percentage changes were reported where relevant. The Shapiro–Wilk test was applied to assess the normality of data distribution, while Levene's and Mauchly's tests were used to evaluate homogeneity of variance and sphericity, respectively. A two-way repeated-measures ANOVAs (4 conditions: CTRL, ISO-1, ISO-2, ISO-3 $\times$ 5 time points: pre-ICA, 3, 6, 9, and 12 min post-ICA) were employed to examine the effects of ICA on CMJ performance variables. Effect sizes for main effects and interactions were calculated using partial eta squared (η^2), categorized as small (0.01–0.059), moderate (0.06–0.137), or large (>0.137). When significant main effects or interactions were identified, Bonferroni-adjusted (p_{bonf}) post hoc comparisons were performed. Additionally, Cohen's d was used to quantify effect sizes for pairwise differences, interpreted as “trivial” ($|d| < 0.20$), “small” ($0.20 \leq |d| < 0.50$), “moderate” ($0.50 \leq |d| < 0.80$), and “large” ($|d| \geq 0.80$) [24].

4. Results

4.1. Jump Height

Repeated measures ANOVA showed no significant main effect of time for absolute scores ($F = 0.923$, $p = 0.451$) or percent changes ($F = 0.281$, $p = 0.839$), and no significant time $\times$ group interactions for either measure (absolute: $F = 0.367$, $p = 0.974$; percent change: $F = 0.444$, $p = 0.910$). Between-condition effects were also non-significant for absolute scores ($F = 0.258$, $p = 0.855$) and percent changes ($F = 0.067$, $p = 0.977$). (Figure 2).

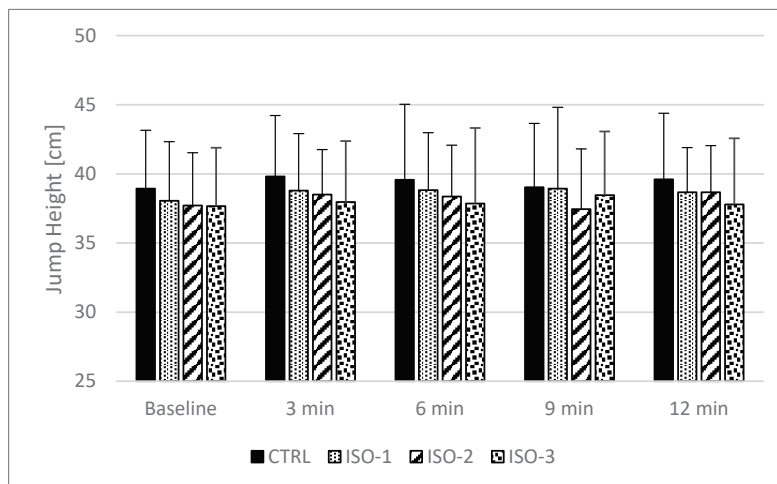


Figure 2. Change in jump height between conditions and time points. CTRL—control condition (without ICA); ISO-1—condition with 1 min rest intervals between ICA sets; ISO-2—condition with 2 min rest intervals between ICA sets; ISO-3—condition with 3 min rest intervals between ICA sets. BL—baseline, 3 min—3rd minute post-ICA, 6 min—6th minute post-ICA, 9 min—9th minute post-ICA, 12 min—12th minute post-ICA.

4.2. Relative Peak Power

A repeated measures two-way ANOVA did not show statistically significant interaction ($F = 0.769$; $p = 0.681$), main effect of time ($F = 0.587$; $p = 0.673$), or condition ($F = 0.177$; $p = 0.911$) for relative PP (Figure 3).

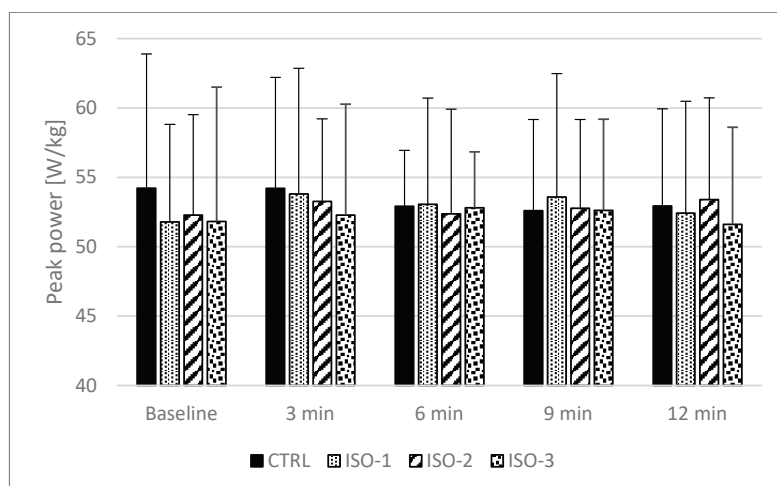


Figure 3. Change in relative peak power between conditions and time points. CTRL—control condition (without ICA); ISO-1—condition with 1 min rest intervals between ICA sets; ISO-2—condition with 2 min rest intervals between ICA sets; ISO-3—condition with 3 min rest intervals between ICA sets. BL—baseline, 3 min—3rd minute post-ICA, 6 min—6th minute post-ICA, 9 min—9th minute post-ICA, 12 min—12th minute post-ICA.

4.3. CMJ Stiffness and CMJ Eccentric Peak Velocity

A repeated measures two-way ANOVA revealed a statistically significant main effect of time on CMJ S ($F = 4.508$, $p = 0.003$, $\eta^2 = 0.231$) and CMJ EPV ($F = 8.542$, $p_{\text{bonf}} < 0.001$, $\eta^2 = 0.363$) (Table 2). However, there were no significant differences between conditions ($F = 0.013$, $p_{\text{bonf}} = 0.998$) or interactions ($F = 1.074$, $p_{\text{bonf}} = 0.385$) for CMJ S, nor significant differences between conditions ($F = 0.774$, $p_{\text{bonf}} = 0.532$) or interactions ($F = 0.770$, $p_{\text{bonf}} = 0.680$) for CMJ EPV. Post hoc comparisons for CMJ S showed a significant decrease at 9th min post-ICA (Mean difference [MD] = -1083 ± 40 [N/m]; Cohen's $d = 0.243$; $p_{\text{bonf}} < 0.001$) compared to pre-ICA. For CMJ EPV, significant decreases were observed at 6th min (MD = -0.22 ± 0.00 [m/s]; Cohen's $d = 0.225$; $p_{\text{bonf}} = 0.042$), 9th min (MD = -0.34 ± 0.04 [m/s]; Cohen's $d = 0.364$; $p_{\text{bonf}} < 0.001$), and 12th min (MD = -0.47 ± 0.23 [m/s]; Cohen's $d = 0.369$; $p_{\text{bonf}} < 0.001$) post-ICA compared to pre-ICA, and at 9th min (MD = -0.17 ± 0.17 [m/s]; Cohen's $d = 0.222$; $p_{\text{bonf}} = 0.047$) and 12th min (MD = -0.29 ± 0.10 [m/s]; Cohen's $d = 0.227$; $p_{\text{bonf}} = 0.039$) compared to 3rd min post-ICA.

Table 2. Changes in countermovement jump stiffness and eccentric peak velocity across conditions and time points.

	Condition	Baseline	3 min	6 min	9 min	12 min	Time	Condition	Time x Condition
Stiffness [N/m]	CTRL	5664 $\pm$ 1240	5368 $\pm$ 1160	5249 $\pm$ 870	5076 $\pm$ 1173 *	5283 $\pm$ 952	$p = 0.003$	$p = 0.998$	$p = 0.385$
	ISO-1	5413 $\pm$ 1112	5438 $\pm$ 1387	5397 $\pm$ 1307	5207 $\pm$ 1237 *	5256 $\pm$ 1275			
	ISO-2	5575 $\pm$ 899	5295 $\pm$ 998	5233 $\pm$ 907	5355 $\pm$ 985 *	5338 $\pm$ 912			
	ISO-3	5304 $\pm$ 953	5320 $\pm$ 770	5413 $\pm$ 970	5235 $\pm$ 849 *	5414 $\pm$ 1214			
Eccentric peak velocity [m/s]	CTRL	-1.27 $\pm$ 0.26	-1.31 $\pm$ 0.16	-1.32 $\pm$ 0.14 *	-1.37 $\pm$ 0.15 *	-1.39 $\pm$ 0.07 *	$p < 0.001$	$p = 0.532$	$p = 0.680$
	ISO-1	-1.21 $\pm$ 0.24	-1.28 $\pm$ 0.08	-1.28 $\pm$ 0.13 *	-1.29 $\pm$ 0.29 *	-1.37 $\pm$ 0.12 *			
	ISO-2	-1.27 $\pm$ 0.06	-1.28 $\pm$ 0.14	-1.32 $\pm$ 0.22 *	-1.29 $\pm$ 0.19 *	-1.26 $\pm$ 0.14 *			
	ISO-3	-1.22 $\pm$ 0.21	-1.27 $\pm$ 0.26	-1.27 $\pm$ 0.28 *	-1.36 $\pm$ 0.17 *	-1.40 $\pm$ 0.20 *			

CTRL—control condition (without ICA); ISO-1—condition with 1 min rest intervals between ICA sets; ISO-2—condition with 2 min rest intervals between ICA sets; ISO-3—condition with 3 min rest intervals between ICA sets. BL—baseline, 3 min—3rd minute post-ICA, 6 min—6th minute post-ICA, 9 min—9th minute post-ICA, 12 min—12th minute post-ICA, *—significant differences between pairwise comparisons, $p < 0.05$.

5. Discussion

The aim of this study was to compare the acute effects of ICA with different rest intervals between sets on subsequent CMJ performance in resistance-trained males. Contrary to the initial hypothesis, no significant improvements in JH or PP were observed in any condition, including ISO-1, ISO-2, ISO-3, and CTRL. Despite the lack of improvements, no decrease in JH or PP was found either, suggesting that neuromotor performance was preserved regardless of the rest duration between sets of ICA. Although at certain time points post-ICA a decrease in S (9th minute post-ICA) and an increase in EPV (6th, 9th, and 12th minutes post-ICA) were observed, similar changes also occurred in the CTRL condition. Therefore, these alterations cannot be attributed specifically to ICA and may reflect general fluctuations in movement mechanics over time rather than intervention-related effects. These findings suggest that 1–3 min rest intervals between ICA sets did not influence JH or PP, transient changes in S and EPV likely reflect compensatory neuromuscular adjustments unrelated to the ICA, possibly serving to maintain jump performance over time. To explore this aspect further, particular attention was given to transient modifications in jump mechanics, which may provide insight into how athletes regulate performance under varying neuromuscular states.

An interesting observation concerned transient alterations in jump mechanics: a decrease in stiffness at the 9th minute and an increase in eccentric phase velocity at the 6th, 9th, and 12th minutes post-ICA. However, similar fluctuations were also present in the control condition, indicating that these patterns more likely reflect time-dependent drift in move-

ment mechanics rather than a true potentiation effect of ICA. Evidence from isolated muscle preparations suggests that lengthening can produce residual force enhancement, whereas subsequent active shortening can induce force depression, effectively reducing force for a given activation [11]. If analogous processes occur *in vivo*, repeated maximal isometric efforts followed by stretch–shortening actions could transiently modulate muscle–tendon stiffness by altering elastic energy storage and release via titin- and cross-bridge-related mechanisms [25]. This aligns with reports that reduced dynamic stiffness can impair force transmission efficiency during jumping [26]. A faster EPV combined with lower S observed in the present study may therefore represent a compensatory strategy to preserve jump height under such constraints, potentially reflecting mild fatigue or altered neuromuscular drive after sustained isometric efforts [27]. It should also be acknowledged that CMJ depth was self-selected to maintain natural movement patterns, which may have allowed subtle adjustments in countermovement amplitude or velocity across trials. Consequently, part of the variability in EPV and S could stem from modifications in jump strategy rather than purely neuromuscular responses. From a physiological standpoint, the absence of a measurable PAPE response may reflect an equilibrium between modest potentiation and low-level fatigue. Although brief maximal isometric actions can transiently enhance calcium sensitivity and motor unit recruitment via myosin regulatory light chain phosphorylation [2,4], the relatively short contraction duration and absence of direct feedback may have limited the degree of motor unit synchronization and overall neural drive. Moreover, insufficient activation of high-threshold motor units and minimal metabolic perturbation could have reduced transient changes in muscle temperature and intramuscular water content, all of which can influence contractile efficiency and power output. Collectively, these physiological and neuromuscular factors likely minimized observable performance gains despite preserved motor function. However, beyond these short-term mechanical fluctuations, another crucial factor that may explain the absence of PAPE responses in our study relates to participant characteristics and training background.

In the present study, no significant differences were observed in CMJ height or power output following ICA, regardless of whether inter-set rest intervals lasted 1, 2, or 3 min. The absence of changes in jumping performance as a function of rest duration may suggest that recovery time between ICA sets is not a primary factor modulating the occurrence of the PAPE effect in resistance-trained participants. Instead, other characteristics of the ICA protocol and their interactions may play a more decisive role.

One potential explanation for the lack of significant effects is the absence of direct monitoring of participants' effort during ICA execution, for example, through maximal isometric force, which in previous studies has been assessed using force platforms [13]. Without an objective evaluation of the generated isometric force, it is plausible that participants did not perform contractions with maximal intent, despite verbal encouragement. As highlighted in previous work [28,29], exerting full effort during isometric exercise is crucial for recruiting high-threshold motor units and eliciting the intended adaptive response to the applied stimulus. Therefore, the use of a force platform would likely represent the most appropriate approach, as it provides a direct and reliable measure of isometric force. Consequently, irrespective of rest duration, the ICA protocol employed in this study may have been insufficient to induce a measurable PAPE effect in explosive lower-limb performance, primarily due to submaximal effort and limited neuromuscular activation. The absence of differences in ISO-1 is consistent with the findings of Jarosz et al. [13], who likewise reported no improvement in jumping performance when applying the same protocol in resistance-trained participants. Furthermore, the lack of significant differences in ISO-3 in the present study corroborates the comparative work of Jarosz et al. [30], which demonstrated that the ISO-3 protocol enhanced jumping performance in highly trained

participants but not in those with only resistance-training experience. This highlights the importance of training status as a key moderator of the PAPE effect. To the authors' knowledge, the ISO-2 protocol has not yet been examined in the literature, which limits the possibility of direct comparisons across studies. Taken together, these findings suggest that while training status moderates the overall effectiveness of different ISO protocols, more subtle, short-term alterations in movement mechanics may still emerge following ICA. Taken together, these findings point to the strong moderating role of training experience and specialization in shaping the PAPE response. A direct comparison with Tsoukos et al. [10] further illustrates how protocol-specific factors likely contributed to the divergent outcomes. In that study, national-level power athletes performed 3×3 s maximal isometric squats at both 90° and 140° knee angles, and a significant enhancement in CMJ height was observed only at the wider 140° position. The use of a shorter muscle length likely reduced fatigue while preserving potentiation mechanisms associated with regulatory light chain phosphorylation. By contrast, the present study maintained a fixed 90° angle and did not include real-time force monitoring, which may have increased the relative contribution of fatigue and reduced the mechanical stimulus necessary to trigger potentiation. Moreover, in a study by Tsoukos et al. [10], participants were national-level male track and field power athletes accustomed to high-intensity stretch–shortening actions, whereas the resistance-trained participants in the current study likely exhibited lower fatigue resistance. Together, these methodological and population-related factors provide a plausible explanation for the absence of a PAPE effect in the present experiment. A comparison with Spieszny et al. [14] further clarifies why our results diverged despite superficially similar ICA structures. In their study, semi-professional handball and soccer players performed $3 \times (3 \times 3$ s) maximal isometric back squats at $\sim 90^\circ$ with 3 min inter-set rest and showed small-to-moderate improvements in both CMJ and SJ at 4–8 min post-ICA. Three protocol elements likely favored potentiation there but not in our cohort: (i) training background: semi-professional, SSC-acclimated athletes vs. our resistance-trained generalists implies higher fatigue resistance and more efficient recruitment of high-threshold motor units; (ii) task similarity: their SJ depth was mechanically constrained to match the ICA angle, improving transfer specificity, whereas in our design only CMJ (self-selected depth) was analyzed; (iii) response sampling: they analyzed the best post-ICA trial (individual-optimal timing), whereas our inference rested on fixed time points and group means. Notably, total contraction time (27 s) and the existence of a 3 min rest condition were aligned across studies; thus, the population specialization and transfer specificity—rather than rest duration per se, are the most parsimonious explanations for the presence of PAPE in Spieszny et al. [14] and its absence here. Nevertheless, methodological aspects of the present study also warrant consideration, as they may have further constrained the observed outcomes.

Despite previous reports demonstrating PAPE following ICA, the present study did not reveal significant improvements in CMJ height or peak power output across any of the ICA conditions. For instance, Jarosz et al. [12] observed significant improvements in CMJ height and relative peak power at 3 and 6 min post-ICA in elite volleyball players, while Spieszny et al. [14] reported enhanced CMJ and SJ performance in semi-professional handball and soccer players. In contrast, our findings are consistent with Jarosz et al. [13], who also found no changes in CMJ following the same ICA protocol in resistance-trained participants, although their analysis of intra-set contraction distribution indicated that shorter, intermittent contractions—performed either as nine 1 s efforts (SUST-1) or three 3 s efforts (SUST-3), enabled the generation of higher Force200 values, which, however, did not translate into improvements in jump performance. Interestingly, Jarosz et al. [12] showed that elite volleyball players-athletes accustomed to frequent explosive stretch–shortening cycle actions demonstrated more favorable responses to ICA than strength-trained but non-

explosive populations, supporting the notion that training background is a decisive factor in whether potentiation outweighs fatigue [10,31]. In line with this, Spieszny et al. [14] reported improvements of 2.7–3.6% in CMJ and ~5% in SJ performance following multiple sets of short isometric squats in semi-professional handball and soccer players, whereas our resistance-trained but non-specialist participants likely lacked the neuromuscular adaptations needed to capitalize on potentiation. High-performance athletes also appear to possess superior fatigue resistance, enabling more effective buffering and clearance of fatigue-related metabolites, which may explain why Doma et al. [32] observed robust PAPE responses in anaerobically trained males, and Guerra et al. [33] in professional male soccer players, but not in cohorts comparable to ours. The findings of Jarosz & Szwarc [30] further substantiate this interpretation, showing that significant improvements in CMJ height occurred only in highly trained athletes, while moderately trained individuals exhibited no measurable benefits. In our study, the lack of improvement may therefore be linked not only to training status but also to participants' younger biological age, shorter training history, and limited experience with isometric contractions, all of which likely constrained their responsiveness to ICA. Our participants, who lacked regular exposure to explosive or isometric modalities and did not represent a defined sport discipline, were likely less efficient in recruiting high-threshold motor units, which may have limited their ability to generate sufficient potentiation to outweigh fatigue. Taken together, the absence of PAPE in this study may thus reflect both protocol-specific factors (e.g., contraction duration, total volume) and participant characteristics (e.g., limited isometric experience, absence of sport specialization, younger age, reduced fatigue resistance). Moreover, the lack of direct verification of force output during ICA further limits the ability to determine whether participants truly performed contractions with maximal intent. These findings reinforce the idea that ICA does not universally induce potentiation, but rather that its effectiveness is contingent upon both methodological design and the adaptive profile of the athlete.

Future research should aim to distinguish between these mechanisms by incorporating objective indicators of fatigue and neuromuscular function (e.g., tensiomyography or biochemical markers such as lactate, creatine kinase, and cortisol). Several methodological limitations should also be acknowledged. First, force output during ICA was not recorded, making it difficult to fully verify participants' effort and potentially attenuating the conditioning stimulus. Second, because participants were moderately trained males, the findings may not generalize to elite or female athletes, in whom PAPE responses might differ [4,9]. Third, only a single ICA configuration at a fixed knee angle (90°) was examined, limiting insight into how joint position and muscle length may influence the potentiation–fatigue balance. Finally, although participants were instructed to abstain from resistance training for 48 h before testing and to maintain similar pre-test routines, other potentially confounding factors, such as daily training load, sleep quality, and nutritional intake, were not directly monitored and could have introduced uncontrolled variability. Accordingly, future studies should employ objective measures of contraction intensity and fatigue and systematically explore the influence of ICA parameters (e.g., joint angle, contraction duration, and set structure) across populations differing in sex, strength, and training background to better clarify the practical relevance of ICA in sport and exercise settings.

6. Conclusions

This study showed that manipulating between-set rest (1, 2, or 3 min) during ICA with total contraction time matched at 27 s across three sets did not acutely enhance CMJ performance, nor jump height, nor peak power in resistance-trained males. Performance was maintained across all conditions, indicating preserved neuromotor function. Notably, a transient decrease in stiffness *S* accompanied by an increase in EPV suggested subtle

shifts in movement mechanics rather than ICA-specific performance effects. Practically, ICA under these parameters is unlikely to acutely improve CMJ in trained individuals, but it also does not impair it; rest intervals may instead modulate jump strategy without altering outcomes.

Author Contributions: Conceptualization, J.J. and A.L.; methodology, J.J. and A.L.; formal analysis, J.J. and A.L.; investigation, A.L., J.J. and P.M.; data curation, A.L.; writing—original draft preparation, A.L.; writing—review and editing, J.J. and M.K.; visualization, A.L.; supervision, M.K.; project administration, A.L.; funding acquisition, M.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee for Scientific Research at the Academy of Physical Education in Katowice (approval no. 3/2021).

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

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s.

Acknowledgments: During the preparation of this work, the authors used ChatGPT-4o Mini (OpenAI, model release September 2025) exclusively to improve the language and readability, with caution. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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

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Article

Effects of Eight-Week Single-Set Resistance Training on Muscle Health, Metabolic Profile and Oxidative Stress in Individuals with Cognitive Impairment

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Abstract

Resistance training (RT) has emerged as an effective strategy to counteract the deleterious effects of aging, improving metabolic health, and preserving functional capacity. However, the impact of low-volume RT on older adults, particularly those with cognitive impairment, remains underexplored. This study investigated the effects of an eight-week low-volume RT program on muscle health, liver function, lipid profile, glycemic control, and oxidative stress markers in individuals with cognitive decline. Twenty-eight participants were assigned to a low-volume RT group (81.0 ± 9.66) and a control group (90.0 ± 10.39 years). The low-volume RT group performed an 8-week RT program (two sessions per week) comprised of one set of 6–12 repetitions at 40–70% 1RM. The control group did not receive the intervention. Before and after the 8 weeks, the biomarkers of muscle health, metabolic profile, and oxidative stress were assessed. The results showed no significant differences between the groups in any biomarker at the baseline or post-test. The intervention group showed a significant increase in serum lactate dehydrogenase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities, all of which remained within normal ranges. No changes were observed in serum creatine kinase activity or the AST/ALT ratio in the intervention group, suggesting that acute-phase responses were occurring rather than tissue damage. Additionally, the intervention group showed a significant increase in high-density lipoprotein cholesterol levels, accompanied by a reduction in the atherogenic index, indicating potential cardiovascular benefits. No significant alterations were detected in the glycemic control and lipid peroxidation markers. These results suggest that low-volume RT can elicit modest, yet clinically meaningful metabolic improvements in individuals with cognitive impairment. Future studies should focus on identifying the minimal effective RT dose needed to optimize the health benefits in this vulnerable population, facilitating long-term adherence to exercise interventions.

Keywords: aging; cognitive impairment; glycemic control; lipid profile; liver function; muscle damage; oxidative stress; training volume; strength training

1. Introduction

Aging is a natural and ubiquitous process that leads to the progressive impairment of multiple functions. Imbalance in antioxidant defenses [1]; metabolic dysfunction, including insulin resistance and changes in lipid profile [2]; cognitive impairment and decline in physical function due to the loss of muscle mass and function (sarcopenia); reduced mobility; and an increased risk of frailty [3] are some of the changes that occur with aging [4].

According to the World Health Organization, population aging is a growing global trend, and by 2050, 22% of the world's population is expected to be over 60 years old [5]. As life expectancy increases, so does the prevalence of chronic diseases, with 80% of older people suffering from at least one disease, and 77% facing two or more diseases [5], putting a strain on healthcare systems and public finances.

Resistance training (RT) is an effective non-pharmacological intervention for delaying and reducing the detrimental effects of aging, thereby improving people's quality of life, while preserving physical and cognitive health [6–9]. RT can have a significant impact on maintaining or improving muscle strength, mobility, muscle recovery, and functional independence [7,10], which are crucial aspects for preserving autonomy and preventing frailty associated with aging [11]. In addition, RT has shown beneficial effects on lipid control [12], oxidative stress [13], and cognitive function [14]. However, in frail, older adults, especially those with cognitive impairment, higher-volume training may reduce adherence and increase fatigue or the risk of injury [15]. For this reason, low-volume protocols, such as single-set resistance training, have been proposed as a safe and more feasible alternative [16]. In individuals with type 2 diabetes, Wan and Su (2024) [17] reported pronounced reductions in glycated hemoglobin (HbA1c) levels following a training regimen consisting of three sets of resistance exercises, showing the clinical relevance of RT for metabolic health. Improvements in selective attention, working memory, and verbal fluency have also been reported [18]. These benefits are mediated by mechanisms such as neuroplasticity, cardiometabolic modulation, glycemic regulation, and reduced inflammation, factors that mitigate the impacts of brain aging [19,20]. In this sense, the beneficial effects of RT on cognitive and brain health have been proposed to be comparable to those of an aerobic training regimen, reinforcing its role not only in strengthening the body, but also in preserving brain health and general well-being [21,22].

Although the RT benefits have been described, there is a paucity of research regarding the impact of low-volume exercise protocols (e.g., a single set or one set per exercise), especially in individuals with cognitive impairment. Most RT programs focus on high-volume training, and the evidence on the effects of low-volume RT is limited, which makes it difficult to understand the physiological adaptations in older populations [23–25]. Several factors, such as physical limitations, fear of injury, and lack of motivation, often prevent older adults from participating in more demanding training programs [26]. Furthermore, the lack of time is also frequently reported as a barrier to engaging in physical exercise programs [16,27]. Considering the reported barriers to physical exercise engagement, a low-volume RT dose can be a practical alternative to overcome these issues, as it has been indicated to be an effective strategy to improve strength- and health-related components [28]. In this context, a low-volume RT protocol offers a time-efficient, low-burden, and highly feasible option for institutionalized individuals, particularly those with cognitive or

functional limitations, where adherence is a critical challenge [29]. Despite its practicality, the physiological effects of such RT approaches remain understudied in vulnerable, older populations. The evidence is scarce regarding the effectiveness of low-volume RT exercise on the biomarkers of muscle health, metabolic profile, and oxidative stress in cognitively impaired individuals.

Therefore, considering the existing research gaps, the current study aimed to investigate the impact of an 8-week low-volume RT regimen on several serum biochemical markers in individuals with cognitive impairment. Specifically, this study evaluated muscle damage upon exercise using markers such as creatine kinase (CK) and lactate dehydrogenase (LDH). Furthermore, we investigated the activity of liver transaminases to monitor liver function [30,31], a crucial aspect given the frailty of the target population of this study [32]. Finally, we investigated the impact of a low-volume RT regimen on their lipid profile (low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, total cholesterol, atherogenic index, and triglycerides), glycemic control parameters (glycated hemoglobin and estimated average glucose), and oxidative stress. We hypothesized that an 8-week low-volume RT intervention would lead to improvements in metabolic and oxidative stress biomarkers in the institutionalized individuals with cognitive impairment. Specifically, we expected increases in the amount of HDL cholesterol, reductions in the total and LDL cholesterol amounts, and a decrease in the atherogenic index, with no significant changes in the markers of liver function (AST and ALT), muscle damage (CK and LDH), and oxidative stress (MDA). If confirmed, our findings would support the safety and efficacy of low-volume RT in this vulnerable population.

2. Materials and Methods

2.1. Experimental Design

This research was part of the TRAIN4BRAIN project, which is registered on ClinicalTrials.gov under ID: NCT06185010 (registered in December 2023, version 2). This study followed an 8-week quasi-experimental design. The participants who could attend the RT sessions at the Sports Sciences Department's gym at the University of Beira Interior were assigned to the low-volume RT group (1SET). Those unable to attend the RT sessions were placed in the control group (CON). Due to institutional constraints and the participants' differing availabilities to travel to the university facilities, group allocation was based on practical feasibility rather than randomization. This approach, although potentially introducing selection bias, was necessary to ensure recruitment and compliance within the logistical limitations of this vulnerable population.

Before the intervention, all the participants completed the Dementia Rating Scale (DRS-2) and Short-Physical-Performance Battery (SPPB) test, and blood samples were collected. Following the baseline procedures, the 1SET group participated in an 8-week RT program with two sessions per week, while the CON group continued their usual daily activities without any RT. At the end of the 8 weeks, all the groups underwent the same blood sample collection. Due to the nature of this study, neither the participants nor the exercise coaches were blinded to the procedures conducted in the intervention. The study's procedures were approved by the Ethics Committee of the University of Beira Interior (approval number: CE-UBI-Pj-2022-065) and were conducted in accordance with the Declaration of Helsinki.

2.2. Subjects' Recruitment and Eligibility Criteria

Between October and November 2023, the participants were recruited from seven residential care facilities located in the Cova da Beira subregion of Portugal. The second author worked closely with the technical directors and the clinical teams of each institution

to identify potential participants. The eligibility requirements included men and women aged 50 or older living in the facility with mild, moderate, or severe cognitive impairment as assessed by the Portuguese version of the DRS-2 [33] and a score of 3 or higher on the SPPB [6]. Individuals were excluded if they had severe physical disabilities (such as being hospitalized, in a wheelchair, or bedridden), advanced dementia (indicating an inability to communicate with the research team and requiring constant care), sustained fractures within the last three months, or were diagnosed with a terminal illness. All the participants gave their informed consent to participate in the study. Figure 1 shows a CONSORT flow diagram. From the one hundred and forty-six institutionalized individuals screened for study eligibility, a total of twenty-eight participants (thirteen in the CON group and fifteen in the 1SET group) were included.

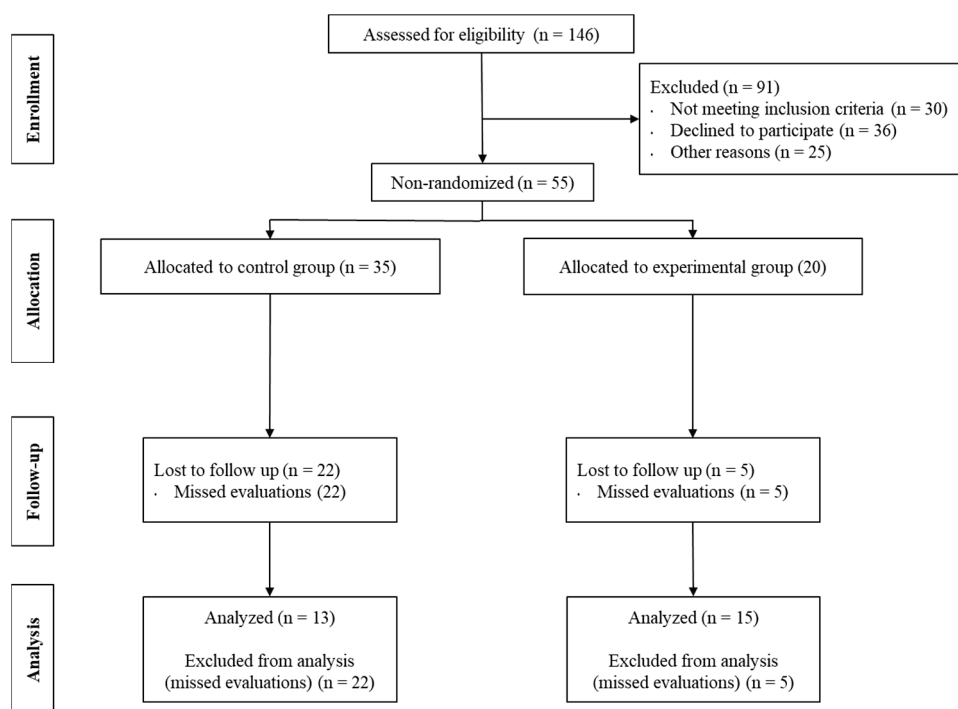


Figure 1. A CONSORT flow diagram of the crossover trial.

2.3. Resistance Training Program

Over an eight-week period, the participants completed two training sessions per week, separated by a 72 h rest period. Each session began with a 10 min warm-up walking on a treadmill at velocities ranging from 1 to 3 km/h. The participants then performed the following sequence of exercises: (1) a leg press, (2) a chest press, (3) chair squats, and (4) medicine ball throws. For the leg and chest press exercises, the participants performed a specific warm-up of one set of five repetitions at 80% of the training weight. The loads for the leg press gradually increased from 50% to 70% of one-repetition maximum (1RM), while for the chest press, the loads increased from 40% to 60% of 1RM during the intervention, as these intensities are associated with optimizing the ability to generate force rapidly in older adults [34,35]. The number of repetitions was 12 in weeks 1 and 2, 10 in weeks 3 and 4, 8 in weeks 5 and 6, and 6 in weeks 7 and 8. In total, 1SET completed 612 repetitions during the RT program. The participants were encouraged to complete all concentric repetitions as quickly as possible and to control the movement during the eccentric phase (2–3 s). The participants rested for 1 min between exercises. The sessions concluded with a 5 min cool-down period of walking at 40% of maximum walking speed, followed by stretching. Table 1 details the characteristics of the RT program. The sessions were supervised by the

second author with the assistance of two undergraduate Sports Sciences students. During all the sessions, the supervisors encouraged the participants to give their maximum effort and closely monitored each session, maintaining a ratio of 1 supervisor to each participant to ensure the correct performance of exercises and maintain safety.

Table 1. Resistance training program.

Exercises	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Leg press (R × %1RM)	12 × 50	12 × 50	10 × 60	10 × 60	8 × 65	8 × 65	6 × 70	6 × 70
Chest press (R × %1RM)	12 × 40	12 × 40	10 × 50	10 × 50	8 × 55	8 × 55	6 × 60	6 × 60
Chair squat (R)	12	12	10	10	8	8	6	6
Medicine ball throw (R × kg)	12 × 1	12 × 1	10 × 1	10 × 1	8 × 1	8 × 1	6 × 1	6 × 1

1RM, one-repetition maximum; R, repetition.

2.4. Blood Sample Collection and Processing

The participants fasted for at least 8 h prior to sample collection. Two tubes were drawn from each participant: one containing a clot activator for serum analysis and another containing an anticoagulant for whole blood analysis. The samples were then transported to the laboratory under refrigeration. The tubes for whole blood analysis were immediately stored at 4 °C until analysis. The tubes containing the clot activator were kept at room temperature for approximately 30 min to allow for clot retraction, and then centrifuged at 3500 rpm for 15 min at room temperature. The resulting supernatant (serum) was carefully collected and stored at 4 °C or −80 °C until analysis.

2.5. Blood Biochemical Analyses

The following blood biochemical parameters were determined: (1) muscle damage and liver function—CK, LDH, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) enzymatic activities; (2) lipid profile—total cholesterol, HDL cholesterol, LDL cholesterol, total cholesterol, atherogenic index, and triglycerides levels; (3) glycemic control—HbA1c levels and mean average glucose; and (4) lipid peroxidation as an indicator of oxidative stress—malondialdehyde (MDA) levels.

All biochemical parameters, except MDA and HbA1c levels, were measured using an Atellica® CH analyzer (Siemens Healthineers, Erlangen, Germany) following standardized procedures as described below. MDA levels were measured using a microplate fluorescence reader SpectraMax® Gemini™ EM Microplate Spectrofluorometer (Molecular Devices, San Jose, CA, USA). HbA1c levels were measured using an Horiba ABX system.

2.5.1. CK Activity Assay

The quantitative determination of serum CK activity was performed using the commercial Atellica® CH Creatine Kinase (CK_L) assay kit (Ref. 11097640, Siemens Healthineers, Erlangen, Germany) according to the manufacturer's instructions. Briefly, the serum samples were mixed with a reaction buffer containing creatine phosphate and adenosine diphosphate at 37 °C, resulting in the formation of adenosine triphosphate (ATP). Then, ATP was coupled to hexokinase/glucose-6-phosphate dehydrogenase, leading to the production of reduced nicotinamide adenine dinucleotide phosphate (NADPH). The concentration of NADPH was quantified by the increase in absorbance at 340/596 nm, which reflects CK enzyme activity.

2.5.2. LDH Activity Assay

The quantitative determination of serum LDH activity was performed using the commercial Atellica® CH Lactate Dehydrogenase L-P (LDLP) assay kit (Ref. 11097594,

Siemens Healthineers) according to the manufacturer's instructions. Briefly, the serum samples were mixed with a reaction buffer containing L-lactate and nicotinamide adenine dinucleotide (NAD) at 37 °C. In the presence of LDH and NAD, L-lactate is converted to pyruvate and NADH (reduced form of NAD). The amount of NADH produced, which is proportional to the enzymatic activity of LDH, was determined by measuring the increase in absorbance at 340/410 nm.

2.5.3. AST Activity Assay

Serum AST enzyme activity was measured using the commercial Atellica[®] CH Aspartate Aminotransferase (AST) assay kit (Ref. 11097607, Siemens Healthineers) following the manufacturer's instructions. Briefly, the serum samples were incubated at 37 °C with a reaction buffer containing L-aspartate and α -ketoglutarate. In the presence of AST, these substrates are converted to L-glutamate and oxaloacetate. In a coupled reaction catalyzed by malate dehydrogenase, oxaloacetate is reduced to malate with the simultaneous oxidation of NADH to NAD⁺. The decrease in NADH concentration was measured by a reduction in absorbance at 340/410 nm, being proportional to the activity of AST in the samples.

2.5.4. ALT Activity Assay

Serum ALT enzyme activity was measured using the commercial Atellica[®] CH Alanine Aminotransferase (ALT) assay kit (Ref. 11097605, Siemens Healthineers) following the manufacturer's instructions. Briefly, the serum samples were incubated at 37 °C with a reaction buffer containing L-Alanine and α -ketoglutarate. ALT catalyzes the formation of L-glutamate and pyruvate, which is subsequently converted to lactate in a coupled reaction catalyzed by lactate dehydrogenase, accompanied by the oxidation of NADH to NAD⁺. The rate of absorbance decreases at 340/410 nm, reflecting the reduction in NADH concentration, which is proportional to ALT enzymatic activity in the samples.

2.5.5. Total Cholesterol

Serum total cholesterol was quantified using the commercial Atellica[®] CH Cholesterol_2 (Chol_2) assay kit (Ref. 11097609, Siemens Healthineers) following the manufacturer's instructions. Briefly, the serum samples were incubated at 37 °C with a reaction buffer in which cholesterol esters were hydrolyzed by cholesterol esterase to release fatty acids and cholesterol. In the presence of oxygen, cholesterol oxidase catalyzes the conversion of cholesterol to cholest-4-en-3-one, producing hydrogen peroxide. Under the catalytic influence of peroxidase, phenol, and 4-aminoantipyrine, a colored complex is formed. The absorbance of this complex at 505/694 nm was measured, which is proportional to the cholesterol concentration in the samples.

2.5.6. HDL Cholesterol

Serum HDL cholesterol was quantified using the commercial Atellica[®] CH HDL Cholesterol (HDLc) assay kit (Ref. 11537213, Siemens Healthineers), according to the manufacturer's instructions. This assay employs a two-reagent format and relies on the Accelerator Selective Detergent methodology, which is based on accelerating the reaction of cholesterol oxidase with non-HDL unesterified cholesterol and selectively dissolving HDL using a specific detergent at 37 °C. A colorimetric reaction was subsequently triggered, and color development was measured at 545/694 nm using an endpoint colorimetric technique. The color produced is directly proportional to the amount of HDL cholesterol present in the sample.

2.5.7. Triglycerides

Serum triglycerides were quantified using the commercial Atellica[®] CH Triglycerides_2 (Trig_2) assay kit (Ref. 11537222, Siemens Healthineers) following the manufacturer's instructions. Briefly, the serum samples were mixed with a reaction buffer at 37 °C, which initiates sequential enzymatic reactions catalyzed by lipoprotein lipase, glycerol kinase, glycerol-3-phosphate oxidase, and peroxidase. The formation of the end-product quinoneimine is directly proportional to the amount of triglycerides in the sample and was measured at 505/694 nm using an endpoint colorimetric technique.

2.5.8. LDL Cholesterol

Serum LDL cholesterol was calculated from the quantified serum concentrations of total cholesterol, HDL cholesterol, and triglycerides using the Friedewald equation [36]:

$$\text{LDL-cholesterol} = \text{total cholesterol} - \text{HDL-cholesterol} - (\text{triglycerides}/5) \quad (1)$$

2.5.9. Atherogenic Index

The atherogenic index was calculated using the measured the serum levels of total cholesterol and HDL cholesterol [37] according to the following equation:

$$\text{Atherogenic index} = \text{total cholesterol}/\text{HDL-cholesterol} \quad (2)$$

2.5.10. Glycated Hemoglobin

HbA1c concentration was determined in the whole blood samples using a commercially available HbA1c reagent set (Ref. H7546, Horiba Medical, Montpellier, France) and high-performance liquid chromatography with the Horiba ABX system. All the procedures were performed in accordance with the manufacturer's instructions. The method is based on an antigen–antibody interaction to directly quantify HbA1c in whole blood. Briefly, total hemoglobin and HbA1c were adsorbed onto latex particles, to which an anti-human HbA1c monoclonal antibody was added, forming a complex with latex-HbA1c. A polyclonal IgG antibody was then able to bind to the monoclonal antibody, inducing agglutination. The extent of agglutination, which is proportional to the amount of HbA1c adsorbed onto the surface of latex particles, was measured by absorbance at 660 nm. HbA1c concentration was then determined using a calibration curve.

2.5.11. Estimated Average Glucose

Estimated average glucose was calculated from the measured HbA1c concentrations as described above using the formula established by the A1c-Derived Average Glucose (ADAG) study [38]:

$$\text{Estimated average glucose (mg/dL)} = 28.7 \times \text{HbA1c (\%)} - 46.7 \quad (3)$$

2.5.12. Malondialdehyde

Serum MDA, a by-product of lipid peroxidation, was quantified using the commercial TBARS (TCA Method) assay kit (Ref. 700870, Cayman Chemical, Ann Arbor, MI, USA) following the manufacturer's instructions. Briefly, the samples were mixed with a reaction buffer containing thiobarbituric acid (TBA) and trichloroacetic acid (TCA) and incubated at 90–100 °C for 1 h to allow for the formation of MDA-TBA adducts. After a 10 min cooling period to stop the reaction, the mixture was centrifuged at 1600× *g* for 10 min at 4 °C. The resulting supernatant was carefully transferred to an opaque multiwell plate, and fluorescence was measured at an excitation wavelength of 530 nm and an emission wavelength of 550 nm. MDA concentrations were calculated based on a standard curve.

2.6. Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics Version 29.0.1.0. We assessed data normality using the Shapiro–Wilk test. We performed a Mann–Whitney test to assess the baseline differences between the groups in age, height, and body mass. For the normally distributed variables, we conducted ANCOVA with age as a covariate to analyze the differences between the groups at pre- and post-intervention in terms of the biomarkers of muscle health, metabolic profile, and oxidative stress. We selected age as a covariate to adjust for its potential confounding effect. The non-parametric equivalent test (Quade ANCOVA) was used for the data that were not normally distributed. We used a paired-sample t-test (for normally distributed variables) and the Wilcoxon test (for non-normally distributed variables) to analyze the differences within the groups over time in terms of the biomarkers of muscle health, metabolic profile, and oxidative stress. We report the results as the mean $\pm$ standard deviation (SD) for the normally distributed data and the median with interquartile range (Q1 and Q3) for the non-normally distributed data. We calculated the Hedge’s g effect size for the normally distributed variables to assess the magnitude of the differences, and it was interpreted as small ($g < 0.30$), medium ($g: 0.30–0.69$), or large ($g: \geq 0.70$). We conducted post-hoc power analysis using G*Power software (v3.1.9.2, Dusseldorf, Germany) for the normally distributed variables using the statistical test “ANCOVA: Fixed effects, main effects and interactions”. Power analysis included the effect size (f), an alpha level of 0.05, a sample size of 28, a numerator of 1, two groups, and one covariate. The graphs were created using GraphPad Prism version 8.0.1.

3. Results

This study included twenty-eight institutionalized individuals aged between 57 and 95 years. Of these, thirteen participants were allocated to CON, and fifteen to 1SET. The participants in the 1SET group completed the intervention with an attendance rate of 88%. No adverse events (e.g., injuries, nausea, and dizziness) were reported during the intervention. Table 2 presents the demographic and anthropometric characteristics of the participants in both the CON and 1SET groups. The data include age, sex distribution, height, body mass, and the level of cognitive impairment. No statistically significant differences were observed between the CON and 1SET groups for age ($p = 0.061$), height ($p = 0.286$), or body mass ($p = 0.901$).

Table 2. Participants’ demographic and anthropometric characteristics.

		CON ($n = 13$)	1SET ($n = 15$)
Age (years)	Mean $\pm$ SD	90.00 $\pm$ 10.39	81.00 $\pm$ 9.66
	Min–max	57–95	58–93
Sex (n)	Female	9	6
	Male	4	9
Height (m)		1.58 $\pm$ 0.08	1.53 $\pm$ 0.09
Body mass (kg)		66.40 $\pm$ 11.21	67.90 $\pm$ 10.74
Cognitive Impairment (DRS-2, n)	Mild	2	4
	Moderate	1	4
	Severe	10	7

CON: control group; 1SET: low-volume RT group.

3.1. Effect of 8-Week Low-Volume RT Intervention on Muscle Health and Liver Function

No significant differences were observed between the groups in CK and LDH activities at the baseline (CK: $p = 0.682$; LDH: $p = 0.548$) or post-test (CK: $p = 0.782$; LDH: $p = 0.776$).

Figure 2 shows no significant changes over time in CK activity in either group. In contrast, SET1 showed a moderate increase in LDH activity, from 194.07 ± 34.68 U/L to 219.87 ± 46.00 U/L, although the values remained within the normal reference range (120–246 U/L). No significant changes were observed in the CON group (from 204.0 (177.0, 235.0) U/L to 198.0 (183.5, 252.5) U/L) in LDH.

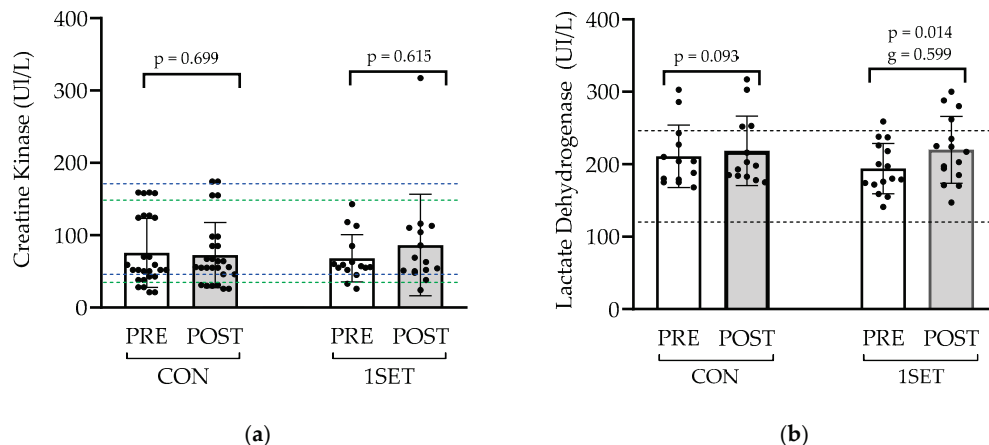


Figure 2. Dot plots showing effects of intervention on creatine kinase (a) and lactate dehydrogenase (b). Significance values are reported for within-group comparisons. Effect sizes (g) only apply to normally distributed variables. Dashed lines represent reference ranges. Blue and green dashed lines indicate creatine kinase reference values for men (46–171 U/L) and women (34–145 U/L), respectively; black dashed line indicates reference range for lactate dehydrogenase (120–146 U/L). Clinically meaningful thresholds are also indicated where applicable.

No significant differences were observed between the groups in AST and ALT activity at the baseline (AST: $p = 0.728$, $g = 0.287$; ALT: $p = 0.692$, $g = 0.187$) or post-test (AST: $p = 0.363$, $g = 0.441$; ALT: $p = 0.975$, $g = 0.044$). Figure 3 shows a significant increase in AST activity in the 1SET group, from 20.73 ± 4.23 U/L to 24.80 ± 4.71 U/L, and in ALT activity, from 16.27 ± 4.43 U/L to 19.27 ± 6.32 U/L. These changes occurred within the clinical reference range. No significant changes were observed in the CON group for either enzyme (AST: from 22.08 ± 4.89 U/L to 22.38 ± 5.94 U/L; ALT: from 17.46 ± 7.80 U/L to 18.92 ± 8.75 U/L). There were no significant changes in the AST/ALT ratio in either group. In the 1SET group, the ratio increased from 1.29 (1.12, 1.43) to 1.33 (1.22, 1.50), while in the control group, it changed from 1.25 (1.10, 1.74) to 1.29 (1.03, 1.40). All the values remained above one and within normal physiological limits, with no significant between-group differences (baseline: $p = 0.803$; post-test: $p = 0.308$). The post-hoc power analysis of AST (effect size $f = 0.225$) and ALT (effect size $f = 0.022$) revealed statistical powers of 0.21 and 0.05, respectively.

3.2. Effect of 8-Week Low-Volume RT Intervention on Lipid Profile and Glycemic Control

No significant differences were observed between the groups for total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, or atherogenic index at the baseline (total cholesterol: $p = 0.221$, $g = 0.576$; HDL: $p = 0.596$; LDL: $p = 0.195$, $g = 0.603$; triglycerides: $p = 0.994$; atherogenic index: $p = 0.258$, $g = 0.578$) or post-test (total cholesterol: $p = 0.077$, $g = 0.789$; HDL: $p = 0.845$; LDL: $p = 0.120$, $g = 0.690$; triglycerides: $p = 0.282$; atherogenic index: $p = 0.126$, $g = 0.698$). Figure 4 shows a significant and moderate increase in the HDL cholesterol levels in the 1SET group, from 43.0 (35.0, 46.0) mg/dL to 45.0 (42.0, 52.0) mg/dL, reaching values within the normal reference range. Additionally, a significant and moderate reduction in the atherogenic index was observed in the 1SET group (from 3.44 ± 0.81 to 3.05 ± 0.59), as well as a significant and small reduction in the CON group (from

4.02 ± 1.13 to 3.69 ± 1.16). No significant changes were detected in the total cholesterol, LDL cholesterol, and triglyceride levels in either group after the intervention. The post-hoc power analysis of total cholesterol (effect size $f = 0.395$), the atherogenic index (effect size $f = 0.349$), and LDL cholesterol (effect size $f = 0.345$) revealed statistical powers of 0.52, 0.43, and 0.42, respectively.

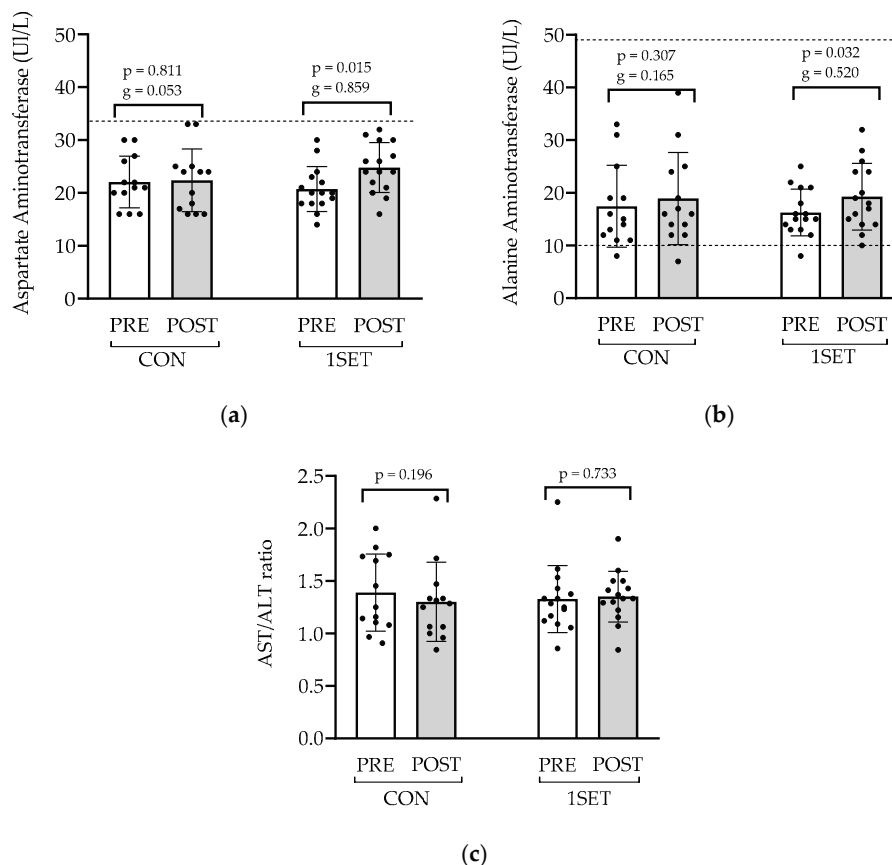


Figure 3. Dot plots showing effects of intervention on aspartate aminotransferase (AST; (a)), alanine aminotransferase (ALT; (b)), and AST/ALT ratio (c). Significance values are reported for within-group comparisons. Effect sizes (g) only apply to normally distributed variables. Dashed lines represent reference ranges. Black dashed lines indicate clinical reference ranges for AST (<34 U/L) and ALT (10–49 U/L) for both men and women. Clinically meaningful thresholds are also indicated where applicable.

No significant differences were observed between the groups in HbA1c or the estimated average glucose levels at the baseline (HbA1c: $p = 0.732$, $g = 0.065$; estimated average glucose: $p = 0.742$, $g = 0.059$) or post-test (HbA1c: $p = 0.508$, $g = 0.283$; estimated average glucose: $p = 0.499$, $g = 0.287$). Figure 5 shows that the intervention did not result in significant changes in HbA1c and the estimated glucose levels in the 1SET (HbA1c: from 6.08 ± 0.39% to 6.14 ± 0.45%; estimated average glucose: from 127.73 ± 11.23 mg/dL to 129.60 ± 12.99 mg/dL) and CON groups (HbA1c: from 6.05 ± 0.62% to 5.99 ± 0.56%; estimated average glucose: from 126.85 ± 17.66 mg/dL to 125.31 ± 16.12 mg/dL). The HbA1c values were within the normal reference range (<6.5%) in both the groups. The estimated average glucose levels, derived from HbA1c, reflected the same pattern. The post-hoc power analysis of HbA1c (effect size $f = 0.141$) and estimated average glucose (effect size $f = 0.143$) revealed a statistical power of 0.11 for both the outcome measures.

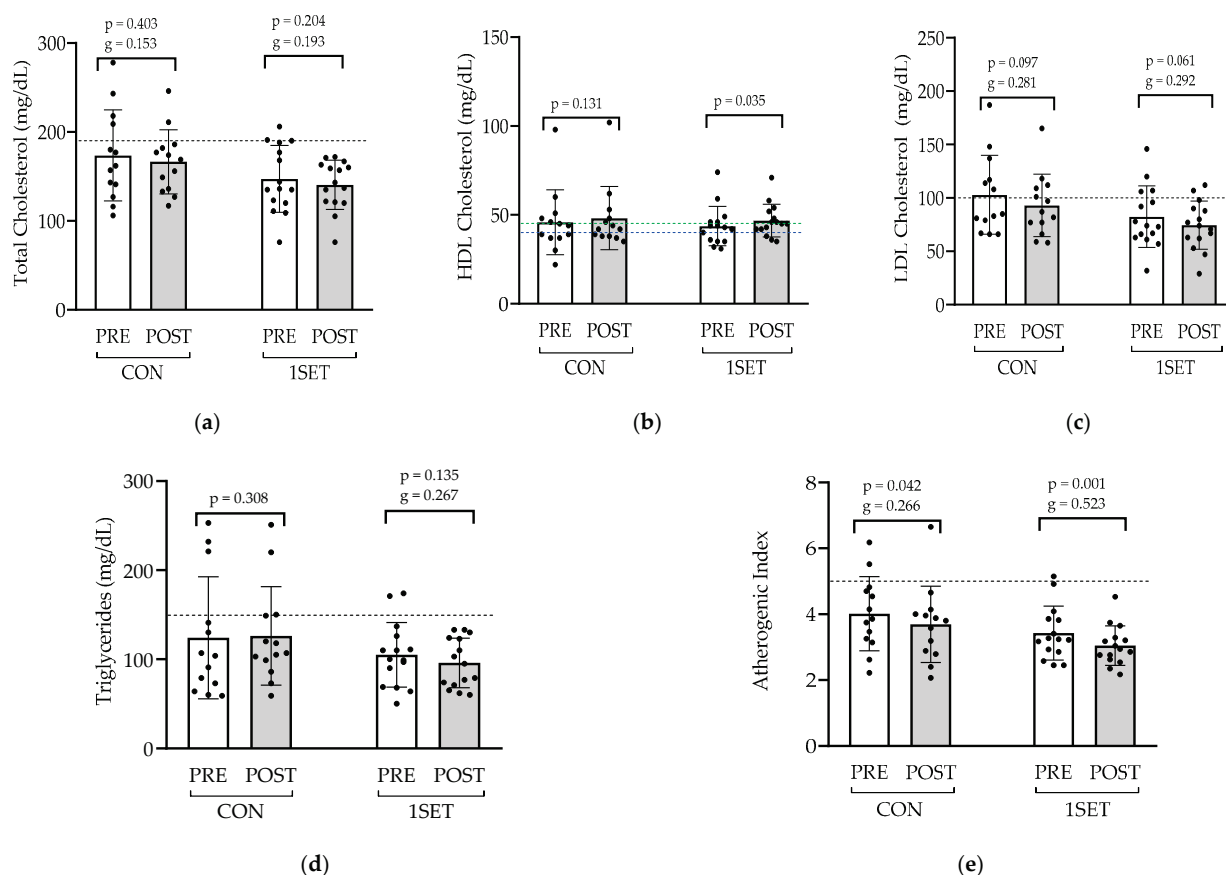


Figure 4. Dot plots showing effects of intervention on total cholesterol (a), HDL cholesterol (b), LDL cholesterol (c), triglycerides (d), and atherogenic index (e). Significance values are reported for within-group comparisons. Effect sizes (g) only apply to normally distributed variables. Dashed lines represent reference ranges. Blue and green dashed lines indicate HDL cholesterol reference values for men (>40 mg/dL) and women (>45 mg/dL), respectively; black dashed lines indicate reference values for total cholesterol (<190 mg/dL), LDL cholesterol (<100 mg/dL), triglycerides (<150 mg/dL), and atherogenic index (<5.00) for both sexes. Clinically meaningful thresholds are also indicated where applicable.

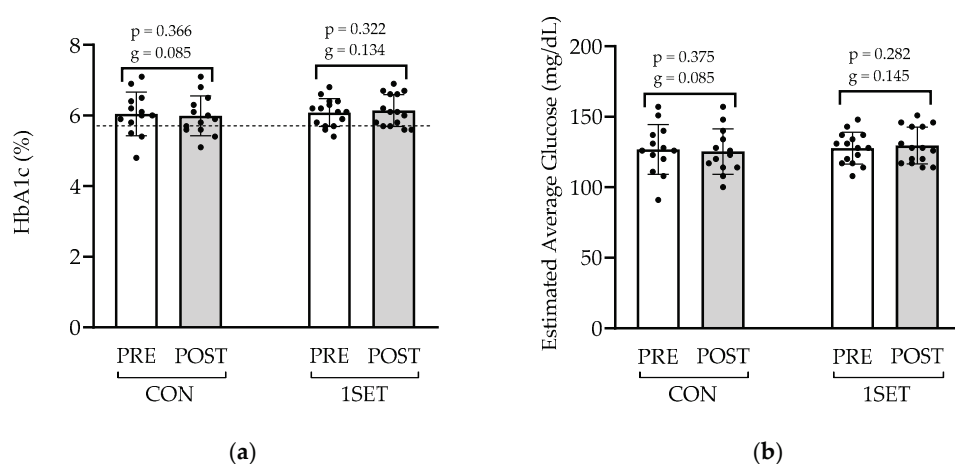


Figure 5. Dot plots showing effects of intervention on (a) glycated hemoglobin (HbA1c) and (b) estimated average glucose. Significance values are reported for within-group comparisons. Dashed lines indicate clinical reference values for both sexes for HbA1c (<5.7%). Estimated average glucose is shown solely as value derived from HbA1c, which is parameter with direct clinical relevance. Clinically meaningful thresholds are also indicated where applicable. Corresponding *p*-values and effect sizes (g) are shown above bars.

3.3. Effect of 8-Week Low-Volume RT Intervention on Oxidative Stress

No significant differences were observed between the groups in the MDA levels at the baseline ($p = 0.845$) or post-test ($p = 0.681$). Figure 6 shows no significant changes in the MDA levels after the intervention in the 1SET (from $2.20 \pm 1.18 \mu\text{M}$ to $1.86 \pm 0.54 \mu\text{M}$) and CON (from $2.43 (1.03, 3.07) \mu\text{M}$ to $2.08 (1.06, 2.54) \mu\text{M}$) groups. The mean baseline MDA levels were within the normal reference range for human plasma (from 0.26 to $3.94 \mu\text{M}$).

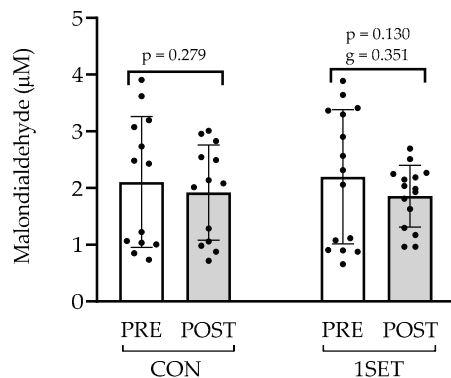


Figure 6. Dot plots showing effects of intervention on serum malondialdehyde (MDA) levels. Significance values are reported for within-group comparisons. Effect sizes (g) only apply to normally distributed variables.

4. Discussion

RT is increasingly recognized as an effective non-pharmacological approach that promotes several health benefits and may contribute to improving people's quality of life, especially in older and more vulnerable populations [7]. The present study aimed to investigate the safety and potential metabolic and antioxidant benefits of a single-set low-volume RT exercise in institutionalized individuals with cognitive impairment. Our results demonstrated that a low-volume RT protocol applied twice a week for 8 weeks was sufficient to elicit significant improvements in specific biochemical health indicators without causing liver dysfunction or muscle damage.

4.1. Muscle Damage and Liver Function Markers

One of the primary concerns regarding the RT protocol used in the present study was ensuring its safety for muscle health and liver function, particularly given the highly vulnerable and functionally limited population enrolled in this study. Although we observed increases in the activities of LDH, AST, and ALT, all the values remained within the normal range. These enzymatic changes likely reflect acute, exercise-induced responses rather than tissue injury or liver dysfunction. The transient elevations in the liver function markers suggest acute physiological adaptations to exercise rather than pathological processes, as further supported by the stable levels of CK activity, a more specific marker of muscle damage [39,40]. Other studies in the literature have shown that exercise may induce transient elevations in liver transaminase levels [41–43]. Therefore, we evaluated the impact of low-volume RT intervention on liver function parameters, including AST and ALT enzyme activities, as well as the AST/ALT ratio (Figure 3). This ratio is an indicator of liver function, but it can also be affected by muscle damage, as AST is found in both liver and muscle tissues, while ALT is primarily liver-specific. Given that AST is expressed in skeletal muscle, the AST/ALT ratio serves as a useful tool for distinguishing between the liver and muscular sources of enzyme fluctuations [30,31]. Notably, the AST/ALT ratio remained unchanged pre- and post-intervention and between the 1SET and CON groups post-intervention, reinforcing the interpretation that the observed increases are physiologi-

cal adaptations rather than indicators of tissue injury or liver dysfunction. Furthermore, the previous studies have shown that RT can induce transient increases in muscle enzymes, which reflect acute metabolic responses and muscle adaptation [44]. Further investigation into the underlying mechanisms is needed to better understand the clinical relevance of these enzyme fluctuations.

4.2. Lipid Profile and Atherogenic Index

Although ANCOVA revealed no significant differences in the total cholesterol levels between the groups after the intervention, a trend towards a reduction was observed in both the groups. This trend suggests that low-volume RT intervention may have a potentially beneficial effect on the total cholesterol levels, which could be confirmed in studies with larger samples or longer duration. There was a significant increase in the serum HDL cholesterol levels and a reduction in the atherogenic index after the 8-week training period relative to the pre-intervention values. These findings suggest that even low-volume RT interventions can induce beneficial effects on lipid metabolism. These changes are clinically relevant since HDL is known to protect the cardiovascular system by facilitating reverse cholesterol transport and modulating inflammatory activity in the vascular endothelium [45]. These effects are especially important in the older population, where preserving cardiovascular health and mitigating inflammatory processes are the key to reducing the atherosclerotic risk and promoting healthy aging [46]. Our findings align with those of other studies published in the literature, which have also reported improvements in lipid parameters following RT interventions, even with low-volume protocols [12,47]. For instance, a randomized controlled trial demonstrated that RT improved the lipid profiles in obese older women following a 12-week intervention [48]. Similarly, the meta-analysis of various exercise modalities demonstrated significant improvements in lipid profiles among older populations following RT exercise (8 and 10 weeks) [49]. Furthermore, another study found that even a low-volume RT protocol effectively reduced cardiovascular risk factors in untrained older women, emphasizing the potential benefits of RT interventions, even with low-volume approaches, over a 6-month period [50].

Surprisingly, a reduction in the atherogenic index was also observed in the CON group, raising important questions about the specificity of the intervention's effects. This finding raises a significant concern regarding the potential influence of confounding variables in the present study. Although the CON group did not participate in structured training, spontaneous alterations in daily routines, such as modest increases in informal physical activity (e.g., walking within the facility), unmonitored dietary modifications (including changes in nutrient and caloric intake or meal timing), and adjustments in medication regimens (e.g., the initiation or dosage adjustments of lipid-lowering drugs), may have contributed to the unexpected reduction in the atherogenic index, thereby limiting the interpretation and generalization of the results. Moreover, the small sample size may amplify the effects of individual variability, potentially magnifying the impact of isolated changes within the group. Informal reports from staff indicated that at least one participant underwent medication adjustments during the study period. Future studies should incorporate systematic and objective monitoring methods, such as detailed dietary records, accelerometry for physical activity assessment, and comprehensive medication logs, to better control for potential confounding variables. However, it should be noted that implementing such protocols for older populations with cognitive impairment may be particularly challenging, requiring a high level of involvement and sustained commitment from residential institutions, caregivers, and health professionals. Therefore, the design of training protocols must strike a balance between the need for controlled conditions and the feasibility of executing the protocol in real-world environments.

4.3. Glycemic Control

Regarding glycemic control, the absence of significant changes in HbA1c and the estimated average glucose levels following RT may be attributed to the characteristics of the RT protocol, namely its duration (8 weeks) and weekly frequency (two sessions per week). While RT has been shown to acutely enhance insulin sensitivity, particularly in the hours following exercise [51], sustained improvements in glycemic markers such as HbA1c typically require longer-term interventions. Some studies have demonstrated that longer RT protocols, typically ranging from 12 to 24 weeks and involving higher volumes, produce measurable improvements in HbA1c levels in older adults with type 2 diabetes [52–55]. For instance, Castaneda et al. (2002) reported a significant reduction in HbA1c in older adults after 16 weeks of RT, showing the potential long-term benefits of resistance exercise on glycemic control [53]. Similarly, Ibañez et al. (2005) found improvements in insulin sensitivity following a 16-week RT program in older men, which translated into the better control of HbA1c [56]. Moreover, Misra et al. (2008) demonstrated that RT positively impacted glycemic markers, including HbA1c, in older individuals with metabolic syndrome after 12 weeks of intervention [52]. Since HbA1c reflects the average blood glucose over the two months preceding its measurement, short training periods may be insufficient to elicit measurable changes in this parameter [57].

4.4. Oxidative Stress Marker

Considering that the aging process is associated with increased oxidative stress, which has been implicated in the pathogenesis of neurodegenerative diseases [58], we investigated whether our RT protocol could modulate oxidative stress by assessing the circulating levels of MDA, a biomarker of lipid peroxidation. No significant changes in MDA concentrations were observed following the intervention, nor were there any differences between the 1SET and CON groups at the end of the study. The lack of increase in lipid peroxidation suggests that the RT protocol was not detrimental in promoting oxidative damage, an important consideration when working with older populations. Some studies in the literature report a decrease in oxidative stress levels after 12 or 18 weeks of RT [59,60], while others report no changes even with antioxidant supplementation over 24 weeks [61]. Exercise intensity may also impact oxidative stress. Vincent et al. (2002) investigated the effects of a 6-month RT program with varying intensities [62]. Their results showed a 14% reduction in MDA levels in a group that trained at 50% 1RM, while the group that used 80% 1RM showed a reduction of 18%. In summary, although our RT protocol did not significantly alter the MDA levels, the lack of increased lipid peroxidation suggests that the intervention was safe from an oxidative stress standpoint. Taken together with the previous findings, it is plausible that both exercise duration and intensity play critical roles in modulating oxidative stress. It is essential to acknowledge that although MDA is a widely utilized marker of lipid peroxidation, its limited specificity and inability to detect oxidative damage to other molecules, as well as the variations induced by external factors, represent significant limitations. Therefore, to achieve the more comprehensive assessment of oxidative stress, future studies should include a broader panel of both oxidative and antioxidant biomarkers. The previous studies have shown that resistance exercise can either increase or decrease oxidative stress, depending on the specific characteristics of the training protocols, and possibly the individual's training status [63–66]. Therefore, the impact of our low-volume RT protocol on oxidative stress was evaluated by measuring the serum levels of MDA, a by-product of lipid peroxidation and a widely used marker of oxidative stress in cells and tissues (Figure 6). These results suggest that an 8-week low-volume RT protocol does not increase oxidative stress and may be considered a safe training approach for the population under study.

4.5. Methodological Considerations and Study Limitations

When interpreting the present findings, it is essential to acknowledge the methodological limitations that may have influenced the outcomes. First, the small sample size substantially reduced the statistical power to detect subtle effects and limited the generalizability of the results to broader populations. This is particularly important given the interindividual variability often observed in clinical and metabolic parameters. Second, the quasi-experimental design, without random assignment, may have introduced a potential risk of selection bias. The participants were allocated based on practical considerations, including institutional constraints, individual availability, and ease of transport to the training facility. Although this allocation method was not random, there is a risk that unmeasured differences between the groups could have influenced the outcomes. While this approach boosted participant adherence, which is key in studies involving vulnerable populations, it may have compromised the internal validity more compared to that of a randomized controlled trial. Third, the duration of the intervention (8 weeks) may have been insufficient to elicit significant changes in specific metabolic and functional outcomes. Adaptations related to lipid metabolism, glycemic control, and oxidative stress often require longer training periods to present robust physiological effects. Fourth, there was no systematic monitoring of the participants' spontaneous physical activity outside the intervention sessions. This lack of control, especially in the CON group, introduces the possibility that untracked variations in daily activity may have influenced the metabolic outcomes, limiting the interpretation of intervention effects. Finally, we did not consistently track medication throughout this study. Changes in medication, such as the initiation, discontinuation, and dose adjustment of lipid-lowering or antidiabetic drugs, could have significantly influenced the biochemical markers measured. To strengthen the validity and applicability of future research, we recommend the inclusion of a larger randomized sample; extended intervention durations; and the implementation of objective, systematic methods to track dietary intake, physical activity (e.g., via accelerometry), and medication adherence. These methodological improvements would allow for clearer attribution of observed effects to the intervention and enhance confidence in the intervention's efficacy.

4.6. Summary and Future Directions

In summary, our findings suggest that a low-volume RT dose can promote modest, yet clinically meaningful improvements in the patients' metabolic profile without significant changes in the commonly used markers of oxidative stress, liver function, and muscle damage in individuals with cognitive impairment. These findings are particularly important for vulnerable populations, in which safety is the primary concern. Future randomized controlled studies should aim to clarify the relative contributions of training volume, intensity, and duration in optimizing health benefits, particularly in vulnerable populations where safety and adherence are the key concerns. Additionally, age, specific clinical conditions, and baseline physical status should also be considered to design more tailored and effective intervention approaches.

5. Conclusions

The reluctance of older adults to engage in more demanding training programs can be attributed to several factors, such as physical limitations, the fear of injury, and reduced motivation. The findings of this study suggest that preliminary, but meaningful health benefits may be achievable in older adults with cognitive impairment through low-volume RT exercises of a short duration, such as eight weeks. The protocol appeared to be safe with respect to muscle health and liver function, while showing potential improvements in their lipid profile. Although additional benefits might be expected with longer interventions

(e.g., improved glycemic control and reduced oxidative stress), future studies should optimize training characteristics, while maintaining low training volumes. These strategies may be particularly relevant since low-volume protocols can enhance long-term adherence to training programs, potentially resulting in sustained health benefits in this population. These findings suggest that low-volume RT protocols may be a promising approach in long-term care facilities, particularly for older adults with cognitive impairment. Given their simplicity, safety, and minimal resource requirements, such interventions could be feasibly integrated into routine care to support metabolic health and functional maintenance in this vulnerable population. In summary, the current study provides preliminary, yet promising evidence that an 8-week low-volume RT dose may offer metabolic benefits in older adults with cognitive impairment. However, further randomized trials and a larger sample size are needed to confirm these findings and better define the scope of potential benefits.

Author Contributions: Conceptualization, M.C.M., M.I., D.E. and C.P.F.; methodology, M.C.M., D.E. and C.P.F.; formal analysis, M.L., D.L.M. and C.N.; investigation, M.L. and N.F.; resources, D.A.M., M.C.M., H.P.N. and D.E.; data curation, M.L., N.F. and D.L.M.; writing—original draft preparation, M.L., N.F. and D.L.M.; writing—review and editing, N.F., D.L.M., M.C.M., H.P.N., M.I., D.E. and C.P.F.; visualization, M.L. and C.P.F.; supervision, M.C.M., D.E. and C.P.F.; project administration, M.C.M., D.E. and C.P.F.; funding acquisition, D.A.M., M.C.M. and D.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Portuguese Institute of Sport and Youth, I.P. (IPDJ) [TRAIN4BRAIN project; reference number: CP/0542/DDT/2023], “la Caixa” Foundation with the project SR24-00179, and by the National Funds by FCT—Foundation for Science and Technology under the following project UID/04045: Research Center in Sports Sciences, Health Sciences, and Human Development, as well as grant numbers BII5 CIDESD-UBI UIDB/04045/2020 (M.L.), and PRT/BD/154638/2023 (N.F.).

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Beira Interior (approval number: CE-UBI-Pj-2022-065; date of approval: 15 November 2022).

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

Data Availability Statement: The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: We sincerely thank all the residential care facilities (Santa Casa da Misericórdia da Covilhã; Lar de São José, Covilhã; Associação Centro Social Sagrado Coração Maria, Ferro; Centro Social de Nossa Senhora da Conceição, Vila do Carvalho; Centro Social Vales do Rio; Centro de Convívio e Apoio à 3.^a Idade, Tortosendo) and the participants who agreed to be part of this study.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of this study; in the collection, analyses, or interpretation of data; in the writing of this manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

1RM	One-Repetition Maximum
ADAG	A1c-Derived Average Glucose
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
CK	Creatine Kinase
CON	Control

DRS-2	Dementia Rating Scale-2
HbA1c	Hemoglobin A1c
HDL	High-Density Lipoprotein
LDH	Lactate Dehydrogenase
LDL	Low-Density Lipoprotein
MDA	Malondialdehyde
NAD	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
R	Repetition
RT	Resistance Training
SD	Standard Deviation
1SET	Single Set
SPPB	Short-Physical-Performance Battery
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid-Reactive Substances
TCA	Trichloroacetic Acid

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Article

Acute Heterogeneous Changes in Muscle and Tendon Viscoelastic Properties Following a VO₂max Treadmill Test in High-Level Male Soccer Players

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Abstract

Background: This study aimed to investigate the acute changes in muscle and tendon viscoelastic properties in response to a progressive treadmill VO₂max test among professional male soccer players. **Methods:** Bilateral assessments at five sites—the Achilles tendon (AT), biceps femoris, semitendinosus, rectus femoris (RF), and sternocleidomastoid (SCM)—measured tone (oscillation frequency), dynamic stiffness, logarithmic decrement (elasticity), stress relaxation time, and creep. Each site was probed five times and values averaged. Repeated-measures ANOVA (Time × Side) with Bonferroni correction tested pre- to post-exercise changes; Pearson’s *r* examined associations with VO₂max. **Results:** Significant Time effects (all *p* < 0.05) were observed for RF frequency ($\eta_p^2 = 0.226$), RF creep ($\eta_p^2 = 0.144$), AT stiffness ($\eta_p^2 \approx 0.035$), AT frequency ($\eta_p^2 = 0.035$), and SCM frequency ($\eta_p^2 = 0.037$). Post-exercise, right AT stiffness fell by 65 ± 14 N/m (*p* = 0.015), while left AT stiffness rose by 22 ± 9 N/m (*p* = 0.015). RF stiffness decreased by 28 ± 6 N/m (*p* < 0.001) and tone by 1.2 ± 0.3 Hz (*p* < 0.001), with creep ($+0.08 \pm 0.02$; *p* < 0.001) and relaxation time ($+1.5 \pm 0.7$ ms; *p* < 0.001) increasing. SCM tone declined by 0.8 ± 0.4 Hz (*p* = 0.010). Baseline RF properties—frequency (*r* = −0.597), stiffness (*r* = −0.59), relaxation time (*r* = 0.53), and creep (*r* = 0.48)—correlated moderately with VO₂max (all *p* < 0.05). **Conclusions:** These findings suggest that viscoelastic adaptations to exhaustive aerobic exercise are tissue- and side-specific, and that rectus femoris viscoelastic properties may serve as potential indicators of endurance readiness.

Keywords: aerobic capacity; muscle stiffness; portable myometry; running; football

1. Introduction

The publication rate for research focused on human body stiffness in the literature of sports science and medicine is rapidly increasing [1,2], most likely because stiffness has been associated with increased athletic performance [1,3,4]. Stiffness traces back to Hooke’s law, which holds that the force required to deform an object equals the spring constant multiplied by the displacement distance [5,6]. Muscle and tendon stiffness indicate longitudinal tissue viscoelastic properties as assessed by tissue resistance to passive stretching [7].

It is primarily responsible for transferring and absorbing motion energy in a variety of sporting activities [8]. Furthermore, muscle and tendon stiffness can objectively indicate tissue condition [9] and fatigue [10]. For example, Yanagisawa et al. [9] utilized real-time ultrasound elastography to demonstrate that the stiffness of the gastrocnemius muscle increased immediately after dynamic exercise, indicating acute fatigue-related changes in tissue condition. Likewise, Andonian et al. [10] applied shear-wave elastography to track quadriceps tendon stiffness before, during, and after an extreme ultra-marathon, observing progressive increases in stiffness that paralleled the accumulation of muscle fatigue. Kalkhoven et al. found that increased medial gastrocnemius muscle stiffness appears to be beneficial for athletic performance in soccer players [11]. It is therefore important to accurately assess the stiffness of specific muscles and tendons based on the specificity of different sports disciplines in order to monitor changes in muscle and tendon stiffness both acutely and chronically.

Recent findings by Krzysztofik et al. [12] highlight the relevance of this assessment in the context of post-activation performance enhancement (PAPE). Their study demonstrated that various back squat activation protocols significantly affect both jumping performance and tissue stiffness. Specifically, reductions in AT and vastus lateralis stiffness were observed following different conditioning activities (e.g., high-load squats and velocity-based protocols), while jump height was acutely enhanced. These results emphasize that intensity, movement velocity, and training volume are critical variables when aiming to optimize performance without inducing excessive fatigue. Notably, a single set of squats at 60% 1 RM with a 10% velocity loss proved effective in acutely improving jump performance, while limiting increases in training volume and preserving tendon integrity.

In professional soccer, athletes routinely subject their muscle-tendon units to extreme loads [13], placing them at elevated risk for both acute injury and cumulative fatigue that can compromise performance and career longevity [14,15]. Despite advances in training-load monitoring, real-time assessment of tissue mechanical properties remains largely confined to laboratory settings and chronic adaptations, and the immediate viscoelastic responses of muscle and tendon to maximal aerobic exercise—critical indicators of acute fatigue and tissue readiness [16–18]—have not been systematically investigated in elite players. To address this gap, we applied the MyotonPRO [19], a handheld myometer validated for in vivo measurement of muscle and tendon mechanics (Myoton AS, Tallinn, Estonia), during the post-VO₂max window, to characterize acute, tissue- and side-specific viscoelastic changes in professional soccer players. We assessed tone, stiffness, elasticity, relaxation time and creep in the Achilles tendon, biceps femoris, semitendinosus, rectus femoris and sternocleidomastoid before and immediately after a progressive treadmill effort to exhaustion. Accordingly, the objective of the present study was to investigate the acute, tissue- and side-specific changes in muscle and tendon viscoelastic properties in professional male soccer players following a progressive treadmill VO₂max test, as measured by the MyotonPRO device. We hypothesized that a VO₂max test would induce heterogeneous, tissue- and side-specific alterations—with the rectus femoris and Achilles tendon showing the strongest associations between their baseline viscoelastic properties and VO₂max—suggesting their potential as acute indicators of endurance readiness and informing strategies for injury prevention and performance optimization.

2. Materials and Methods

2.1. Study Design

The study was designed to investigate changes in the muscle viscoelastic properties of the Achilles tendon, biceps femoris muscle, semitendinosus muscle, rectus femoris muscle, and sternocleidomastoid muscle, specifically tension (oscillation frequency in Hz) and

stiffness (N/m), under the influence of progressive treadmill effort to exhaustion. Muscle viscoelastic properties for both legs were measured using a Myoton device at two time points: at rest before and immediately after the completion of the measurement of VO_2max .

2.2. Participants

The study involved 21 highly trained male soccer players classified based on training status and performance caliber according to the classification of McKay et al. [20] (Table 1). The inclusion criteria were as follows: (a) male gender; (b) age between 18 and 40 years; (c) no cardiovascular diseases, such as unstable coronary artery disease, unstable arrhythmia, or severe heart failure; (d) no respiratory diseases, such as chronic obstructive pulmonary disease (COPD); (e) absence of muscle injuries (leading to absence from training for more than 4 weeks) for at least 6 months prior to the start of the study (based on a preliminary medical examination evaluated by the team doctor). The experimental session was conducted during the off-season period.

Table 1. Descriptive characteristics of the study participants.

Characteristic	M ± SD
Age [years]	24 ± 5
Body mass [kg]	75 ± 7
Body fat [%]	12.1 ± 2.7
Body height [cm]	180 ± 6
Resistance training experience [years]	7.9 ± 2.2
VO_2max [$\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$]	54.9 ± 3.7

M—Mean, SD—Standard deviation.

Participants were informed about potential risks and benefits associated with their participation in the project, as well as their right to withdraw from the study at any time without providing a reason for their decision. The participants signed a written consent form to participate in the study. All stages of the study were conducted at the Academy of Physical Education in Katowice, in the Human Functional Research Laboratory. The experimental project was approved by the Bioethics Committee for Scientific Research (3/2021, approval date 27 May 2021), following the ethical standards of the 1983 Helsinki Declaration. None of the participants withdrew from the study.

2.3. Testing Protocol

Participants were asked not to perform any resistance exercises for 24 h prior to the start of the experimental session. Additionally, they were advised to maintain their regular dietary habits and refrain from the consumption of any supplements or stimulants, except for habitual supplementation such as creatine in the week preceding the experiment. At the beginning of the experimental session, body composition was assessed using a multi-channel bioelectrical impedance analysis in a laboratory setting using the InBody 720 device (InBody, Tokyo, Japan). The first resting measurement of the muscle viscoelastic properties of the Achilles tendon, biceps femoris muscle, semitendinosus muscle, rectus femoris muscle, and sternocleidomastoid muscle, were directly taken before the commencement of VO_2max measurement. All measurements of the viscoelastic properties of the muscles were performed in the same room as the VO_2max measurement. All tests were conducted by the same person trained to operate the Myoton device.

The second sample was taken immediately after the completion of the VO_2max measurement. The reliability between the trials (within the session) of a selected muscle group (2 trials per participant, $N = 21$, total measurements = 42) was tested using the intraclass correlation coefficient (ICC) model. Domholdt's classification scales (1993) were

applied to interpret the ICC: very high = 1.00–0.90; high = 0.89–0.70; moderate = 0.69–0.50; low = 0.49–0.26. This indicates that the ICC value across the time points was in a range of 0.71–0.94, with the exception of BF (0.56). A high reliability coefficient indicates that the applied tests represent a consistent measurement of data regarding the viscoelastic properties of muscles among soccer players.

2.4. Progressive Exercise on the Treadmill

The exercise was conducted on a Pulsar treadmill (HP-Cosmos, Nussdorf-Traunstein, Germany), starting at a speed of 6 km/h and 0 degrees incline. The treadmill speed was progressively increased by 2 km/h every 3 min of exercise until voluntary exhaustion [21]. During the test, heart rate, minute ventilation (VE), oxygen consumption (VO_2), and carbon dioxide exhalation (CO_2) were continuously measured breath-by-breath using a stationary ergo-spirometer, the Metalyzer 3B (Cortex, Isernhagen, Germany). $\text{VO}_{2\text{max}}$ was determined based on a reduced or plateaued VO_2 with increasing speed ($\text{VO}_2 \leq 150 \text{ mL/min}$ above peak VO_2). Capillary blood samples from the fingertip were collected to assess lactate concentration (LA) (Biosen C-line Clinic, EKF-Diagnostic GmbH, Barleben, Germany) during the 30-s break between treadmill speed increments, as well as at 3, 6, 9, and 12 min during the post-exercise recovery period to determine lactate utilization. Resting capillary blood samples and post-exercise blood samples were also used to determine acid-base balance and hemoglobin oxygen saturation (RapidLab 248, Bayer Diagnostics, Leverkusen, Germany).

2.5. Measurement of Muscle Viscoelastic Properties

MyotonPRO (Myoton AS, Tallinn, Estonia) is a non-invasive device monitoring superficial mechanical deformation of soft tissues by delivering a brief mechanical impulse and recording the resulting damped oscillations via an integrated accelerometer. It was used to bilaterally assess the following muscle viscoelastic properties: Achilles tendon, biceps femoris muscle, semitendinosus muscle, rectus femoris muscle, and sternocleidomastoid muscle. The participants were lying on their back, side, or stomach (depending on the command given by the specialist for proper sample collection) on a physiotherapy couch and rested for 3 min before the measurements of the viscoelastic properties of the muscles were taken (Figure 1). Measurement sites on each muscle were located using a measuring tape and marked with a skin-safe waterproof marker. A pillow was placed under the head, and a unique rolling cushion was placed under the lower leg and straightened foot to facilitate relaxation. One series of five individual Myoton measurements for each muscle group (5 points) was measured separately for the left and right leg. Additionally, to better understand the issue, the functionality of the dominant and non-dominant lower limbs was also determined. The dominant leg for a specific player was determined based on the information provided by the player in the questionnaire. Measurements comprised five dependent variables: dynamic stiffness ($\text{N}\cdot\text{m}^{-1}$), representing the resistance of the tissue to deformation; natural oscillation frequency (Hz), reflecting passive muscle tone; logarithmic decrement (unitless), indexing the rate of damping and thus tissue elasticity; mechanical stress relaxation time (ms), indicating the temporal viscous response under constant deformation; and creep (unitless), denoting progressive deformation under sustained load [22]. Myoton's accelerometer was set to 3200 Hz, and the average value for analysis was obtained from five consecutive measurements (0.4 N for 15 ms) [2].



Figure 1. Myotonometry of Achilles tendon procedure.

2.6. Statistical Analysis

All statistical analyses were performed using Jamovi (version 2.3.21; The Jamovi Project, Sydney, Australia). Data normality was assessed using the Shapiro–Wilk test, while the Levene test was used to examine the homogeneity of variances. To verify the assumption of homogeneity of covariance matrices, descriptive statistics are presented as means, standard deviations (SD), and 95% confidence intervals (CI). A 2-way repeated-measures ANOVA was conducted to assess differences in the variables of interest. Effect sizes for main effects and interactions were reported as partial eta squared (η_p^2) and classified as small (0.01–0.059), moderate (0.06–0.137), and large (>0.137). A Bonferroni correction was applied to account for multiple comparisons. Correlation analyses were performed using Pearson’s linear correlation coefficient, with values from the dominant leg baseline included in the analysis. The magnitude of correlation was interpreted as follows: 0.00–0.10 (negligible), 0.10–0.39 (weak), 0.40–0.69 (moderate), 0.70–0.89 (strong), and 0.90–1.00 (very strong). The threshold of significance was set at $\alpha = 0.05$. Statistical significance was set at $p < 0.05$ for all tests. The level of significance was interpreted as follows: $p < 0.05$ indicating moderate evidence against the null hypothesis; $p < 0.01$ providing strong evidence against the null hypothesis; $p < 0.001$ demonstrating very strong evidence against the null hypothesis. The results are presented in grouped charts, with 95% (CI) displayed as error bars. Correlation analyses are visualized using scatter plots, incorporating density marginals, a linear regression line, and standard error shading to illustrate the strength and direction of correlation between variables.

3. Results

A parametric analysis of variance was selected for the analysis as the Shapiro–Wilk test indicated p -values > 0.05 for almost all variables, confirming the normality of the distributions (Table 2). Additionally, the Levene’s test for homogeneity of variances of Side factor also yielded p -values > 0.05 across all variables, supporting the assumption of homogeneity. Since the repeated measures design included only two levels, the assumption of sphericity was inherently satisfied in this case.

Table 2. Descriptive statistics and ANOVA results for CREEP Variable.

Measure	Side	PRE		POST		ANOVA Effects		
		M $\pm$ SD (95% CI)	Shapiro–Wilk (p)	M $\pm$ SD (95% CI)	Shapiro–Wilk (p)	Time	Side	Time $\times$ Side
AT	R	0.61 $\pm$ 0.16 (0.53, 0.68)	0.79 (<0.001)	0.67 $\pm$ 0.16 (0.60, 0.75)	0.82 (0.001)		$p > 0.05$	
	L	0.59 $\pm$ 0.08 (0.55, 0.63)	0.96 (0.61)	0.61 $\pm$ 0.09 (0.56, 0.65)	0.96 (0.49)			
BF	R	1.09 $\pm$ 0.10 (1.05, 1.13)	0.94 (0.20)	1.09 $\pm$ 0.09 (1.05, 1.13)	0.93 (0.16)		$p > 0.05$	
	L	1.11 $\pm$ 0.10 (1.06, 1.15)	0.94 (0.21)	1.10 $\pm$ 0.07 (1.07, 1.14)	0.98 (0.97)			
S	R	1.10 $\pm$ 0.09 (1.07, 1.14)	0.95 (0.38)	1.11 $\pm$ 0.10 (1.07, 1.16)	0.96 (0.49)		$p > 0.05$	
	L	1.08 $\pm$ 0.08 (1.04, 1.12)	0.97 (0.80)	1.09 $\pm$ 0.09 (1.05, 1.13)	0.99 (0.98)			
RF	R	1.11 $\pm$ 0.11 (1.06, 1.15)	0.94 (0.18)	1.18 $\pm$ 0.09 (1.15, 1.22)	0.98 (0.88)	$p < 0.001$	$p > 0.05$	$p > 0.05$
	L	1.10 $\pm$ 0.12 (1.05, 1.16)	0.95 (0.37)	1.19 $\pm$ 0.10 (1.14, 1.23)	0.96 (0.46)			
MOS	R	1.17 $\pm$ 0.21 (1.07, 1.27)	0.98 (0.94)	1.17 $\pm$ 0.20 (1.08, 1.26)	0.92 (0.09)		$p > 0.05$	
	L	1.16 $\pm$ 0.17 (1.08, 1.23)	0.95 (0.39)	1.24 $\pm$ 0.13 (1.18, 1.30)	0.98 (0.97)			

Data are presented as mean $\pm$ standard deviation (SD) with 95% confidence intervals (CI) and median values. Shapiro–Wilk test results (W , p) indicate the normality of data distribution. AT—Achilles tendon; BF—Biceps femoris; S—Semitendinosus; RF—Rectus femoris; MOS—sternocleidomastoid.

Significant differences were observed for the main effect of time for the CREEP variable for RF ($F = 43.45$; $p < 0.001$; $\eta_p^2 = 0.14$). No significant interaction was found for TIME POINT $\times$ Side ($F = 0.07$; $p = 0.79$). The between-side analysis for the Side factor showed no significant differences ($F = 2.68 \times 10^{-4}$; $p = 0.99$). Post hoc comparisons with Bonferroni correction identified a statistically significant change in the variable from pre- to post-intervention, with a Δ of -0.08 ($t = -6.59$; $p < 0.001$). Results are illustrated in Figure 2a.

No significant differences were found for the RT variable (all $F < 1.0$, $p > 0.05$) (Table 3).

The analysis of the DEC variable for MOS revealed a significant main effect for TIME POINT ($F = 6.67$; $p = 0.014$; $\eta_p^2 = 0.039$) (Table 4). The interaction between TIME POINT $\times$ Side approached significance ($F = 4.05$; $p = 0.051$; $\eta_p^2 = 0.024$). The Side factor in the between-side analysis showed no significant effect ($F = 0.37$; $p = 0.547$). Post hoc comparisons confirmed a significant pre-post difference ($\Delta = -0.06$; $t = -2.58$; $p = 0.014$). Results are presented in Figure 2c.

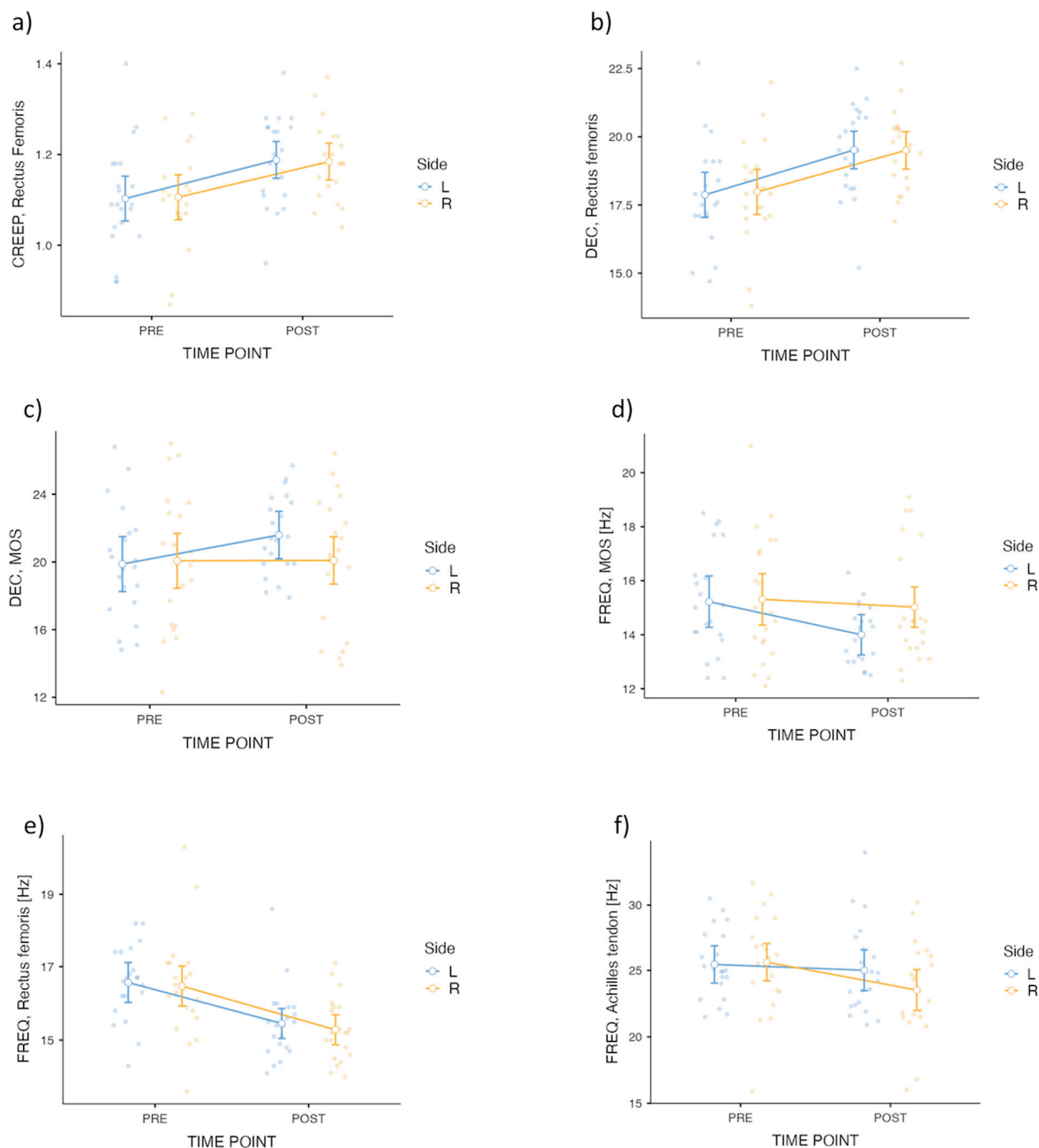


Figure 2. ANOVA visualization for CREEP, DEC and FREQ variables of Rectus femoris, MOS and Achilles Tendon. Group means ($\pm$ SEM) and individual data points for CREEP, DEC, and FREQ variables are presented for PRE and POST time points, separated by Side (Left [L], Right [R]). Each subplot corresponds to a specific variable and tissue: (a) CREEP of the Rectus femoris, (b) DEC of the Rectus femoris, (c) DEC of the MOS, (d) FREQ of the MOS [Hz], (e) FREQ of the Rectus femoris [Hz], and (f) FREQ of the Achilles tendon [Hz]. Data visualization includes individual data points and condition-specific means with standard error bars.

The analysis of the FREQ variable for AT (Figure 2f) revealed a significant main effect for TIME POINT ($F = 6.17$; $p = 0.017$; $\eta_p^2 = 0.035$) (Table 5). The interaction between TIME POINT $\times$ Side was not significant ($F = 2.64$; $p = 0.112$). The between-side analysis for the Side factor showed no significant effect ($F = 0.54$; $p = 0.467$). Post hoc comparisons confirmed a statistically significant pre-post difference ($\Delta = 1.27$; $t = 2.48$; $p = 0.017$). The

analysis of the FREQ variable for RF (Figure 2e) revealed a significant main effect for TIME POINT ($F = 38.69$; $p < 0.001$; $\eta_p^2 = 0.226$). The interaction between TIME POINT $\times$ Side was not significant ($F = 0.042$; $p = 0.838$), and the between-side analysis showed no significant effect for the Side factor ($F = 0.226$; $p = 0.637$). Post hoc comparisons confirmed a significant improvement in the variable from pre- to post-intervention, with a Δ of 1.15 ($t = 6.22$; $p < 0.001$). The analysis of the FREQ variable for MOS (Figure 2d) revealed a significant main effect for TIME POINT ($F = 7.30$; $p = 0.010$; $\eta_p^2 = 0.037$). The interaction between TIME POINT $\times$ Side was not significant ($F = 2.82$; $p = 0.101$), and the between-side analysis showed no significant effect for the Side factor ($F = 1.10$; $p = 0.301$). Post hoc comparisons confirmed a statistically significant pre–post difference, with a Δ of 0.75 ($t = 2.70$; $p = 0.01$).

Table 3. Descriptive statistics and ANOVA results for RT variable.

Measure	Side	PRE		POST		ANOVA Effects		
		M $\pm$ SD (95% CI)	Shapiro–Wilk (<i>p</i>)	M $\pm$ SD (95% CI)	Shapiro–Wilk (<i>p</i>)	Time	Side	Time $\times$ Side
AT	R	9.41 $\pm$ 2.85 (8.11, 10.71)	0.77 (<0.001)	10.53 $\pm$ 2.70 (9.30, 11.76)	0.81 (<0.001)		$p > 0.05$	
	L	9.18 $\pm$ 1.38 (8.55, 9.81)	0.97 (0.74)	9.43 $\pm$ 1.53 (8.73, 10.13)	0.96 (0.46)			
BF	R	17.64 $\pm$ 1.71 (16.86, 18.42)	0.95 (0.40)	17.77 $\pm$ 1.57 (17.05, 18.49)	0.93 (0.11)		$p > 0.05$	
	L	18.08 $\pm$ 1.76 (17.27, 18.88)	0.94 (0.22)	18.18 $\pm$ 1.50 (17.50, 18.86)	0.94 (0.22)			
S	R	18.03 $\pm$ 1.48 (17.35, 18.70)	0.96 (0.52)	18.34 $\pm$ 1.77 (17.54, 19.15)	0.97 (0.69)		$p > 0.05$	
	L	17.53 $\pm$ 1.41 (16.88, 18.17)	0.98 (0.88)	17.80 $\pm$ 1.55 (17.09, 18.50)	0.99 (0.99)			
RF	R	17.98 $\pm$ 1.87 (17.13, 18.83)	0.96 (0.48)	19.50 $\pm$ 1.43 (18.86, 20.15)	0.97 (0.81)		$p > 0.05$	
	L	17.87 $\pm$ 1.88 (17.02, 18.73)	0.95 (0.39)	19.51 $\pm$ 1.68 (18.75, 20.28)	0.96 (0.56)			
MOS	R	20.07 $\pm$ 4.02 (18.24, 21.90)	0.97 (0.77)	20.09 $\pm$ 3.85 (18.34, 21.84)	0.94 (0.17)		$p > 0.05$	
	L	19.88 $\pm$ 3.31 (18.37, 21.38)	0.97 (0.77)	21.59 $\pm$ 2.32 (20.54, 22.64)	0.97 (0.63)			

Data are presented as mean $\pm$ standard deviation (SD) with 95% confidence intervals (CI) and median values. Shapiro–Wilk test results (*W*, *p*) indicate the normality of data distribution. AT—Achilles tendon; BF—Biceps femoris; S—Semitendinosus; RF—Rectus femoris; MOS—sternocleidomastoid.

Table 4. Descriptive statistics and ANOVA results for DEC variable.

Measure	Side	PRE		POST		ANOVA Effects		
		M $\pm$ SD (95% CI)	Shapiro–Wilk (<i>p</i>)	M $\pm$ SD (95% CI)	Shapiro–Wilk (<i>p</i>)	Time	Side	Time $\times$ Side
AT	R	0.98 $\pm$ 0.16 (0.91, 1.05)	0.94 (0.21)	0.99 $\pm$ 0.18 (0.91, 1.07)	0.91 (0.05)		$p > 0.05$	
	L	1.07 $\pm$ 0.21 (0.97, 1.17)	0.95 (0.28)	1.13 $\pm$ 0.21 (1.03, 1.22)	0.95 (0.31)			

Table 4. Cont.

Measure	Side	PRE		POST		ANOVA Effects		
		M ± SD (95% CI)	Shapiro–Wilk (p)	M ± SD (95% CI)	Shapiro–Wilk (p)	Time	Side	Time × Side
BF	R	1.44 ± 0.23 (1.34, 1.54)	0.96 (0.53)	1.42 ± 0.14 (1.35, 1.48)	0.92 (0.07)		$p > 0.05$	
	L	1.29 ± 0.19 (1.20, 1.37)	0.94 (0.20)	1.31 ± 0.20 (1.22, 1.40)	0.91 (0.05)			
S	R	1.45 ± 0.16 (1.38, 1.53)	0.96 (0.44)	1.39 ± 0.15 (1.33, 1.46)	0.96 (0.46)		$p > 0.05$	
	L	1.53 ± 0.16 (1.46, 1.60)	0.90 (0.04)	1.49 ± 0.14 (1.43, 1.56)	0.94 (0.17)			
RF	R	1.30 ± 0.18 (1.22, 1.38)	0.96 (0.46)	1.24 ± 0.23 (1.14, 1.35)	0.95 (0.37)	$p > 0.05$	$p = 0.031$	$p > 0.05$
	L	1.43 ± 0.24 (1.32, 1.54)	0.93 (0.16)	1.38 ± 0.22 (1.29, 1.48)	0.98 (0.88)			
MOS	R	1.02 ± 0.15 (0.96, 1.09)	0.96 (0.52)	1.04 ± 0.16 (0.96, 1.11)	0.97 (0.76)	$p = 0.014$	$p > 0.05$	$p = 0.051$
	L	0.95 ± 0.13 (0.89, 1.01)	0.92 (0.08)	1.06 ± 0.17 (0.98, 1.13)	0.96 (0.55)			

Data are presented as mean ± standard deviation (SD) with 95% confidence intervals (CI) and median values. Shapiro–Wilk test results (W , p) indicate the normality of data distribution. AT—Achilles tendon; BF—Biceps femoris; S—Semitendinosus; RF—Rectus femoris; MOS—sternocleidomastoid.

Table 5. Descriptive statistics and ANOVA results for FREQ Variable.

Measure	Side	PRE		POST		ANOVA Effects		
		M ± SD (95% CI)	Shapiro–Wilk (p)	M ± SD (95% CI)	Shapiro–Wilk (p)	Time	Side	Time × Side
AT	R	25.60 ± 3.67 (23.90, 27.30)	0.97 (0.61)	23.50 ± 3.57 (21.90, 25.10)	0.97 (0.66)	$p = 0.017$	$p > 0.05$	$p > 0.05$
	L	25.40 ± 2.61 (24.30, 26.60)	0.95 (0.41)	25.00 ± 3.41 (23.50, 26.60)	0.91 (0.06)			
BF	R	16.30 ± 1.33 (15.70, 16.90)	0.96 (0.55)	16.50 ± 1.20 (15.90, 17.00)	0.94 (0.25)		$p > 0.05$	
	L	16.10 ± 1.53 (15.40, 16.80)	0.90 (0.03)	15.90 ± 1.25 (15.30, 16.50)	0.96 (0.58)			
S	R	17.00 ± 1.24 (16.40, 17.50)	0.95 (0.33)	16.60 ± 1.38 (15.90, 17.20)	0.95 (0.31)		$p > 0.05$	
	L	17.10 ± 1.51 (16.50, 17.80)	0.95 (0.30)	17.00 ± 1.26 (16.50, 17.60)	0.86 (0.01)			
RF	R	16.50 ± 1.42 (15.80, 17.10)	0.92 (0.09)	15.30 ± 0.86 (14.90, 15.70)	0.96 (0.46)	$p < 0.001$	$p > 0.05$	$p > 0.05$
	L	16.60 ± 1.01 (16.10, 17.00)	0.97 (0.74)	15.50 ± 0.99 (15.00, 15.90)	0.87 (0.01)			

Table 5. Cont.

Measure	Side	PRE		POST		ANOVA Effects		
		M ± SD (95% CI)	Shapiro–Wilk (p)	M ± SD (95% CI)	Shapiro–Wilk (p)	Time	Side	Time × Side
MOS	R	15.30 ± 2.36 (14.20, 16.40)	0.95 (0.28)	15.00 ± 2.14 (14.10, 16.00)	0.86 (0.01)	p = 0.010	p > 0.05	p > 0.05
	L	15.20 ± 1.94 (14.30, 16.10)	0.94 (0.18)	14.00 ± 1.07 (13.50, 14.50)	0.95 (0.37)			

Data are presented as mean ± standard deviation (SD) with 95% confidence intervals (CI) and median values. Shapiro–Wilk test results (W , p) indicate the normality of data distribution. AT—Achilles tendon; BF—Biceps femoris; S—Semitendinosus; RF—Rectus femoris; MOS—sternocleidomastoid.

The analysis of variance for the STIFF variable for AT revealed a significant main effect for TIME POINT ($F = 6.53$; $p = 0.015$; $\eta_p^2 = 0.035$) (Table 6). The interaction between TIME POINT × Side approached significance but was not statistically significant ($F = 3.05$; $p = 0.089$). The between-side analysis showed no significant effect for the Side factor ($F = 0.270$; $p = 0.606$). Post hoc comparisons confirmed a statistically significant improvement from pre- to post-intervention, with a Δ of 38.6 ($t = 2.55$; $p = 0.015$). The analysis of variance for the STIFF variable for S revealed a significant main effect for TIME POINT ($F = 5.15$; $p = 0.029$; $\eta_p^2 = 0.014$). The interaction between TIME POINT × Side was not significant ($F = 0.010$; $p = 0.921$), and the Side factor in the between-side analysis showed no significant effect ($F = 1.45$; $p = 0.235$). Post hoc comparisons confirmed a significant pre–post improvement, with a Δ of 6.52 ($t = 2.27$; $p = 0.029$). The analysis of the STIFF variable for RF revealed a significant main effect for TIME POINT ($F = 31.92$; $p < 0.001$; $\eta_p^2 = 0.207$). The interaction between TIME POINT × Side was not significant ($F = 0.019$; $p = 0.892$), and the Side factor in the between-side analysis also showed no significant effect ($F = 0.01$; $p = 0.968$). Post hoc comparisons confirmed a significant improvement, with a Δ of 27.6 ($t = 5.65$; $p < 0.001$). Results for STIFF changes are presented in Figure 3.

Table 6. Descriptive statistics and ANOVA results for STIFF variable.

Measure	Side	PRE		POST		ANOVA Effects		
		M ± SD (95% CI)	Shapiro–Wilk (p)	M ± SD (95% CI)	Shapiro–Wilk (p)	Time	Side	Time × Side
AT	R	25.60 ± 3.67 (23.90, 27.30)	0.97 (0.61)	23.50 ± 3.57 (21.90, 25.10)	0.97 (0.66)	p = 0.015	p > 0.05	p > 0.05
	L	25.40 ± 2.61 (24.30, 26.60)	0.95 (0.41)	25.00 ± 3.41 (23.50, 26.60)	0.91 (0.06)			
BF	R	16.30 ± 1.33 (15.70, 16.90)	0.96 (0.55)	16.50 ± 1.20 (15.90, 17.00)	0.94 (0.25)		p > 0.05	
	L	16.10 ± 1.53 (15.40, 16.80)	0.90 (0.03)	15.90 ± 1.25 (15.30, 16.50)	0.96 (0.58)			
S	R	17.00 ± 1.24 (16.40, 17.50)	0.95 (0.33)	16.60 ± 1.38 (15.90, 17.20)	0.95 (0.31)	p = 0.029	p > 0.05	p > 0.05
	L	17.10 ± 1.51 (16.50, 17.80)	0.95 (0.30)	17.00 ± 1.26 (16.50, 17.60)	0.86 (0.01)			

Table 6. Cont.

Measure	Side	PRE		POST		ANOVA Effects		
		M $\pm$ SD (95% CI)	Shapiro–Wilk (<i>p</i>)	M $\pm$ SD (95% CI)	Shapiro–Wilk (<i>p</i>)	Time	Side	Time $\times$ Side
RF	R	16.50 $\pm$ 1.42 (15.80, 17.10)	0.92 (0.09)	15.30 $\pm$ 0.86 (14.90, 15.70)	0.96 (0.46)	$p < 0.001$	$p > 0.05$	$p > 0.05$
	L	16.60 $\pm$ 1.01 (16.10, 17.00)	0.97 (0.74)	15.50 $\pm$ 0.99 (15.00, 15.90)	0.87 (0.01)			
MOS	R	15.30 $\pm$ 2.36 (14.20, 16.40)	0.95 (0.28)	15.00 $\pm$ 2.14 (14.10, 16.00)	0.86 (0.01)	$p > 0.05$	$p > 0.05$	$p > 0.05$
	L	15.20 $\pm$ 1.94 (14.30, 16.10)	0.94 (0.18)	14.00 $\pm$ 1.07 (13.50, 14.50)	0.95 (0.37)			

Data are presented as mean $\pm$ standard deviation (SD) with 95% confidence intervals (CI) and median values. Shapiro–Wilk test results (*W*, *p*) indicate the normality of data distribution. AT—Achilles tendon; BF—Biceps femoris; S—Semitendinosus; RF—Rectus femoris; MOS—sternocleidomastoid.

The analysis revealed no statistically significant effects ($p > 0.05$) for all other variables. No meaningful changes over time, side-related differences, or interactions were observed. However, the DEC variable showed a significant effect for the Side factor in the between-side analysis for RF ($p = 0.031$; Figure 2b), indicating potential side-related differences in these measures. Despite some trends approaching significance, no conclusive differences were found for the remaining parameters.

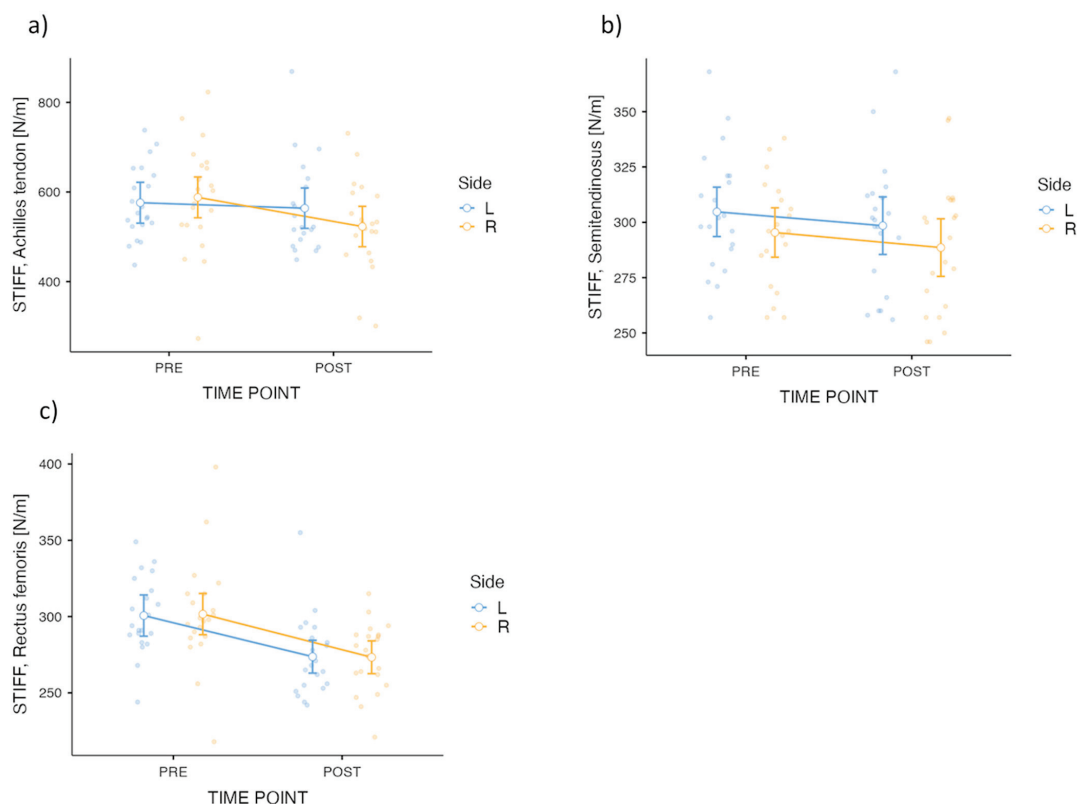


Figure 3. ANOVA visualization STIFF variables of (a) Achilles tendon, (b) Semitendinosus and (c) Rectus femoris. Group means ($\pm$ SEM) and individual data points for STIFF variables are presented for PRE and POST time points, separated by Side (Left [L], Right [R]). Each subplot corresponds to a specific variable and tissue: (a) Achilles tendon, (b) Semitendinosus and (c) Rectus femoris. Data visualization includes individual data points and condition-specific means with standard error bars.

The correlation analysis demonstrated significant relationships between VO_2max and several RF Pre viscoelastic properties (Figure 4). Specifically, VO_2max showed a moderate negative correlation with RF Pre FREQ ($r = -0.60, p < 0.01$) and RF Pre STIFF ($r = -0.58, p < 0.01$), indicating that higher aerobic capacity was associated with lower muscle frequency and stiffness. Conversely, VO_2max was positively correlated with RF Pre RT ($r = 0.53, p < 0.05$) and RF Pre CREEP ($r = 0.48, p < 0.05$). All other correlations between VO_2max and the examined variables were nonsignificant ($p > 0.05$).

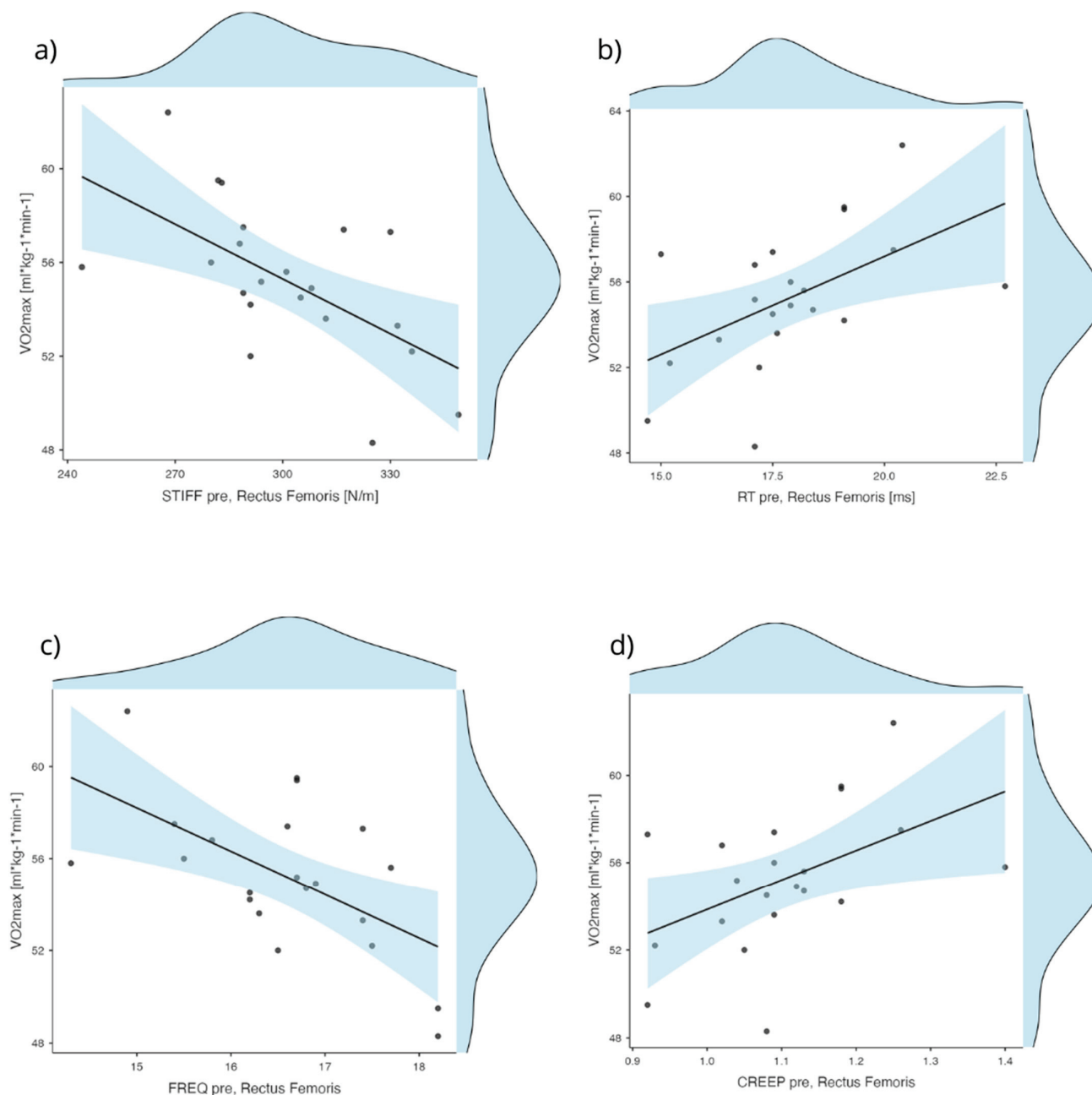


Figure 4. Scatterplots for correlations assumptions. Scatterplots with regression lines and 95% confidence intervals displaying the relationship between VO_2max (mL·kg⁻¹·min⁻¹) and various pre-intervention viscoelastic properties of the Rectus Femoris muscle. Each panel represents a bivariate correlation: (a) STIFF pre for [N/m], (b) RT [ms], (c) FREQ pre [H/z], (d) CREEP pre.

4. Discussion

The main finding of this study was that changes in stiffness were inhomogeneous across different muscle groups among professional soccer players following exhaustive treadmill exercise. Specifically, stiffness significantly increased in the left Achilles tendon (AT) and decreased in the right AT, left sternocleidomastoid (S), and left rectus femoris (RF). Moreover, there was a significant decrease in the state of tension—as indicated by natural oscillation frequency (FREQ)—in the left RF, left S, and right AT, along with a notable reduction in the ratio of relaxation to deformation time (CREEP) for both the left and right RF as well as the left S.

4.1. Heterogeneity of Acute Alterations of Viscoelastic Properties

The heterogeneity of these changes, particularly in AT stiffness, is unexpected. For instance, Farris et al. [23] reported that a 30-min constant-intensity run did not significantly alter Achilles' tendon stiffness. Relative strain and elongation remained constant during the run, and the tendon length while standing was unchanged post-exercise. In contrast, our study found a significant increase in left AT stiffness and a decrease in right AT stiffness, which may be partially attributable to the progressive increase in intensity until exhaustion. An increase in exercise intensity can impose greater stress on the tendon, potentially altering its post-exercise viscoelastic properties [24]. The decrease in right AT stiffness and its reduced oscillation frequency may signal a decline in the tendon's viscoelastic properties [25]. Although the literature on tendon viscoelasticity is limited, fluid flow and structural interactions within the extracellular matrix during loading are likely to be key factors influencing this behavior [18,19]. Additionally, tendinopathy—characterized by reduced stiffness and elasticity—has been linked to diminished explosive performance in athletes [26]. The observed differences in tendon response between legs might also reflect varying viscoelastic conditions, possibly due to disturbed movement patterns or unilateral stress during treadmill running, though movement patterns were not assessed in the current study.

Supporting these observations, several studies have documented heterogeneous responses in viscoelastic properties across muscle groups. Farris et al. [23] underscored that the tendon's response is highly sensitive to both exercise intensity and duration, while De Zee et al. [24] demonstrated that the dynamic viscoelastic behavior of lower extremity tendons is markedly influenced by the specific loading conditions during simulated running. These findings may help explain the asymmetrical changes observed between the dominant and non-dominant limbs in our investigation. The role of muscle fiber composition and neuromuscular recruitment patterns in mediating fatigue-induced changes in tissue stiffness is well documented. Fitts [27] detailed cellular mechanisms of muscle fatigue, indicating that muscles with a higher proportion of fast-twitch fibers might exhibit a different response to exhaustive exercise compared to those dominated by slow-twitch fibers. Similarly, Enoka and Duchateau [28] discussed how neuromuscular factors contribute to fatigue, potentially underpinning the differential stiffness alterations observed among various muscle groups. Clinically, the implications of altered tendon stiffness are significant. Morgan et al. [26] found that decreased tendon stiffness is associated with tendinopathic conditions, which can compromise explosive performance in athletes. These insights emphasize the need for precise post-exercise monitoring of tissue viscoelastic properties, particularly in elite athletes. Moreover, Andonian et al. [10] validated the use of stiffness measurements as objective markers of muscle fatigue, reinforcing the relevance of our methodological approach utilizing the MyotonPRO device.

In summary, the inhomogeneous changes observed in tissue stiffness across muscle groups suggest that factors such as exercise intensity, muscle fiber composition, and neuro-

muscular recruitment patterns may differentially influence the viscoelastic properties of muscles and tendons.

4.2. Viscoelastic Properties of the Rectus Femoris as an Indicator of Readiness for Exhaustive Exercise Testing

The present study revealed notable correlations between baseline MyotonPRO measurements and VO_2max , suggesting that non-invasive assessments of muscle viscoelastic properties may serve as potential indicator of maximal endurance performance. Notably, these significant correlations were observed exclusively in the rectus femoris. Specifically, the rectus femoris demonstrated moderate negative correlations between VO_2max and both natural oscillation frequency ($r = -0.6$, $p < 0.01$) and stiffness ($r = -0.58$, $p < 0.01$), while positive correlations were observed with relaxation time (RT; $r = 0.53$, $p < 0.05$) and creep ($r = 0.484$, $p < 0.05$). These results suggest that soccer players with greater VO_2max exhibit significantly lower resting tone and stiffness in the rectus femoris, implying a more compliant muscle architecture that may enhance elastic energy storage and return during sustained running. This finding is consistent with recent work by Andonian et al. [10], who reported that baseline measures of muscle elasticity can serve as potential indicator of endurance training responsiveness—despite observing no meaningful changes in vastus lateralis viscoelasticity—underscoring the utility of rectus femoris stiffness as a non-invasive biomarker for aerobic performance potential.

The exclusive occurrence of these correlations in the rectus femoris suggests that this muscle group may serve as a particularly sensitive marker for assessing the interplay between muscle viscoelastic properties and endurance performance. Bizzini and Mannion [19] have demonstrated the reliability and sensitivity of the MyotonPRO for quantifying muscle stiffness and tone, underscoring the utility of such measurements. Similarly, Andonian et al. [10] reported that changes in muscle stiffness are closely associated with exercise-induced fatigue, reinforcing the notion that pre-test stiffness levels—particularly in the rectus femoris—could have predictive value regarding subsequent endurance performance. Additionally, foundational work by Fitts [27] and Enoka and Duchateau [28] has described the neuromuscular adaptations during fatigue, which may underpin the observed associations between viscoelastic muscle properties and VO_2max [26,27]. Given these insights, incorporating MyotonPRO assessments of the rectus femoris prior to maximal endurance tests could provide coaches and sports scientists with a non-invasive method to gauge muscle readiness. Such pre-assessment might facilitate the optimization of warm-up protocols, more accurate monitoring of fatigue, and the tailoring of training regimens to enhance performance while reducing injury risk. Future research should further explore the predictive value of these correlations and investigate whether targeted interventions to modulate rectus femoris stiffness can positively influence endurance outcomes.

4.3. Limitations

While the current study provides novel insights into the viscoelastic responses of muscle and tendon tissues following exhaustive treadmill exercise, several limitations should be acknowledged. First, the relatively small sample size of professional male soccer players may limit the generalizability of the findings to other populations, including female athletes and individuals from different sports disciplines. Second, although the MyotonPRO device offers a reliable and non-invasive method for assessing tissue viscoelastic properties, its use may be subject to variability in probe placement and potential measurement error, particularly when evaluating deeper tissue layers. Third, the design of the study does not allow for the evaluation of recovery kinetics or long-term adaptations in viscoelastic properties post-exercise. Additionally, the absence of concurrent kinematic and electromyographic

data restricts an understanding of the neuromuscular mechanisms underlying the observed asymmetrical changes between muscle groups and limbs.

4.4. Practical Implications

The findings of this study have several practical implications for sports scientists, coaches, and clinicians. The use of non-invasive tools such as the MyotonPRO to assess the viscoelastic properties—particularly of the rectus femoris—can serve as a strategic pre-assessment measure to determine an athlete's readiness for exhaustive exercise. By identifying baseline tissue characteristics that correlate with performance metrics like VO_2max , practitioners may tailor warm-up protocols and training regimens to enhance neuromuscular efficiency and reduce the risk of overuse injuries. Furthermore, regular monitoring of muscle and tendon stiffness could inform the design of recovery strategies, ensuring that athletes maintain optimal tissue properties for peak performance. Ultimately, integrating viscoelastic assessments into routine performance evaluations could provide actionable feedback, allowing for timely adjustments to training loads and preventive measures against potential injuries.

5. Conclusions

This study demonstrated that exhaustive treadmill exercise induces heterogeneous changes in the viscoelastic properties of muscle and tendon tissues in professional soccer players. The alterations were both tissue-specific and side-dependent, with significant changes observed particularly in the rectus femoris, Achilles' tendon, and semitendinosus muscles. Among the measured variables, stiffness, frequency, and creep showed the most notable modifications post-exercise. The rectus femoris exhibited significant associations with VO_2max , suggesting its viscoelastic properties may serve as potential indicators of aerobic readiness. These findings highlight the relevance of using non-invasive tools such as the MyotonPRO for the assessment of muscle–tendon mechanics in elite athletes. The data also suggest that monitoring viscoelastic properties prior to maximal endurance testing may provide valuable insights into fatigue, asymmetries, and performance potential. Future research should explore long-term adaptations to training and recovery using this methodology and assess whether interventions targeting specific muscle-tendon units can enhance performance or mitigate injury risk.

Author Contributions: Conceptualization, K.S. and J.J.; methodology, J.J., K.S. and A.T.; software, A.T.; validation, A.T., K.S. and R.R.; formal analysis, M.K.; investigation, M.D.; resources, M.D. and J.J.; data curation, A.T. and R.R.; writing—original draft preparation, K.S. and A.T.; writing—review and editing, K.S., A.T. and L.R.; visualization, A.T. and K.S.; supervision, M.K. and R.R.; project administration, M.K.; funding acquisition, M.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved (number: 3/2021, approval date 27 May 2021) by the Institutional Review Board at the Academy of Physical Education in Katowice.

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

Data Availability Statement: The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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

Abbreviations

The following abbreviations are used in this manuscript:

AT	Achilles Tendon
BF	Biceps Femoris
CI	Confidence Interval
DEC	Logarithmic Decrement (measure of elasticity)
FREQ	Natural Oscillation Frequency (Hz), characterizing muscle tone
HR	Heart rate
ICC	Intraclass Correlation Coefficient
LA	Lactate
MOS	Sternocleidomastoid
PAPE	Post-Activation Performance Enhancement
PRE/POST	Pre-exercise/Post-exercise
RF	Rectus Femoris
R/L	Right/Left
RL	Relaxation Time (ms)
S	Semitendinosus
SD	Standard Deviation
VO ₂	Oxygen consumption
VO ₂ max	Maximal Oxygen Uptake
VE	Minute Ventilation

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Article

Correlation of the Asymmetry Index from the Single-Leg Countermovement Jump with the Asymmetry Index from Isokinetic Strength in Elite Youth Football Players

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Abstract: Football is a sport in which athletes perform many movements using one of their legs, which is characterized as dominant. This differentiation in usage often creates asymmetries between the limbs, which has been noted to be related to the occurrence of injuries as well as negative effects on performance. To assess these asymmetries, vertical jump tests, as well as tests using an isokinetic dynamometer, were employed. The aim of this study was to examine the relationship between the asymmetries observed in the application of the single-leg countermovement jump (SLCMJ) and those observed through isokinetic strength assessment at 60°/s, 180°/s, and 300°/s in high-level youth football players. The study involved 63 elite youth football players. For statistical analysis, correlation analysis and linear regression analysis were used. The results showed a significant positive correlation between the CMJ Bilateral Asymmetry Index and the BAI Anterior Quadriceps (60°/s) ($r = 0.262$, $p = 0.038$, 95% CI [0.016, 0.479]), indicating a small-to-moderate effect size (Fisher's $z = 0.269$, SE = 0.129). Additionally, a significant negative correlation was identified between the CMJ Bilateral Asymmetry Index and the BAI Posterior Hamstrings (60°/s) ($r = -0.319$, $p = 0.011$, 95% CI [-0.525, -0.077]), demonstrating a moderate inverse relationship (Fisher's $z = -0.331$, SE = 0.129). The overall regression model was significant, $F(6,56) = 2.42$, $p = 0.038$, $R^2 = 0.206$, indicating that the predictors collectively explained approximately 20.6% of the variance in the CMJ Bilateral Asymmetry Index. From the findings of this study, we conclude that the SLCMJ asymmetry index cannot replace the asymmetry index from isokinetic strength tests.

Keywords: asymmetry; youth; soccer; isokinetic strength; countermovement jump

1. Introduction

In football, as in most sports involving ball handling, athletes develop a preference for using one of their legs to control the ball. Typically, a football player uses their dominant leg to shoot and pass the ball, while the other leg is considered the non-dominant leg [1]. The choice of the dominant leg often influences the player's position on the field. However, in addition to movements involving the ball, most movements made by football players during matches or training sessions are performed without the ball and are characterized

by the preference for the dominant leg [2,3]. Such movements include changes of direction, take-offs for jumping, feints, and tackles, among others.

Over time, this preference has been shown to lead to asymmetries between the limbs in performance on specific physical tests (e.g., single-leg jumps, isokinetic strength measurements) [4]. When these asymmetries exceed 15%, they have been associated with an increased risk of injury [5,6] as well as a negative impact on athletic performance [7–11]. These asymmetries have been observed at all levels of football players, including professionals, amateurs, and youth players [11–13].

Over the years, various tests have been used to detect asymmetries, such as isokinetic strength measurements [14,15], vertical jump tests [8,16], horizontal jump tests [7,17,18], and strength exercises (e.g., back squat) [19]. However, it should be noted that these tests differ significantly from each other. For example, the assessment using an isokinetic dynamometer focuses on one joint and measures different types of strength depending on the speed of movement, whereas a jump test is a multi-joint test that evaluates the power/jumping ability of the athlete's lower limbs. Therefore, the appropriate test should be chosen depending on the specific goal.

Among these tests, the isokinetic dynamometer is the most reliable for assessing strength asymmetries. However, this test requires specialized personnel for administration, is time-consuming, and has high costs, which means it is not frequently conducted by teams for all their players. In contrast, tests that involve single-leg jumps can be conducted with much more affordable equipment, require less specialized expertise from the evaluator, and are much quicker. Additionally, many jumping tests are part of the weekly routine for football teams. Thus, if there was a relationship between the detection tests (isokinetic evaluation and jump tests), it would be possible to detect not only strength asymmetries but also asymmetries in various forms of strength using a jumping test.

This approach could help identify asymmetries in the lower limbs (across a broader spectrum of physical abilities, maximum strength, power-speed, and strength endurance) as part of the weekly routine for football players, enabling the implementation of individualized programs before these asymmetries affect performance or become a risk factor for injury.

Therefore, the aim of this study was to examine the relationship between the asymmetries observed in the application of the single-leg countermovement jump (SLCMJ) and those observed through isokinetic strength assessment at $60^\circ/\text{s}$, $180^\circ/\text{s}$, and $300^\circ/\text{s}$ in high-level youth football players. We hypothesize that the asymmetries observed in the SLCMJ will be positively correlated with the asymmetries observed in the isokinetic strength evaluation ($60^\circ/\text{s}$, $180^\circ/\text{s}$, and $300^\circ/\text{s}$), and particularly at $300^\circ/\text{s}$, which is an indicator of the lower limb's power-speed.

2. Methods

2.1. Participants

A priori power analysis was conducted to estimate the appropriate sample size for detecting a significant correlation between the CMJ Bilateral Asymmetry Index and isokinetic variables. The analysis was performed using G*Power version 3.1.9.7 [20,21], and the following parameters were applied: a two-tailed test, an α error probability of 0.05, a power ($1-\beta$) of 0.80, and an expected effect size (Cohen's q) of 0.4 [22]. Based on these criteria, the analysis determined that a sample size of 62 participants would be required to achieve sufficient statistical power. To ensure robustness and accommodate any potential exclusions, we decided to include a total of 63 participants in the study.

The study included 63 high-level youth football players. The characteristics of the participants are presented in Table 1.

Table 1. Characteristics of participants.

Variable	Mean	SD
Age (yrs)	15.4	0.9
Height (m)	1.74	0.08
Weight (kg)	64.75	9.23
Body mass index	21.3	1.8
Body fat (%)	11.7	1.7
Lean mass (kg)	57.08	7.92
Fat mass (kg)	7.67	1.73
PHV (yrs)	1.7	0.8

PHV, peak height velocity.

Regarding playing positions, central midfielders (CM) constituted the largest group, comprising 30.2% of the sample, followed by side midfielders (SM) at 27.0%, central defenders (CD) at 19.0%, side defenders (SD) at 11.1%, goalkeepers (GK) at 7.9%, and forwards (F) at 4.8%. Dominance in leg use was also assessed, revealing that 76.2% of the players favored their right leg, while 23.8% were left-leg dominant (Table 2).

Table 2. Frequencies of position and dominant leg.

Position	Counts	% of Total	Cumulative %
GK	5	7.9	7.9%
CM	19	30.2	38.1%
SM	17	27.0	65.1%
CD	12	19.0	84.1%
SD	7	11.1	95.2%
F	3	4.8	100.0%
Right leg	48	76.2	76.2
Left leg	15	23.8	100

GK, goalkeeper; CM, central midfielder; SM, side midfielder; CD, central defender; SD, side defender; F, forward.

To participate, the players had to meet the following criteria: (a) no musculoskeletal injuries in the past two months, (b) no current medication use. All players were fully informed about the study's requirements and provided appropriate consent to participate, with consent from a parent or guardian for players under the age of 18. The investigation was also approved by the institutional review board.

The study took place at the beginning of the preseason during the players' ergometric testing. Specifically, the players were in their first week of training with the team after the transitional period. The participants were members of a high-level academy of a professional football team and had been training systematically for approximately 10 years. The previous year, they had finished in the top positions of their country's domestic championship.

2.2. Procedures

The participants were familiar with the tests, as these were assessments they had performed annually during their ergometric evaluations. All tests were conducted in the morning, between 9:00 and 12:00 AM, in a laboratory setting with stable temperature and humidity conditions (~22 °C, ~40% humidity). Initially, the participants arrived at the lab, and their anthropometric characteristics were measured. This was followed by a 10 min warm-up, consisting of 7 min of cycling on a cycle ergometer (Monark 839, Varberg, Sweden) at 100 Watts and 3 min of stretching exercises for the lower limbs (hamstrings, quadriceps, gastrocnemius, adductors) and arm rotations. The jump test was then conducted, followed

by the isokinetic strength evaluation. Additionally, the participants were allowed to drink water ad libitum to ensure proper hydration during testing.

2.3. Anthropometric Measurements and Assessment of Maturation

To measure body mass, the players removed their clothing and shoes and stood in their underwear. The measurement was taken using an electronic scale with an accuracy of 0.1 kg (Seca 220e, Hamburg, Germany). The same device also included a stadiometer, which was used to measure the players' height with an accuracy of 0.1 cm. To assess body fat percentage, a skinfold caliper (Lange, Beta Technology, Santa Cruz, CA, USA) was used to measure four skinfold sites (biceps, triceps, suprailiac, and subscapular), and Siri's equation [23] was applied to calculate the body fat percentage.

The chronological age at peak height velocity (PHV) of the players was estimated using the equation proposed by Moore and colleagues [24]. Early, average, and late maturation of the children were defined as those players with an estimated chronological age at PHV of less than 13 years of age, 13–15 years of age, and more than 15 years of age [25].

2.4. Single-Leg Countermovement Jump (SLCMJ)

The single-leg countermovement jump was measured using the electronic leap mat Chronojump from Boscosystem (Chronojump, Boscosystem, Barcelona, Spain). The participants stood in an upright position and lifted one leg off the ground with their hands placed on their hips. From this position, they bent the knee of the supporting leg to a self-selected depth, followed by a quick upward vertical movement. Two jumps were performed for each leg, and the best performance was used for analysis. If the jump technique was incorrect, the player was asked to repeat the jump after a one-minute recovery.

2.5. Isokinetic Strength Testing

The isokinetic strength of the hamstring and quadriceps muscles was measured using a Cybex II isokinetic dynamometer (Lumex Inc., Ronkonkoma, New York, NY, USA). For each angular velocity, peak isokinetic torque was recorded simultaneously, and the torque produced by the limb weight and the dynamometer arm was extracted from the collected data. Each participant then sat on the dynamometer in an adjustable chair, with their upper body secured by straps placed diagonally across the chest and around the hips and thighs to minimize any unwanted joint movement. The knee being tested was positioned at 90° of flexion (with 0° representing full knee extension) to align with the axis of the dynamometer lever arm at the distal point of the lateral femoral condyle. The length of the lever arm was adjusted individually for each participant, and the resistance pad was positioned just above the medial malleolus. The other leg remained relaxed, hanging freely without any applied force. Knee movements followed a specific sequence: extension was performed from the 90° flexed position, and flexion was performed from full extension (0°). Participants were instructed to kick their tested leg as hard and fast as possible through the full range of motion (ROM) while keeping their arms crossed over their chest. Three repetitions were performed at each angular velocity, with the peak torque (PT) value being recorded. A 30 s rest period was allowed between repetitions, and a 60 s rest interval was given between assessments at different angular velocities. During the session, participants were encouraged to perform maximal voluntary contractions with verbal positive reinforcement. Data were collected at angular velocities of 60, 180, and 300°/s, and maximal isokinetic strength was identified as the torque produced by the hamstring and quadriceps muscles during movements through their full ROM [26,27]. The ratio of peak torques at each angular velocity reflects the concentric strength ratio between the knee flexors and extensors. The traditional H:Q ratio was calculated by dividing each

participant's highest concentric peak torque for leg flexion by their highest concentric peak torque for leg extension.

2.6. Asymmetry Index

To calculate asymmetry, both in the CMJ (Countermovement Jump) and in isokinetic exercises at angular velocities of $60^\circ/\text{s}$, $180^\circ/\text{s}$, and $300^\circ/\text{s}$, the Bilateral Asymmetry Index was utilized, based on the following equation: $[\text{dominant leg} - \text{non-dominant leg}] / [\text{dominant leg} + \text{non-dominant leg}] \times 100$.

This method assesses inter-limb asymmetry during bilateral CMJs and was previously used [28,29]. To determine the direction of asymmetry during unilateral CMJs, an "IF function" ($\text{IF} \times [\text{non-dominant leg} < \text{dominant leg}, 1, -1]$) was added to the end of the equation, as suggested in previous research [30].

Leg preference during bilateral CMJs was identified through a questionnaire for the identification of dominant and non-dominant limbs, aligned with prior studies, such as those by Pérez-Castilla et al. (2021) [31].

2.7. Statistical Analysis

Initially, G*Power version 3.1.9.7 [20,21] was employed to estimate the required sample size and ensure adequate statistical power for the analysis. Descriptive statistics and correlation analyses were performed using JASP (version 0.19.3.0 for Windows), IBM SPSS Statistics (version 29 for Windows), and Jamovi (version 2.6.23.0 for Windows) [32–35]. In the current analysis, the correlation levels were interpreted as follows: coefficients around $r = 0.10$ were considered small, $r = 0.30$ indicated moderate relationships, and $r = 0.50$ or higher reflected strong correlations, in line with Cohen's effect size guidelines. Effect sizes for the observed correlations were calculated using Fisher's z transformation to provide a standardized measure of relationship strength, accompanied by standard error measurements. Furthermore, linear regression analysis was performed to examine the predictive relationship between the CMJ Bilateral Asymmetry Index and the isokinetic variables. Statistical significance was set at $p < 0.05$.

3. Results

The results regarding the biological maturation of the participants showed that, out of the total of 63 participants, 24 were in the stage of normal maturation, while the rest were all in the stage of early maturation. The characteristics of the youth football players are presented in the next table.

The correlation analysis revealed significant relationships between the CMJ Bilateral Asymmetry Index and specific isokinetic strength variables. A significant positive correlation was observed between the CMJ Bilateral Asymmetry Index and the BAI Anterior Quadriceps ($60^\circ/\text{s}$) ($r = 0.262$, $p = 0.038$, 95% CI [0.016, 0.479]), indicating a small-to-moderate effect size (Fisher's $z = 0.269$, SE = 0.129). This finding indicates a modest but meaningful association between bilateral asymmetry and the anterior quadriceps performance at lower angular velocities.

In contrast, a significant negative correlation was identified between the CMJ Bilateral Asymmetry Index and the BAI Posterior Hamstrings ($60^\circ/\text{s}$) ($r = -0.319$, $p = 0.011$, 95% CI [-0.525, -0.077]), demonstrating a moderate inverse relationship (Fisher's $z = -0.331$, SE = 0.129). This suggests that greater bilateral asymmetry in the CMJ is associated with reduced performance in the posterior hamstrings at this angular velocity.

No significant correlations were found between the CMJ Bilateral Asymmetry Index and the other variables, including BAI Anterior Quadriceps at $180^\circ/\text{s}$ ($r = 0.138$, $p = 0.280$, 95% CI [-0.114, 0.373]) and $300^\circ/\text{s}$ ($r = 0.121$, $p = 0.347$, 95% CI [-0.131, 0.358]), or the BAI

Posterior Hamstrings at 180°/s ($r = 0.162$, $p = 0.205$, 95% CI $[-0.090, 0.394]$) and 300°/s ($r = -0.107$, $p = 0.404$, 95% CI $[-0.346, 0.145]$).

These findings indicate specific relationships between bilateral asymmetry and muscle performance at angular velocities of 60°/s, indicating distinct roles for the anterior quadriceps and posterior hamstrings (Table 3 and Figure 1).

Table 3. Pearson correlation results between CMJ Bilateral Asymmetry Index (BAI) and muscle strength asymmetries at various angles.

Variable	Pearson's r	p	95% CI Lower	95% CI Upper
BAI Anterior Quadriceps (60°/s)	0.262 *	0.038 *	0.016	0.479
BAI Anterior Quadriceps (180°/s)	0.138	0.280	−0.114	0.373
BAI Anterior Quadriceps (300°/s)	0.121	0.347	−0.131	0.358
BAI Posterior Hamstrings (60°/s)	−0.319 *	0.011 *	−0.525	−0.077
BAI Posterior Hamstrings (180°/s)	0.162	0.205	−0.090	0.394
BAI Posterior Hamstrings (300°/s)	−0.107	0.404	−0.346	0.145

* $p < 0.05$.

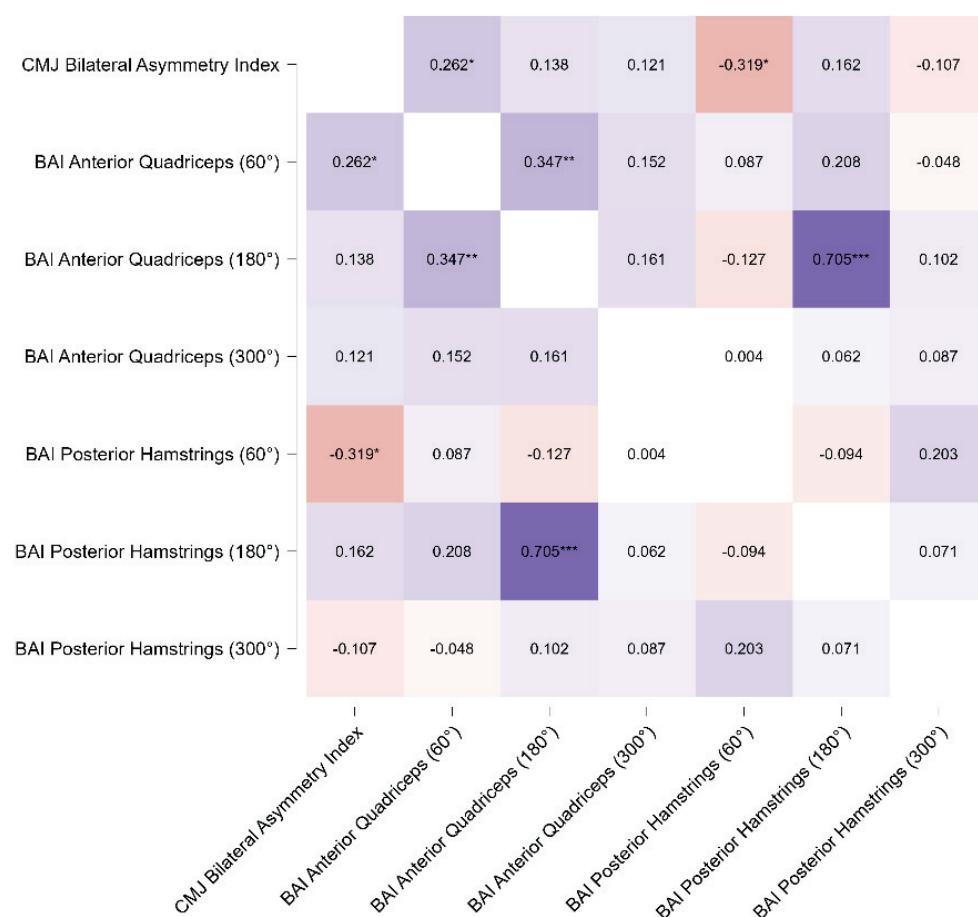


Figure 1. Pearson's correlation matrix of CMJ Bilateral Asymmetry Index (BAI) and isokinetic variables across different angular velocities. The heatmap displays Pearson's correlation coefficients (r) between the CMJ Bilateral Asymmetry Index and biomechanical variables, including BAI Anterior Quadriceps (60°/s, 180°/s, 300°/s) and BAI Posterior Hamstrings (60°/s, 180°/s, 300°/s). Significant correlations are indicated with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Warmer colors indicate positive correlations, while cooler colors represent negative correlations.

Moreover, a multiple linear regression analysis was conducted to explore the relationship between the CMJ Bilateral Asymmetry Index and the biomechanical predictors, including BAI Anterior Quadriceps (60°/s, 180°/s, 300°/s) and BAI Posterior Hamstrings (60°/s,

180°/s, 300°/s). The overall regression model (Table 3) was significant, $F(6,56) = 2.42$, $p = 0.038$, $R^2 = 0.206$, indicating that the predictors collectively explained approximately 20.6% of the variance in the CMJ Bilateral Asymmetry Index. The regression coefficients revealed that BAI Anterior Quadriceps (60°/s) was a significant positive predictor of the CMJ Bilateral Asymmetry Index, $B = 0.208$, $SE = 0.094$, $t(56) = 2.211$, $p = 0.031$, with a standardized estimate of $\beta = 0.288$ and a 95% confidence interval of [0.027, 0.549]. Conversely, BAI Posterior Hamstrings (60°/s) demonstrated a significant negative relationship with the CMJ Bilateral Asymmetry Index, $B = -0.203$, $SE = 0.074$, $t(56) = -2.723$, $p = 0.009$, with a standardized estimate of $\beta = -0.340$ and a 95% confidence interval of [-0.589, -0.090]. The other predictors, including BAI Anterior Quadriceps (180°/s), BAI Anterior Quadriceps (300°/s), BAI Posterior Hamstrings (180°/s), and BAI Posterior Hamstrings (300°/s), did not show statistically significant contributions to the model ($p > 0.05$) (Table 4). These results highlight the specific influence of BAI Anterior Quadriceps and BAI Posterior Hamstrings at 60°/s angular velocity on bilateral asymmetry during CMJ performance.

Table 4. Linear regression results for CMJ Bilateral Asymmetry Index.

Predictor	B	SE	95% CI (B)	t	p	β	95% CI (β)
Intercept	3.8571	0.7940	2.2665	5.4477	4.858	<0.001	—
BAI Anterior Quadriceps (60°/s)	0.2083	0.0942	0.0196	0.3970	2.211	0.031 *	0.2878
BAI Anterior Quadriceps (180°/s)	-0.0479	0.0684	-0.1850	0.0891	-0.701	0.486	-0.1258
BAI Anterior Quadriceps (300°/s)	0.0627	0.0835	-0.1046	0.2300	0.750	0.456	0.0916
BAI Posterior Hamstrings (60°/s)	-0.2029	0.0745	-0.3523	-0.0536	-2.723	0.009 *	-0.3396
BAI Posterior Hamstrings (180°/s)	0.0499	0.0542	-0.0587	0.1585	0.921	0.361	0.1552
BAI Posterior Hamstrings (300°/s)	-0.0154	0.0620	-0.1396	0.1088	-0.248	0.805	-0.0308

Note: $F(6,56) = 2.42$, $p = 0.038$, $R^2 = 0.206$ * Statistically significant predictors (* $p < 0.05$). CI = Confidence Interval. B represents unstandardized coefficients, SE is the standard error, β denotes standardized coefficients, and 95% confidence intervals (CI) are provided for both B and β .

4. Discussion

The results of the study partially confirmed the initial hypothesis. Specifically, a positive correlation was observed between the asymmetry in the SLCMJ and the BAI Anterior Quadriceps (60°/s), as well as a negative correlation between the asymmetry in the SLCMJ and the BAI Posterior Hamstrings (60°/s). No correlations were observed with strength measurements at other angular velocities. Additionally, the regression analysis showed that the BAI Anterior Quadriceps (60°/s) was a significant positive predictor of the CMJ Bilateral Asymmetry Index, while the BAI Posterior Hamstrings (60°/s) demonstrated a significant negative relationship with the CMJ Bilateral Asymmetry Index.

As mentioned earlier, asymmetry in isokinetic strength above 10% has been reported as a potential risk factor for injury [36,37], although there are studies that set the risk threshold at 15% [5,6]. Research interest has also focused on the effect of asymmetry on football players' performance in physical performance indicators [38,39]. However, it seems that the results of these studies are not clear-cut, and the impact of asymmetry appears to depend on how it is assessed and the individual involved [40]. Therefore, it is evident that there is considerable room for further study on this topic.

In the present study, the participants were adolescents, and it is well known that biological maturation can significantly affect performance in physical ability tests [41,42]. The results of this study showed that approximately one-third of the participants were at a stage of normal maturation, while the remaining two-thirds were at a stage of early maturation. This observation is likely due to the fact that the participants are members of a professional football team academy. Often, biologically mature football players outperform their peers (normal maturation, delayed maturation) in physical abilities, which leads to them being selected by team scouts and coaches. This is likely why the majority of

participants in this study were at an early maturation stage, and there were no participants in the late maturation stage. However, although the maturation stage of the participants was assessed, the effect of maturation level on the asymmetry indices and performance of the participants was not examined. This fact is mentioned as a limitation of the study.

Maximum strength is an important factor for jumping ability [43]. Specifically, a positive correlation between strength and jumping ability has been reported [44,45]. Strength improvement positively affects the rate of force development, which is particularly crucial for fast movements such as jumping [46,47]. It has been observed that vertical jump height can be explained by knee extensor and flexor strength by approximately 30% [36]. Thus, deficiencies in strength could affect jumping performance.

In one of the earliest studies on this subject [29], researchers investigated the relationship between bilateral asymmetry in isokinetic knee strength and the single-leg jump. The results indicated that the asymmetry index at $180^\circ/\text{s}$ of isokinetic strength was positively correlated with the bilateral asymmetry index for the maximum flexion angle and the mean knee joint torque during single-leg jumps. In the present study, as previously mentioned, a positive correlation was observed between the asymmetry in the SLCMJ and the asymmetry in the isokinetic strength of the quadriceps at $60^\circ/\text{s}$, and a moderate negative correlation was found between the asymmetry in the SLCMJ and the asymmetry in the isokinetic strength of the hamstrings at $60^\circ/\text{s}$. More recent studies also report correlations between asymmetry indices in different tests [30], though these correlations were characterized as “poor” in some cases. This may be due to the use of an isometric strength test, as opposed to the current study, which utilized isokinetic strength assessment across the full range of motion of the joint at three different angular velocities.

The literature includes studies that found no correlations between the asymmetry indices used. For example, Loturco et al. (2018) [48] studied whether asymmetries in isokinetic strength ($60^\circ/\text{s}$ and $300^\circ/\text{s}$) were related to asymmetries in jump tests (squat jump, CMJ). Their results showed no correlations except for one between squat jump asymmetry and knee flexion at $60^\circ/\text{s}$. In a later study [39], asymmetry indices derived from the Abalakov test, a change of direction test, and an iso-inertial power test were found to be unrelated. A lack of correlations between asymmetry indices was also reported in another study [38]. The limited correlations observed suggest that multiple and varied tests are needed to detect asymmetries in football players. Furthermore, since the above studies involved both genders and participants of different ages and skill levels, it appears that the lack of relationship between asymmetry indices across different tests is more related to the nature of the parameter being assessed and less to the characteristics of the sample [39].

As mentioned earlier, the results of the regression analysis in this study showed that the quadriceps and hamstring asymmetry indices could explain 20.6% of the variance in the CMJ Bilateral Asymmetry Index. In this case, an R-squared value of 20.6% suggests that approximately 20.6% of the variation in the dependent variable (SLCMJ asymmetry) can be explained by the independent variables included in the model, while the remaining 79.4% may be attributed to factors not captured by the model or random variation. Although this is not a very high proportion, it can still provide useful insights depending on the context and domain of the analysis.

This study has certain limitations. The participants were only male athletes in the developmental age group, meaning the results cannot be generalized to both genders or to different skill levels. The effect of biological maturation on the asymmetry indices has not been examined. Additionally, to detect asymmetry in the SLCMJ, equipment that calculates jump height based on flight time was used. The use of a force plate would provide more detailed and accurate data. Furthermore, only isokinetic strength assessments of the knee flexors and extensors were conducted. Therefore, future research involving both

genders and utilizing a force plate, as well as isokinetic strength evaluations of hip muscles, which significantly affect jump performance [49,50], may uncover stronger correlations and relationships between variables.

5. Conclusions

The results showed that no significant correlations were observed between the two asymmetry indices across all their parameters, meaning that the isokinetic dynamometer asymmetry index cannot be replaced by the SLCMJ asymmetry index. The asymmetries observed in football players depend on the physical ability being assessed, and a combination of tests is required to form a complete picture of the player. Coaches and rehabilitation professionals should use both methods (SLCMJ asymmetry index and isokinetic strength asymmetry index) for a comprehensive assessment of lower-limb asymmetry in young football players. More specifically, the SLCMJ asymmetry index should be used for jumping ability, while the corresponding asymmetry indices should be used for isokinetic strength.

Author Contributions: Y.M., T.I.M., A.M. and A.S. designed the study and provided critical feedback on the manuscript; A.M., A.E.K., G.A., R.K. and V.K. collected and processed. C.B., L.V. and E.S. analyzed the data. Y.M., A.S. and T.I.M. revised the first draft; A.S. and Y.M. conducted the statistical analysis. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The Ethics Committee of the School of Physical Education and Sport Science at Thessaloniki hereby approved the study (App. No. 188/2024).

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

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: The authors thank the players of the team who participated in the study.

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

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Article

Acute Effects of Isometric Conditioning Activity with Different Set Volumes on Countermovement Jump Performance in Highly Trained Male Volleyball Players

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Abstract: Background: This study aimed to evaluate the impact of different set volumes and durations of isometric conditioning activity (ICA) (a single 9 s set vs. three 9 s sets, totaling 27 s) on countermovement jump (CMJ) performance in highly trained male volleyball players. Understanding these effects is essential for optimizing warm-up protocols, particularly before competitions or high-intensity training sessions, to maximize performance outcomes. Methods: The 14 participants completed three conditions: one set of ICA (9 s) and three sets of ICA (27 s), each involving maximal isometric contractions in the half-back squat position, as well as a control condition without ICA. Approximately 3 min pre-ICA and at 3, 6, and 9 min post-ICA, the CMJ performance was assessed. Results: The results showed a significant interaction between CMJ height ($p = 0.002$) and relative peak power ($p = 0.004$) with the three-set condition. Post hoc comparisons indicated a significant increase in CMJ height at 3 min ($p = 0.018$; effect size [ES]: 0.868) and 6 min ($p = 0.044$; ES: 0.808), and a relative peak power output at 3 min ($p = 0.005$; ES: 0.874) and 6 min ($p = 0.034$; ES: 0.745) post-ICA compared to pre-ICA. No significant interactions or main effects were found for the modified reactive strength index or CMJ contraction time. Conclusions: The findings suggest that a three-set ICA with a total duration of 27 s may acutely improve CMJ height and relative peak power output in highly trained volleyball players.

Keywords: PAPE; post-activation performance enhancement; resistance training; sports performance

1. Introduction

Post-activation performance enhancement (PAPE) refers to a short-term improvement in athletic performance following the implementation of specific conditioning activities (CAs) preceding a subsequent sport task [1]. According to Kalinowski et al. [2], two key factors that determine the successful implementation of PAPE effects in training settings

are exercise specificity and achieving an optimal balance between fatigue and potentiation [3,4]. Therefore, these CAs typically involve high-intensity isotonic or isometric exercises performed prior to high-velocity movements with similar biomechanical patterns, such as heavy squats before vertical jumps [4]. Existing research suggests that the most likely mechanism explaining the short-term improvement in neuromuscular efficiency is attributed to the phosphorylation of myosin regulatory light chains and the increased recruitment of higher-order motor units [5]. Additionally, PAPE may be associated with an increase in muscle temperature, reduced pH levels, enhanced blood flow, and fluid circulation within muscle fibers [6]. In sports such as volleyball, an increased jump height directly benefits technical actions like setting, spiking, serving, and blocking [7], which are crucial for achieving success in a volleyball game [8].

Isometric conditioning activities (ICAs) are widely employed in both research and athletic training due to their effectiveness in enhancing power performance [9–11]. Additionally, they are simple to implement and can be integrated into various training plans without the need for specialized equipment. The PAPE effect induced by ICA depends, among other factors, on the number of sets and the total duration of isometric contraction [9,12]. However, the literature still lacks definitive guidelines regarding the optimal number of sets and total ICA duration that maximize power output performance. While some studies suggest that even a brief total ICA duration of 3–9 s may suffice to enhance CMJ performance ($3.8 \pm 1.2\%$) in highly trained athletes [10], others highlight the need for longer protocols involving a greater number of sets and extended total ICA durations. Moreover, Spieszny et al. [1] demonstrated that three sets of ICA, each consisting of three repetitions of 3 s contractions with a cumulative ICA duration of 27 s, resulting in a significant improvement in CMJ height ($2.7\% (\pm 3.7\%)$) at the fourth minute and $3.6\% (\pm 5.7\%)$ at the eighth-minute post-ICA). The same protocol used by Krzysztofik et al. [4] also resulted in a significant enhancement of CMJ performance. Similarly, the study by Lum et al. [13] observed an increase in barbell velocity during the power clean in each set when evaluating a protocol consisting of three sets of ICA, each including three repetitions of maximum voluntary isometric contractions. However, the study by Lum et al. [14] assessed the PAPE effect at only one time point, immediately after each ICA set, and utilized a different type of ICA, such as a power clean starting position, as well as a post-ICA test, namely the power clean. Therefore, it is important to emphasize that studies to date have primarily examined only one specific set configuration (three sets of three maximum attempts) and one total ICA duration without direct comparisons [1,4,10]. This approach limits the ability to determine the optimal number of sets—and consequently, the total ICA duration—for maximizing PAPE effects assessed across several time points following the intervention of all ICA sets in highly trained athletes. In contrast, Seitz and Haff [3] indicated that single sets of CA for stronger individuals promote superior neuromuscular adaptations than multiple sets of CA, a phenomenon particularly relevant to highly trained athletes. As highlighted by Seitz and Haff [3] and Hodgson et al. [5], these mechanisms may be linked to the increased phosphorylation of myosin light chains, enhanced motor unit recruitment, and athletes' greater tolerance to an induced loading [15]. This effect could similarly apply to ICA protocols. It is worth considering that the use of ICA may lead to different outcomes compared to traditional isotonic CA due to factors such as reduced muscle damage, decreased fatigue levels, or other differences in neuromuscular adaptations. These factors suggest that the results obtained from studies on isotonic CA may not necessarily be fully applicable to ICA. Therefore, it is important not to rely solely on the existing literature on isotonic CA but rather to conduct direct comparisons of the effectiveness of various series volumes (one set vs. three sets) and the total duration of ICA (9 s vs. 27 s) in order to better understand the adaptive mechanisms that may occur during ICA implementation.

Consequently, this study aims to fill this gap by directly comparing the effects of different volumes of sets (one set vs. three sets) and total durations of ICA (9 s vs. 27 s) on CMJ performance in highly trained athletes, measured at multiple time points post-ICA, in comparison to a control condition (CTRL). We hypothesized that both protocols would increase CMJ performance at all measurement points (3, 6, and 9 min), with the greatest effect achieved at 6 min; this is typically when the peak response occurs [6]. Additionally, we expected the three-set protocol to produce a greater improvement in CMJ height compared to the one-set protocol.

2. Materials and Methods

2.1. Experimental Approach to the Problem

The study was conducted using a randomized crossover design, in which each participant completed 3 experimental sessions to compare the acute effects of pushing isometric muscle action (PIMA) isometric squats (serving as ICA) on post-activation CMJ performance changes. The participants were randomly assigned to one of two experimental conditions with varying numbers of ICA sets and the total duration of ICA or a control condition (without CA). Each condition consisted of a distribution of 3 isometric contractions lasting 3 s, with a micro-break of less than 1 s between the contractions. The total ICA duration for the first condition, with 1 set of ICA, was 3 contractions per 3 s = 9 s (ISO-1), while the second condition included 3 sets of ICA, 3 contractions per 3 s = 27 s (ISO-3). Additionally, a control condition (CTRL) without any ICA was included. CMJ measurements were taken approximately 3 min before CA and 3, 6, and 9 min after completing all ICA sets, depending on the condition. In CTRL conditions, measurements were taken at the same time points but without the application of ICA (Figure 1).

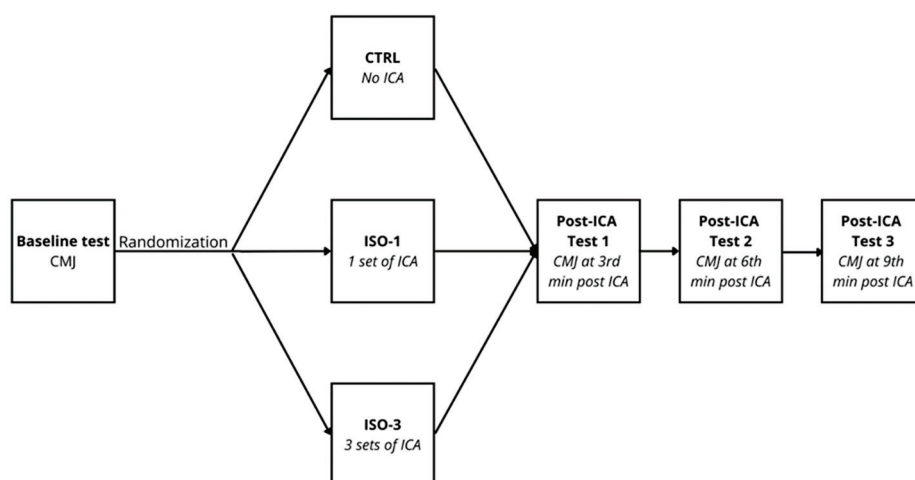


Figure 1. Study design flowchart. CMJ—countermovement jump; ICA— isometric conditioning activity. CTRL—control condition (without CA); ISO-1—condition with 1 set of ICA; ISO-3—condition with 3 sets of ICA.

2.2. Participants

The study involved 14 highly trained male volleyball players (Table 1) [16]. The inclusion criteria were as follows: (a) a minimum of 5 years of experience in volleyball training; (b) performing at least 3 strength training sessions per week over the past 5 years; and (c) no musculoskeletal injuries (resulting in a training break longer than 4 weeks) for at least 6 months prior to the start of the study. Participants were instructed to abstain from any resistance training exercises within 48 h before the commencement of the experimental session. Additionally, they were advised to maintain their regular dietary habits and refrain from the consumption of any supplements or stimulants during the week preceding the

experiment. During the experimental session, body composition was assessed using multi-frequency bioelectrical impedance analysis conducted under laboratory conditions with the InBody 770 device (InBody 770 Biospace Co., Ltd., Seoul, Republic of Korea). Participants were informed about the potential risks and benefits associated with their participation in the study, as well as their right to withdraw from the study at any time without providing a reason before providing their written informed consent for participation. The study was conducted using a randomized crossover design, where each participant remained unaware of the assigned experimental condition. Randomization was carried out using the random.org generator, assigning a unique number and sequence for each participant's session. After random assignment to a study condition, participants remained unaware of the further course of the experiment. Also, detailed information about the study's objectives and expected outcomes was not disclosed to them. The entire research protocol was conducted at the Academy of Physical Education in Katowice, Poland. The experiment received formal approval from the Bioethics Committee for Scientific Research (03/2021) at the Academy of Physical Education in Katowice in accordance with the ethical principles outlined in the Declaration of Helsinki of 1983.

Table 1. Descriptive characteristics of the study participants.

Age [years]	26 ± 4
Body mass [kg]	91 ± 13
Body fat [%]	10.1 ± 2.3
Body height [cm]	196 ± 8
Resistance training experience [years]	8.9 ± 4.0
Relative of 1RM BS [kg/bm]	1.49

1RM—one repetition maximum; BS—back squat; bm—body mass.

A sample size estimation using G*Power software (version 3.1.9.2, Dusseldorf, Germany) showed that to provide >80% power with a significance level of 0.05, correlation among the repeated measures of 0.5 in this study design ($n = 14$; two-way repeated-measures ANOVA, within factors) and an effect size of approximately $d = 0.5$ are required.

2.3. Familiarization Session

All experimental sessions were conducted between 9:30 and 11:30 AM. At least 3 days prior to the first experimental session, participants underwent a familiarization session, which included ICA and CMJ. Each session began with a standard warm-up, including a 5 min cycling session. The sequential exercises included air squats (10 repetitions), forward lunges (10 repetitions), leg swings (10 repetitions), jumping jacks (10 repetitions), and CMJ (5 repetitions). These exercises were performed as part of the athletes' usual warm-up routine for both training and competition. The session was followed by dynamic exercises, all conducted under the supervision of a certified strength and conditioning coach to ensure comprehensive preparation. Afterward, each participant performed 1 set of ICA, consisting of 3 repetitions with a 3 s maximal isometric contraction, followed by 3 CMJ attempts after a 3 min rest period following the completion of the set. Each participant was assigned an individualized rack height for performing the ICA, determined by the height of the barbell pins on the rack. The participants held a barbell on their shoulders, which was secured by the barbell pins to ensure isometric muscle action. The squat depth was set to a 120-degree angle at the knee joint, determined by an experienced coach using a goniometer (EasyAngle, Meloq AB, Stockholm, Sweden). An experienced weightlifting coach standardized body positioning to ensure a vertical torso posture across all conditions. Upon the researcher's command, participants were instructed to "push the barbell vertically upward as hard and

as fast as possible”, pressing their back against the barbell and pushing their feet against the floor. During the CMJ, the squat depth was not restricted.

2.4. Experimental Sessions

After the warm-up (identical to the familiarization session), participants performed a baseline assessment of CMJ performance. After a rest period of approximately 3 min, participants performed PIMA isometric squats as the ICA, depending on the condition, or no ICA (CTRL), in a randomized order. Individual adjustments for the barbell height during ICA were consistent with the familiarization session.

Depending on the assigned conditions, the athletes performed the following activities:

- CTRL—the control condition consisted of maintaining light physical activity, where participants were required to walk freely around the gym where the study took place for the time equivalent to performing the entire ICA set (10 s).
- ISO-1—1 set of ICA consisting of 3 repetitions of 3 s maximal isometric contractions, with a total ICA duration of 1 set per 9 s = 9 s, was performed.
- ISO-3—3 sets of ICA, each consisting of 3 repetitions of 3 s maximal isometric contractions, with a total ICA duration of 3 sets per 9 s = 27 s, including 3 min rest intervals between sets, was performed.

In accordance with the protocol, CMJ measurements in the CTRL condition were conducted at the same time points but without the application of ICA (Figure 1).

2.5. Measurement of Countermovement Jump Performance

Jump performance was assessed using a force platform (Force Decks, Vald Performance, Brisbane, Australia), which is recognized as a valid and reliable tool for measuring the kinematics of vertical jumps [13]. Each participant performed 3 repetitions of CMJ without arm swinging approximately 3 min before the ICA as a baseline and at 3, 6, and 9 min after each ICA. During the measurements, participants started in a standing position with their hands on their hips and maintained a straight-back posture to minimize angular displacement of the hips. They were instructed to remain as still as possible for more than 1 s before initiating the downward movement. Participants then descended into a squat position to a predetermined depth before performing a maximal-effort vertical jump. They were required to land in the same position from which they started, centrally on the force platform. Jump height (JH) was calculated based on the center of mass velocity at takeoff using the impulse–momentum relationship (imp–mom) [cm]. Furthermore, relative peak power output (PP), the modified reactive strength index (RSI_{mod}), and contraction time (CT) were also assessed.

3. Statistical Analyses

All statistical analyses were performed using JASP (Version 0.18.3; JASP Team, University of Amsterdam, The Netherlands) and were shown as means with standard deviations ($\pm$ SD). Statistical significance was set at $p < 0.05$. The normality of data distribution was verified using Shapiro–Wilk tests, while the assumption of variance sphericity was verified using Mauchly’s test. The two-way repeated measures of ANOVA (3 conditions [CTRL; ISO-1; ISO-3] $\times$ 4 time points [pre-ICA; 3rd, 6th, and 9th min post-ICA]) were used to investigate the influence of ICA on JH, PP, CT, and RSI_{mod} in the CMJ. When a significant interaction or main effects were found, post hoc tests with Bonferroni correction (p_{bonf}) were used to analyze pairwise comparisons. The effect sizes were determined by Cohen’s d , which was characterized as “trivial” ($d < 0.20$), “small” ($0.20 \leq d < 0.50$), “moderate” ($0.50 \leq d < 0.80$), or “large” ($d \geq 0.80$).

4. Results

The Shapiro–Wilk test did not indicate any violation of the normality assumption for any of the variables ($p > 0.062$ for all variables). Similarly, Mauchly’s test did not reveal any violation of the sphericity assumption ($p > 0.074$ for all variables).

4.1. Jumping Performance

A repeated measure two-way ANOVA showed a significant interaction for JH ($p = 0.002$; $\eta^2 = 0.226$) and PP ($p = 0.004$; $\eta^2 = 0.213$). The post hoc comparisons for interaction showed a significant increase in JH and PP in the ISO-3 condition at 3 and 6 min post-ICA compared to pre-ICA for JH (mean difference [MD] = 2.7 ± 3.2 cm; Cohen’s $d = 0.75$; $p_{\text{bonf}} = 0.018$ for 3 min, and MD = 2.5 ± 3.9 cm; Cohen’s $d = 0.68$; $p_{\text{bonf}} = 0.044$ for 6 min) and for PP (MD = 3.4 ± 2.3 [W/kg]; Cohen’s $d = 0.53$; $p_{\text{bonf}} = 0.005$ for 3 min, and MD = 2.9 ± 2.1 [W/kg]; Cohen’s $d = 0.43$; $p_{\text{bonf}} = 0.034$ for 6 min) (Table 2).

Table 2. Change in jump height and relative peak power between time points and conditions. BL—baseline; 3 min—3rd-minute post-ICA; 6 min—6th-minute post-ICA; 9 min—9th-minute post-ICA; *—significant difference in comparison to baseline value within the condition; $p < 0.05$.

	Baseline (Jump Height and Peak Power)	3 min (Jump Height and Peak Power)	6 min (Jump Height and Peak Power)	9 min (Jump Height and Peak Power)
CTRL	39.5 ± 3 cm, 52.5 ± 3.4 [W/kg]	38.1 ± 3.1 cm, 51.2 ± 4.2 [W/kg]	39.5 ± 2.4 cm, 52.1 ± 4.2 [W/kg]	38.6 ± 2.7 cm, 51.9 ± 3.7 [W/kg]
ISO-1	39.9 ± 2.1 cm, 53.9 ± 3.3 [W/kg]	38.6 ± 3 cm, 52.1 ± 4.0 [W/kg]	39.7 ± 3 cm, 53.1 ± 3.7 [W/kg]	40.1 ± 2.9 cm, 52.7 ± 3.8 [W/kg]
ISO-3	38.5 ± 3.8 cm, 52.5 ± 3.8 [W/kg]	41.2 ± 3.4 cm *, 54.6 ± 4.1 [W/kg] *	41.0 ± 3.6 cm *, 54.1 ± 3.6 [W/kg] *	39.9 ± 3.9 cm, 53.0 ± 4.6 [W/kg]

4.2. Modified RSI

A repeated measures two-way ANOVA did not show a significant interaction ($p = 0.441$; $\eta^2 = 0.070$), the main effect of time ($p = 0.120$; $\eta^2 = 0.137$), or condition ($p = 0.078$; $\eta^2 = 0.178$) for RSI_{mod} (Figure 2).

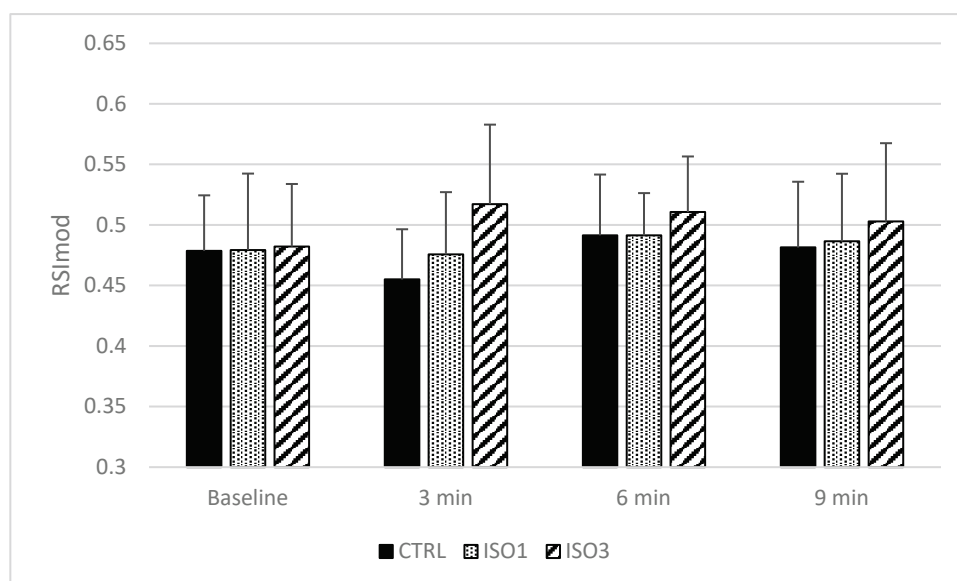


Figure 2. Change in RSI modified between conditions and time points in ISO-3. CTRL—control condition (without ICA); ISO-1—condition with 1 set of ICA; ISO-3—condition with 3 sets of ICA. BL—baseline; 3 min—3rd-minute post-ICA; 6 min—6th-minute post-ICA; 9 min—9th-minute post-ICA.

4.3. Contraction Time

A repeated measures two-way ANOVA did not show a significant interaction ($p = 0.758$; $\eta p^2 = 0.042$), the main effect of time ($p = 0.451$; $\eta p^2 = 0.065$), or condition ($p = 0.335$; $\eta p^2 = 0.081$) for the contraction time (Figure 3).

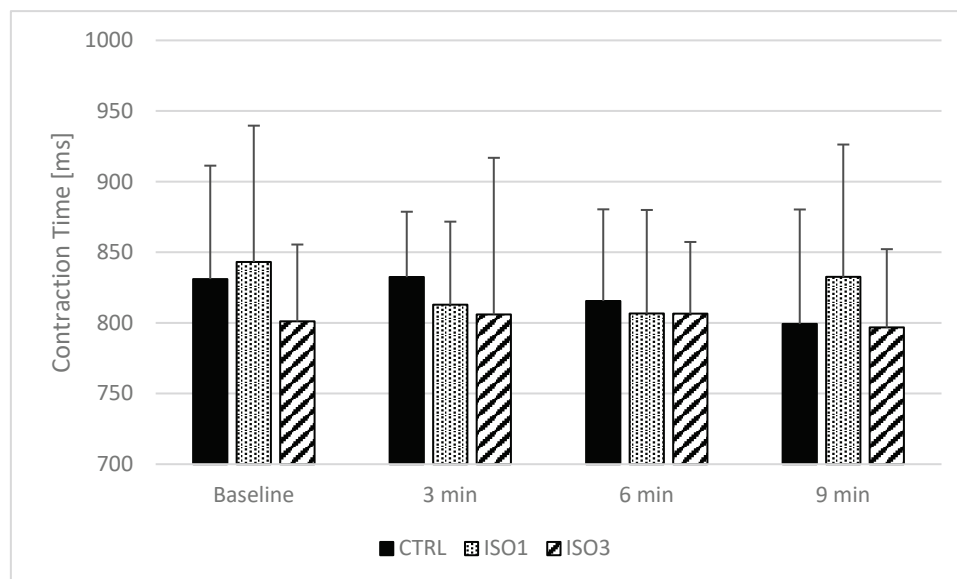


Figure 3. Change in contraction time between the conditions and time points in ISO-3—condition with 3 sets of ICA. BL—baseline; 3 min—3rd-minute post-ICA; 6 min—6th-minute post-ICA; 9 min—9th-minute post-ICA.

5. Discussion

The aim of this study was to compare different ICA volumes on CMJ performance among highly trained male volleyball players. The results demonstrated a significant increase in JH and PP from the baseline to 3 and 6 min after the ISO-3 condition, which consisted of three sets lasting 9 s each (total duration: 27 s), partially confirming the stated hypotheses. Contrary to expectations, no significant differences in JH were observed after the ISO-1 condition (single set lasting 9 s). The results emphasize that to increase the JH and PP output during CMJ in highly trained athletes, the ICA protocol should consist of three sets lasting 9 s each rather than a single set. Moreover, it should be noted that despite the lack of significant differences in RSImod and CT between the studied conditions, RSImod in the ISO-3 condition approached significance, which is also important in the context of interpreting the results. Although it did not reach full statistical significance, this suggests that changes in this parameter may potentially influence the CMJ outcomes, which should be considered in future studies.

The results of our study, which indicate an increase in JH and PP following the ISO-3 condition, align with previous findings by Spieszny et al. [1], which also highlight the beneficial impact of three sets of ICA on CMJ performance. According to the previously established hypotheses, ICA leads to different outcomes compared to traditional isotonic CA, as Seitz and Haff [3] suggested that single sets of CA in stronger individuals promote superior neuromuscular adaptations compared to multiple sets of CA. These differences may stem from the fact that isometric contractions are characterized by lower metabolic costs compared to concentric contractions, potentially enhancing athletic performance temporarily without inducing additional fatigue [17]. As noted by Balshaw et al. [18] and Jenkins et al. [19], a single ICA set may fail to provide a sufficient stimulus to fully recruit high-threshold motor units, which would explain the absence of significant differences in CMJ performance observed after a single ICA set in our study. However, it is not

only the number of sets but also the total duration of the effort that contributes to the volume of ICA, which appears to be a significant factor determining the effectiveness of ICA protocols in improving jumping performance and achieving the PAPE effect. Similarly to isotonic training, where time under tension (TUT) is used as a volume indicator in strength training [20], isometric total duration can also serve as a volume marker in the ICA protocol, determining how long the effort lasts, irrespective of the number of contractions performed. This is emphasized by Allen et al. [21], who reported that muscle damage induced by isometric contractions is primarily associated with the total duration of contractions rather than the number of contractions. Consequently, it can be hypothesized that the total duration of ICA may determine the occurrence of other adaptive changes observed with TUT in isotonic training, similar to the previously analyzed number of sets. However, further research is needed to identify the changes in neuromuscular and metabolic mechanisms occurring in muscles in relation to the total duration of ICA. Our results indicate that a total ICA duration of 27 s within three sets of ICA leads to a significant improvement in JH and PP, consistent with the findings of Spieszny et al. [1]. Conversely, the lack of significant improvement observed with a shorter ICA duration of 9 s partially corresponds to the findings of French et al. [12], who also did not report an improvement in CMJ height within one set of ICA. However, these results contradict the findings of Tsoukos et al. [10], who demonstrated a positive effect of the same ICA duration within three sets of ICA on CMJ height. These discrepancies may stem from differences in experimental protocols, such as the number of sets and the distribution of ICA within individual sets. In our study, we compared two durations of ICA—9 s and 27 s—within one and three sets of ICA protocols, with each set comprising three repetitions of 3 s contractions. By contrast, French et al. [12] analyzed a single contraction lasting 9 or 15 s within one set of ICA, while Tsoukos et al. [10] investigated single contractions lasting 3 s performed in three sets of ICA. Therefore, when analyzing the impact of ICA on the occurrence of the PAPE effect, it seems reasonable to examine not only the number of sets or the total duration of ICA but also the interdependence of these two parameters.

The ratio of the number of sets to the distribution of ICA, as studies have shown, is of significant importance. However, it seems that it cannot be analyzed without assessing the duration of the rest interval between ICA sets. Our findings indicate that a 3 min rest interval between ICA sets is a significant factor contributing to the increase in CMJ height, consistent with the findings of Spieszny et al. [1]. This suggests that longer rest intervals are particularly important in protocols involving a greater number of sets or extended ICA durations. Conversely, research by Tsoukos et al. [10] suggests that shorter 1 min rest intervals may be effective only in the context of fewer sets and shorter ICA durations. Physiological mechanisms such as phosphocreatine resynthesis and lactate clearance [22,23] justify the need for longer rest intervals to sustain high performance during subsequent ICA sets. However, variations in the literature and methodological differences underscore the need for further research to precisely define the optimal rest interval parameters in ICA protocols for enhancing CMJ height.

The level of training experience determines the occurrence of the PAPE effect [24]. Similarly, this may apply to the use of ICA, as although the parameters previously described indicate their significant impact, these studies confirm a relationship between training experience and the level of muscular strength [25]. As suggested by Tsoukos et al. [10] and Kilduff et al. [26], both muscular strength and a higher percentage of fast-twitch fibers in highly trained athletes, resulting from the increased phosphorylation of myosin light chains [15], are positively correlated with improvements in CMJ height and PP, which may explain the positive responses observed under the ISO-3 condition in this study. Additionally, highly trained athletes exhibiting better resistance to fatigue, a greater

buffering capacity, and improved motor unit synchronization [27,28] may have been better prepared to capitalize on the benefits of the ISO-3 condition. The increased buffering capacity and enhanced resistance to skeletal muscle damage suggest that athletes with high training levels are better adapted to endure intense stimuli. As reported by Tillin & Bishop [18], this translates to a prolonged PAPE effect, which is directly associated with higher firing frequencies and greater motor unit recruitment, possibly explaining the positive responses observed in this study. Therefore, it seems that highly trained participants require greater training stimulus and, thus, a higher number of sets and extended ICA duration with longer rest intervals between sets to improve both CMJ height and PP.

One of the limitations of this study is the lack of fatigue monitoring. A potential assessment of physiological factors, such as biochemical blood analysis (including lactate, creatine kinase, and cortisol), as well as the use of methods like tensiomyography or electromyography could potentially confirm whether a relationship exists between PAPE and fatigue and provide new insights into the obtained results. Additionally, the small sample size presents a limitation, as it may affect the generalizability of these findings. These limitations highlight the need for further comparative analyses in order to determine the interdependence of the number of sets, the total duration of ICA, and the rest interval duration, also considering other populations (e.g., athletes from different sports or training levels), all aimed at maximizing the PAPE effect. Such studies could aid in developing an optimal protocol and clarifying potential outcomes despite the various methodological differences described in the literature.

6. Conclusions

The presented study demonstrates that three sets with a total ICA duration of 27 s, with 3 min rest intervals between sets, have a significant effect on CMJ height and relative peak power output in highly trained male volleyball players compared to CTRL. However, contrary to the hypothesis, a single set with a total ICA duration of 9 s did not have a significant impact on CMJ performance. Furthermore, no significant differences were observed between different set volumes (one set vs. three sets) with varying total ICA durations—9 s (one set with three contractions of 3 s) vs. 27 s (three sets with three contractions of 3 s)—on CMJ height, suggesting that three sets with a total ICA duration of 27 s may have practical implications for improving both CMJ height and peak power output in highly trained male volleyball players. Based on these findings, the authors suggest the inclusion of three sets of ICA with 3 min rest intervals in warm-up protocols, especially before competitive events or intense training sessions, in order to optimize performance outcomes.

Author Contributions: Conceptualization, J.J. and M.K.; methodology, J.J. and D.G.; software, I.S.; validation, D.G. and P.E.; formal analysis, I.S. and P.E.; investigation, J.J. and D.G.; writing—original draft preparation, J.J.; writing—review and editing, J.J., M.W., D.L., P.E. and M.K.; supervision, M.W. and M.K.; project administration, M.W. and M.K.; funding acquisition, M.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The experiment received formal approval from the Bioethics Committee for Scientific Research (03/2021) at the Academy of Physical Education in Katowice, in accordance with the ethical principles outlined in the Declaration of Helsinki of 1983.

Informed Consent Statement: Written informed consent was obtained from all participants.

Data Availability Statement: The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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

Abbreviations

The following abbreviations are used in this manuscript:

ICA	Isometric conditioning activity
CMJ	Countermovement jump
PAPE	Post-activation performance enhancement
CTRL	Control condition
ISO-1	Condition with one set of ICA
ISO-3	Condition with three sets of ICA
PIMA	Pushing isometric muscle action
JH	Jump height
PP	Relative peak power output
RSImod	Reactive strength index modified
CT	Contraction time

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Article

Effect of 10-Week Plyometric Training on Anaerobic Performance and Biomechanical Properties of the Muscles in Football Players: Randomized Controlled Trial

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Abstract: This study investigated the effects of a 10-week plyometric training program on sprint performance, reactive power, and biomechanical muscle properties in soccer players. Twenty soccer players were randomly assigned to an experimental group ($n = 10$) or a control group ($n = 10$). Both groups maintained their regular weekly training, with the experimental group performing additional plyometric sessions twice weekly. Pre- and post-intervention assessments included 5 m and 30 m sprint times, Reactive Strength Index (RSI), and biomechanical properties (tension, stiffness, elasticity) of the rectus femoris (RF) and vastus lateralis (VL). The experimental group demonstrated significant improvements in 5 m ($p < 0.01$; ES = 1.44) and 30 m ($p < 0.01$; ES = 1.11) sprint times and RSI ($p < 0.05$; ES = 0.87). No significant changes were observed in muscle tension, stiffness, or elasticity at the group level. However, correlations indicated that higher baseline elasticity in the VL was linked to greater 5 m sprint improvements, while changes in RF elasticity were negatively associated with 5 m sprint gains. These findings suggest that plyometric training effectively enhances short-distance sprint performance and reactive power in soccer players. Although group-level biomechanical properties did not change significantly, individual variability in muscle elasticity may modulate training outcomes, supporting the integration of plyometric exercises into soccer training regimens.

Keywords: functional performance; physical activity; myotonometry; drop jump; sprint; stiffness

1. Introduction

Plyometric training is a widely used method that utilizes a biomechanical mechanism called the stretch-shortening cycle [1]. In this way, we can develop the ability of the neuromuscular system to generate the maximum force in the shortest time possible [2]. Plyometric exercises are characterized by high speed, short duration, and maximum effort, requiring the activation of motor units associated with fast-twitch type II muscle fibers [3]. A study that used a plyometric exercise protocol consisting of 10 sets of 10 jumps in opposing movements showed that this type of exercise caused preferential damage to type II muscle fibers. These results concluded that plyometric training also causes more significant hypertrophy of type II muscle fibers than type I [4]. It usually involves various jumps, leaps, or ballistic throws with the shortest possible contact phase with the ground/object

and the most significant intention of jumping or throwing the object. Such training is a common strategy in various sports disciplines [5]. The impact of plyometric training on skeletal muscle hypertrophy is often described as relatively minor. In a comprehensive review of neuromusculoskeletal adaptations and performance to plyometric training from 2010, Markovic and Mikulic [1] concluded that plyometric exercises can potentially induce muscle hypertrophy and are generally weaker than resistance-induced.

Plyometric training can positively affect professional and amateur soccer players [6]. Positive effects have also been observed in the form of increased strength among men and women, provided that it is performed with appropriate progression, respecting the principles of tissue adaptation time and at a sufficiently high intensity [7]. In many sports, the sprint component is crucial in the context of reaching a specific spot on the field faster than the opponent [8], as well as, in the case of soccer, winning the running duel for the ball or reorganizing the formation in defensive play. It has also been shown that sprinting (45%) and jumping (16%) are the two motor actions of a football player that most often lead to goal-scoring situations [9].

Moreover, it has been confirmed that combining plyometrics and body mass, including counter-movement jumps, depth jumps, and squat jumps, increases vertical jump height [7]. This type of training increases neuromuscular coordination by training the nervous system, thereby enabling the muscles' stretch-shortening cycle [10]. Properly programmed plyometric training positively affects sprint values, with athletes improving their times over distances of 20 and 30 m. Moreover, no significant differences were found in intensity and the addition of external resistance; however, regarding the results achieved, the selection of exercises proved significant.

Combining several exercises yielded better results than prescribing only one type of plyometric exercise [7,11]. Exercises aimed at reactive strength, such as the drop jump, which involves jumping off a platform and then jumping upwards with maximum intent, also significantly improved RSI values [12]. Increased pennation angle, tendon thickness, and increased stiffness are the changes in parameters observed after applying plyometric training [13].

Adaptive changes during training also concern the biomechanical properties of muscles, such as muscle tension (F), dynamic stiffness (S), and elasticity (D). Deficits in these properties can cause weakened athletic performance and be a reason for injuries in athletes [14]. In the case of the quadriceps muscles, they are one of the risk factors for developing knee joint disorders [15]. The primary function of tendons is to store and transmit the mechanical force of muscle contraction to the bones [16]. High values of dynamic stiffness in tendons due to adaptation to the type of training should allow an individual to withstand greater mechanical loads.

The study examined whether significant biomechanical changes occur in the femoris muscles following a training program, using the Myoton device for measurement. Consequently, a study enhances the range of measurement techniques available by incorporating this device. There is a need for more evidence regarding the methodology of this training, the types of exercises, and the assessment of changes in the biomechanical properties of muscles and their impact on the athlete's motor skills. Therefore, this experimental study aimed to assess the impact of a 10-week plyometric training program on running speed, selected parameters of explosive muscle strength, and changes in the biomechanical properties of the quadriceps muscles (muscle tension, flexibility, muscle stiffness) in amateur football players.

2. Materials and Methods

2.1. Participants

The G* power (v3.1.9.6, Kiel University, Kiel, Germany) software was employed to determine the a priori sample size. Recruiting at least 16 participants was necessary to achieve an effect size level of 0.6 ($\alpha = 0.05$; power = 0.96) [17]. Twenty male football players ($n = 20$) from the KS Polonia Łaziska-Górne football club competing in the IV league (age: 23.5 ± 10.5 years, BMI: 23.97 ± 3.96 kg/m², training experience: 14.5 ± 5.95 years), were randomly divided into two groups: the experimental group (eG-n = 10) and the control group (cG-n = 10) according to the following inclusion criteria: age 16–38 years, with a minimum of 5 years of training experience, training at least 3 times a week. The different ages and experiences of the players taking part in the study represented most of the football teams. All players had current medical examinations, allowing them to participate in football competitions. Exclusion from the examination concerned elevated blood pressure occurring before the examination (pressure >140/90 mmHg) in individuals treated after injuries, damage, or unspecified skin and myofascial musculoskeletal system changes. Exclusion could occur at any time during the study at the participant's request. All participants actively and regularly participated in training sessions and competitions during the study. The study was approved by the ethics committee of the Polish Physiotherapy Association (RESOLUTION No. 3 March 2024 dated 27 March 2024) and conducted by the Helsinki Declaration.

2.2. Study Design

The study was designed as a prospective experimental study with a control group (Figure 1). The experimental group underwent a 10-week plyometric training program. In contrast, the control group did not undergo plyometric training and followed a standard football training program. The group assignment was done through simple 1:1 randomization with a random sequence using the website [ranomizer.org](https://www.ranomizer.org). The group assignment was independent of the treatment time and the research staff. Each participant underwent an introductory intervention, plyometric training, receiving a 10-min briefing seven days before the study.

The control group ($n = 10$) followed the previously planned training by the coach for the duration of the study (10 weeks), which was typically football training encompassing the physical, technical, and tactical preparation of the football team players (Table 1). The experimental group ($n = 10$) incorporated a plyometric training protocol into their planned training, having previously conducted an introductory session with a research assistant who explained the exercises, demonstrated proper movement patterns, and provided feedback on the performed exercises. Each athlete participating in the study warmed up according to their training habits for about 10 min. The athletes performed the plyometric training protocol twice a week before the central part of the training. For 10 weeks with a minimum of 48 h between training sessions, they performed 60 to 72 jumps by the end of the protocol during one session in 2–4 sets. The break between each series was 120 s. Each competitor was equipped with a heart rate watch to ensure that their heart rate returned to readiness values. The intensity with which the athletes performed the exercises was increased; at the beginning of the protocol, the hurdles they jumped over and the box they jumped off were lower, and by the end, they were higher. The exercises included two-footed hurdles in a straight line, multiple jumps, and a drop from a platform with a reactive rebound. The training protocol is presented in Table 2. The training was conducted on the field and in the indoor hall at the Municipal Stadium in Łaziska Górne, at 3 Sportowa Street. Sprint measurements, reactive strength indicators, and tissue properties were conducted in the exact location in the afternoon from 5:00 PM to 6:00 PM. For each subject, the same

conditions for task execution and measurements were maintained. All participants were tested under the same conditions (5:00 PM to 6:00 PM). The essential characteristics of the research group are presented in Table 3. All participants underwent the following measurements: sprint time for 10 and 30 s (s), reactive strength index (RSI-[$\text{m}\cdot\text{s}^{-1}$]), muscle tension (F -[$\text{N}\cdot\text{Hz}$]), stiffness (S -[N/m]), and elasticity (D -[$\text{N}\cdot\text{N}$]). The measurements were taken during the following periods: (1) before the implementation of the programmed “pre” training; (2) in the 10th week of the programmed “post” training.

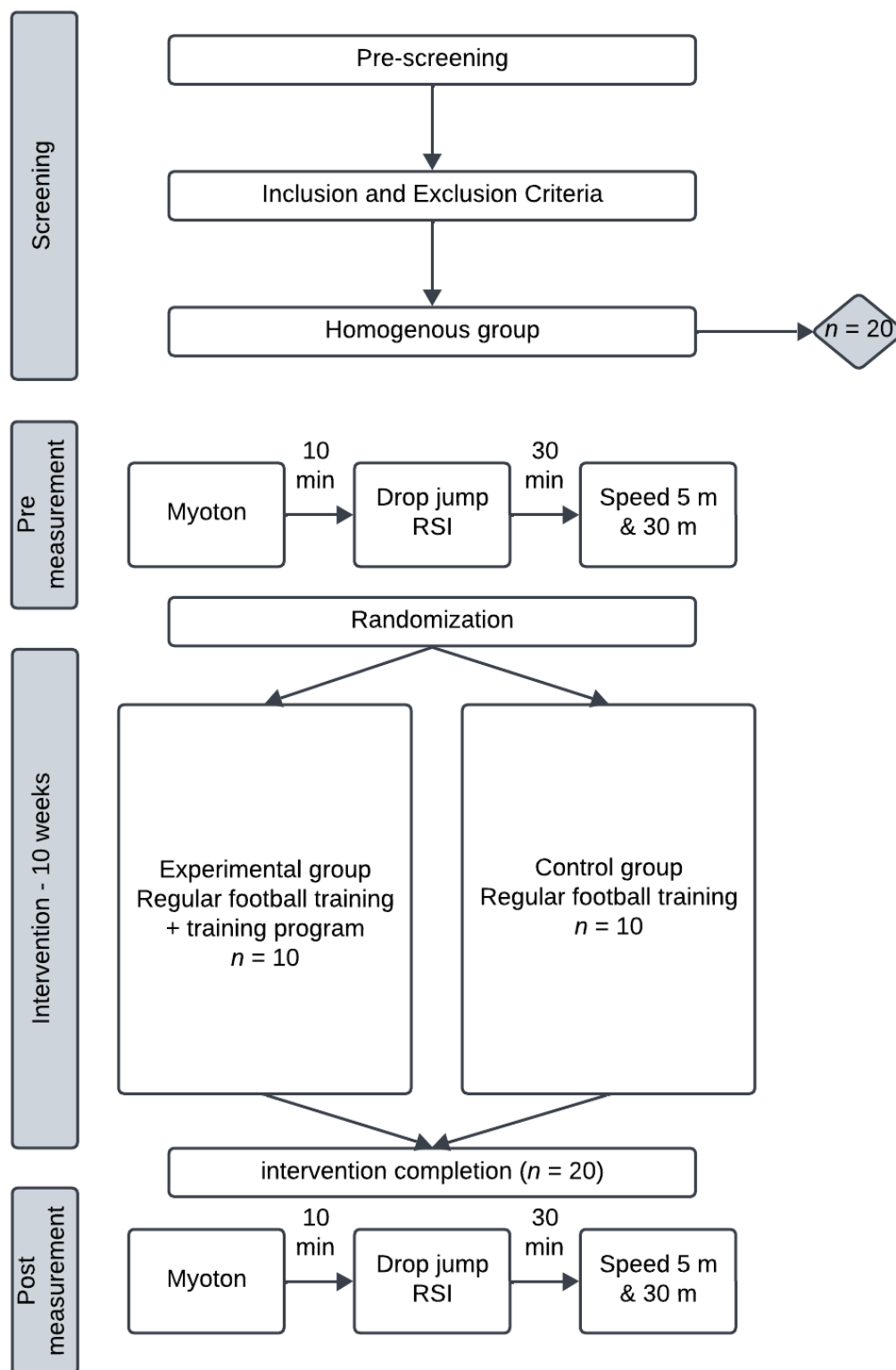


Figure 1. Research protocol.

Table 1. Standard training program followed by the control and experimental groups.

Day of the Week	Type of Training	Exercise	Workload/Intensity	Number of Repetitions/Working Time	Number of Series
Tuesday	Aerobic capacity and power with football elements	Running warm-up	$\leq 60\%$ HRmax	15 min	1
		Continuous run	70–80% HRmax	16 min	2
		Games in small groups, e.g., “rondo”	$\leq 50\%$ HRmax	10 min	1
		Patterns of play in offense or defense	$\leq 50\text{--}80\%$ HRmax	15 min	1
		Small football games on a scaled-down field	-	8 min	2
		Stretching + mobility	$\leq 50\%$ HRmax	5 min	1
Wednesday	Muscle strength and football tactics	Running warm-up	$\leq 60\%$ HRmax	15 min	1
		Barbell glute bridge	60% 1RMmax	$\times 10$	4
		“Plank” position	-	1 min	4
		Dumbbell split squat	60% 1RMmax	$\times 10$ per side	4
		Tactics and shooting	$\leq 50\text{--}80\%$ HRmax	15 min	1
		Formations movement	$\leq 50\text{--}80\%$ HRmax	15 min	1
Thursday	Glycolytic capacity and power + set pieces	Stretching + mobility	$\leq 50\%$ HRmax	5 min	1
		Running warm-up	$\leq 60\%$ HRmax	15 min	1
		Shuttle run	90–100% Vmax	30 s	6
		Set pieces	$\leq 50\%$ HRmax	20 min	1
Friday/Saturday	Friendly game/league game	Stretching + mobility	$\leq 50\%$ HRmax	5 min	1
		Football game		45–90 min	1

HRmax—maximal heart rate.

Table 2. Training program implemented by the experimental group.

Exercise	Number of Jumps	Number of Series	Rest Time Between Series	Intensity	Comment
Hurdles jump	8	3	120 s	Maximum	Hurdles set at the height of 30 cm, successively overcome reactively with the shortest possible contact of the feet with the ground
Multi-jumps	10	3	120 s	Maximum	The athlete performs the longest possible multi-jumps from a short run
Drop jump	6	3	120 s	Maximum	Reactive vertical jump after jumping off a box set at a height of 40 cm

Table 3. Essential characteristics of study group ($n = 20$).

Variable	Mean $\pm$ SD	Range
Age (year)	22.45 $\pm$ 3.67	16–30
Height (cm)	181 $\pm$ 4	173–192
Weight (kg)	78.15 $\pm$ 7.76	64–93
BMI (kg/m ²)	23.82 $\pm$ 1.81	20.45–26.86
Training experience. (year)	14 $\pm$ 2.6	10–20

2.3. Measures

2.3.1. Sprint

For sprint time measurements according to the “gold standard” [18], fully automatic photoelectric cells (Microgate Witty System, Bolzano, Italy, 2019) were used (Figure 2). According to various studies, a setup at intervals of 0 m, 5 m, and 30 m on a natural grass football field does not constitute a significant difference in measurements compared to hard surfaces [19,20] (Figure 3). Each athlete performed two trials at the maximum possible speed, with complete rest between trials. Previously, each athlete warmed up individually for about 10 min, where each ended the warm-up with a few faster sprints as a precaution before the upcoming test. All athletes were trained in the starting procedures, which are strictly standardized—a standing start and push-off from the more muscular trailing leg (Figure 4). The photoelectric cells measured the sprint time over two segments: the time for 5 m and the time for 30 m expressed in seconds.

**Figure 2.** Microgate Witty System photocells are spaced at the measurement site.

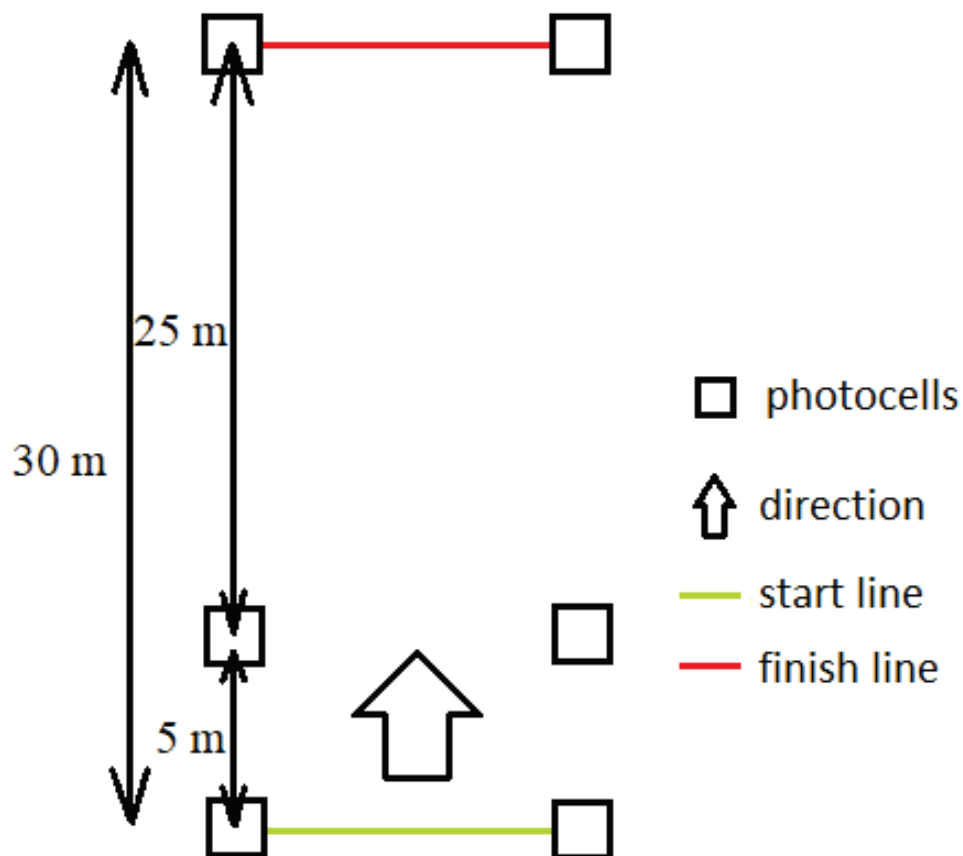


Figure 3. Schematic of sprint measurements.

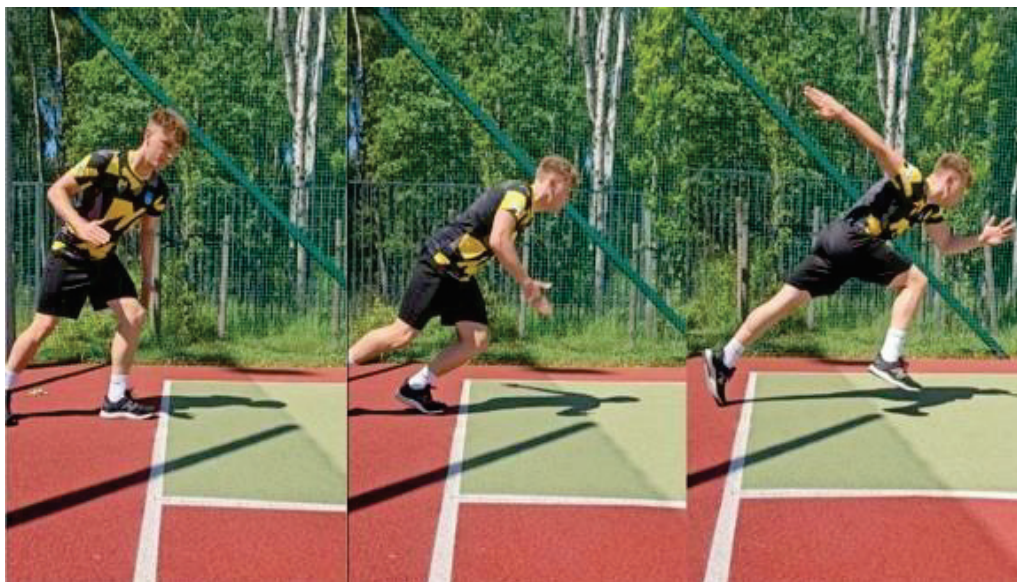


Figure 4. Procedure for sprint measurements.

2.3.2. Reactive Strength Index (RSI)

Reactive Strength Index (RSI) RSI describes a person's ability to transition from eccentric to concentric muscle contraction quickly and aims to assess the athlete's reactive strength—muscle strength. RSI was determined using the drop-jump method on the Optojump Next System. The drop jump is reliable for assessing RSI and related kinematic parameters (42). The athletes had to jump down from a height of 40 cm and, upon landing, perform a vertical jump with maximum intent (Figure 5). The entire procedure was

performed with hands placed on the hips. Before the study, participants warmed up by doing ten squats and passive thigh muscle stretching for one minute. A research assistant trained all athletes in jump technique, and three attempts were made before measurements were taken. The test included two different drop heights and assessed how high the athlete could raise their center of mass. To calculate the RSI for each participant, we used the equation $RSI = \text{jump height [m]} / \text{contact time [s]}$ [21].

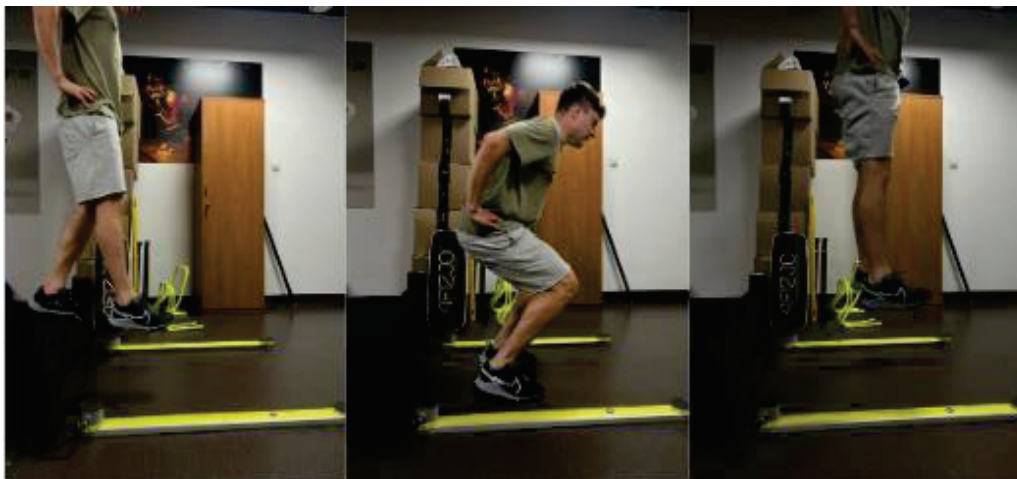


Figure 5. Measurement of reactive strength with Optojump.

2.3.3. Biomechanical Properties of Muscles

Biomechanical properties were measured using a myometer (MyotonPRO AS, Myoton Ltd., Tallinn, Estonia, 2021). Myoton is a digital device with a body and a deep probe ($\varnothing$ 3 mm). An initial pressure (0.18 N) is applied to the surface through the probe, which causes the material underneath to compress. Next, the device releases a mechanical impulse (0.4 N, 15 ms) that temporarily deforms the medium. Myometry is a reliable measurement method and allows for the detection of differences in physical properties compared to stretched muscle fibers [22]. The measurement method involves recording the damped natural vibrations of soft biological tissue in the form of an acceleration signal and then simultaneously calculating the parameters of the stress state and biomechanical properties. Thanks to the device, we can assess, among other things, the state of resting muscle tone (F), which is defined as the suppressed EMG signal and dynamic stiffness (S). Stiffness assessed using myotonometry is based on the theory of free oscillations and results from the natural oscillations of tissues in response to brief mechanical exposure of the skin. Tissues can also regain their original shape after deformation. This property, measured in this study, is called elasticity (D). The greater the elasticity, the faster the tissue returns to its original shape. Measurements with the myotonometer were performed in a standardized, supine resting position. The myotonometer was applied perpendicularly to the tissue (Figure 6). The broadest cross-sectional area of the muscles was determined and marked, meaning that the measurement location for the rectus femoris muscle was the midpoint of the distance from the anterior superior iliac spine to the upper border of the patella. For the vastus lateralis muscle, the segment's midpoint is from the patella's upper edge to the greater trochanter [14,23] (Figure 7).



Figure 6. Measurement of muscle properties with the MyotonPRO device.

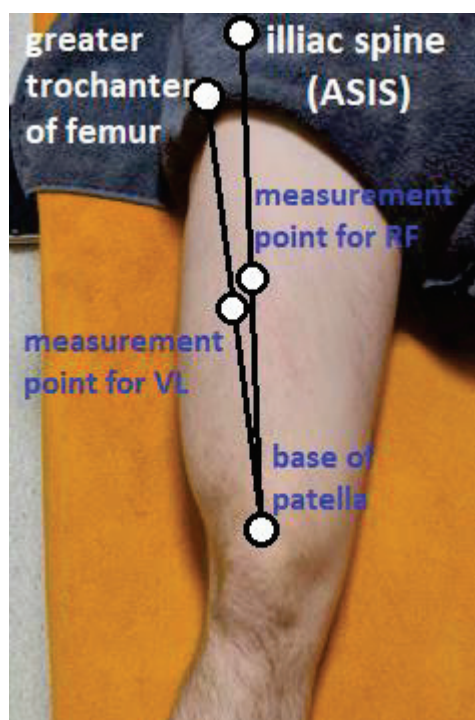


Figure 7. Presentation of measurement points for tissue properties. RF—rectus femoris (rectus femoris muscle) VL—vastus lateralis (vastus lateralis muscle).

2.4. Statistical Analysis

Statistical analyses were performed using Statistica 13.1. (StatSoft, Tulsa, OK, USA) and PRISM 9.4.1. (GraphPad, La Jolla, CA, USA). Results are presented as means with standard deviations and 95% confidence intervals. Normality, homogeneity, and sphericity

of variance of sample data were verified using Shapiro–Wilk, Levene, and Mauchly tests, respectively. Differences between the variables considered were compared using ANOVA with repeated measures. In the case of a significant main effect, the Bonferroni post hoc test was used for post hoc comparisons. The association's strength in the variance analysis was calculated using η^2 . The strength of the effect was calculated using the coefficient η^2 . The strength of the effect was classified as weak if η^2 ranged from 0.01 to 0.059, medium from 0.06 to 0.137, and large > 0.137 . For pairwise comparisons, effect sizes (ES) were determined by Cohen's d and characterized as significant ($ES > 0.8$), moderate ($0.5 < ES < 0.8$), small ($0.20 < ES < 0.49$), and trivial ($ES < 0.05$). Spearman's rank correlation coefficient was used for the correlation analysis. Statistical significance for differences was set at $p < 0.05$. Basic descriptive statistics are presented in Table 4.

Table 4. Basic descriptive statistics.

	Experimental Group		Control Group	
	Pre	Post	Pre	Post
	M $\pm$ SD (−95%; +95%)	M $\pm$ SD (−95%; +95%)	M $\pm$ SD (−95%; +95%)	M $\pm$ SD (−95%; +95%)
RSI ^{†,*}	1.48 $\pm$ 1.42 (0.15; 0.40)	1.69 $\pm$ 1.68 (0.17; 0.46)	1.19 $\pm$ 1.35 (0.22; 0.58)	1.24 $\pm$ 1.28 (0.21; 0.57)
5 m [s] [†]	1.09 $\pm$ 1.11 (0.04; 0.12)	1.01 $\pm$ 1.02 (0.03; 0.10)	1.10 $\pm$ 1.12 (0.05; 0.15)	1.06 $\pm$ 1.07 (0.04; 0.13)
30 m [s] [†]	4.30 $\pm$ 4.33 (0.10; 0.28)	4.13 $\pm$ 4.18 (0.10; 0.28)	4.36 $\pm$ 4.43 (0.14; 0.38)	4.28 $\pm$ 4.30 (0.09; 0.25)
RF F [Hz]	15.61 $\pm$ 15.30 (0.88; 2.36)	15.45 $\pm$ 15.25 (0.81; 2.16)	15.39 $\pm$ 15.55 (0.59; 1.58)	15.21 $\pm$ 15.20 (0.60; 1.61)
RF S [N/m]	281.90 $\pm$ 274.00 (26.91; 71.45)	271.90 $\pm$ 274.00 (22.87; 60.71)	275.70 $\pm$ 275.50 (12.28; 32.60)	273.90 $\pm$ 276.00 (12.27; 32.58)
RF D [N/m] [†]	1.51 $\pm$ 1.54 (0.17; 0.47)	1.40 $\pm$ 1.40 (0.19; 0.53)	1.42 $\pm$ 1.43 (0.20; 0.53)	1.38 $\pm$ 1.33 (0.16; 0.43)
VL F [Hz]	18.74 $\pm$ 3.31 (2.52; 4.84)	15.87 $\pm$ 14.38 (1.43; 3.79)	19.02 $\pm$ 18.40 (2.47; 6.55)	17.04 $\pm$ 15.56 (1.41; 3.76)
VL S [N/m]	346.30 $\pm$ 321.50 (52.00; 138.03)	291.60 $\pm$ 264.48 (26.07; 69.19)	345.40 $\pm$ 333.00 (35.71; 94.78)	311.70 $\pm$ 291.90 (19.03; 50.52)
VL D [N/m]	1.59 $\pm$ 1.47 (0.11; 0.29)	1.35 $\pm$ 1.14 (0.19; 0.52)	1.70 $\pm$ 1.47 (0.01; 0.31)	1.53 $\pm$ 1.40 (0.12; 0.33)

RF—rectus femoris; VL—vastus lateralis; F—tension; S—stiffness; D—elasticity; [†]—intragroup difference; *—intergroup difference.

3. Results

The first part of the analysis involved intergroup comparisons. In most cases, the data distributions are normal ($p > 0.05$), and in a few cases, they are close to normal. Therefore, a parametric analysis of variance with repeated measures was used. Due to extreme asymmetries in the data distribution, a non-parametric equivalent was used for some of the viscoelastic properties of muscle.

Significant differences were found for the main effects of the 5 m variable pre-post ($F = 16.476$; $p < 0.01$; $\eta^2 = 0.47$). In contrast, no significant differences were found for the group effect ($F = 1.090$; $p > 0.05$) and the higher-order pre-post*group interaction ($F = 2.128$; $p > 0.05$). To determine which groups had significant differences, further analyses were

conducted using Bonferroni's post-hoc multiple comparison tests. Significant differences were found in the 5 m [s.] variable after and before the intervention in the experimental group ($p < 0.01$; $df = -0.087$ (-8.6%); $ES = 1.44$). These results are supported by the data presented in Figure 8.

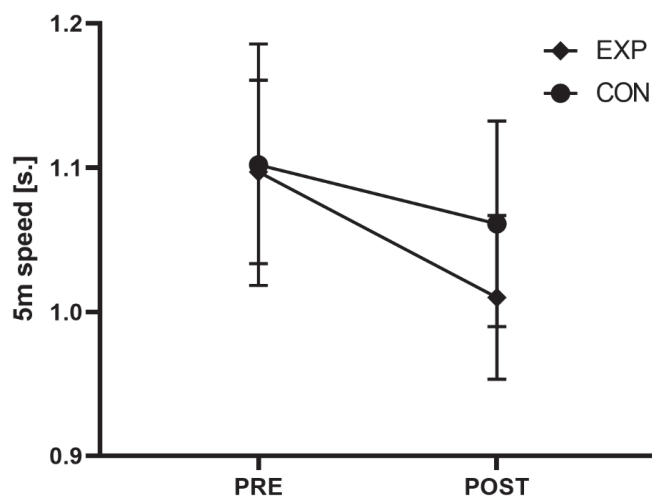


Figure 8. Intergroup comparison of 5 m speed.

Significant differences were found for the main effects of the 30 m variable pre-post ($F = 37.81$; $p < 0.01$; $\eta^2 = 0.67$). In contrast, no significant differences were found for the effect group ($F = 2.14$; $p > 0.05$). However, for the interaction group*pre-post, analysis of variance allowed us to reject the null hypothesis of no difference ($F = 5.5$; $p < 0.05$; $\eta^2 = 0.23$). There was a significant difference in the 30m variable after and before the intervention in the experimental group ($p < 0.01$; $df = -0.1720$ (-4.15%); $ES = 1.11$) after applying Bonferroni post-hoc multiple comparison tests. These findings are further supported by the data illustrated in the accompanying Figure 9.

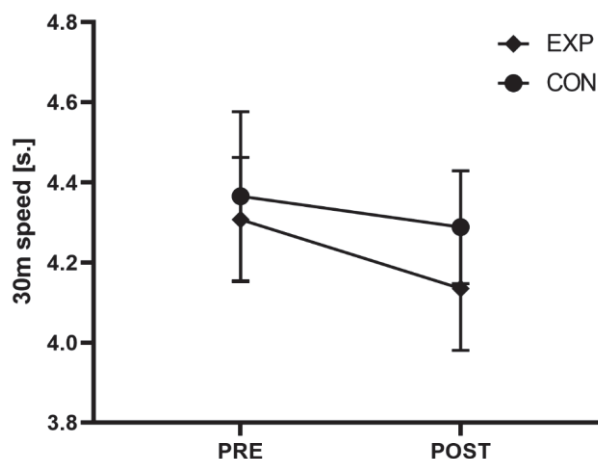


Figure 9. Intergroup comparison of 30 m speed.

Analysis of the dependent variable RSI results showed significant differences for the group ($F = 10.15$; $p < 0.01$, $\eta^2 = 0.36$) and pre-post ($F = 8.58$; $p < 0.01$; $\eta^2 = 0.32$) factors. There were no significant differences in the group*post-pre interaction ($F = 3.33$; $p < 0.01$). There was a significant difference in the RSI variable after and before the intervention in the experimental group ($p < 0.05$; $df = 0.2070$ (14.3%); $ES = 0.87$) after using Bonferroni's post-hoc multiple comparison tests. These findings are further supported by the data illustrated in the accompanying Figure 10.

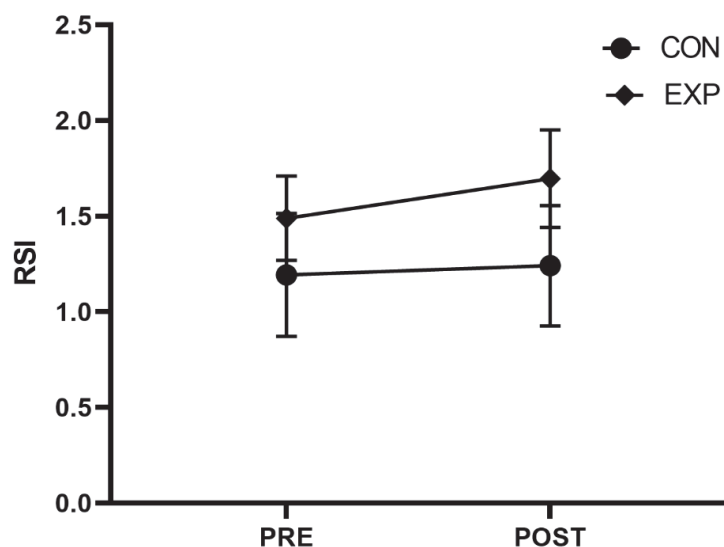


Figure 10. Intergroup comparison of RSI.

Significant differences were found for the main effects of the RF elasticity (D) variable pre-post ($F = 11.76$; $p < 0.01$; $\eta^2 = 0.39$). In contrast, no significant differences were found for the effect group ($F = 2.43$; $p > 0.05$), and for the group*pre-post ($F = 0.35$; $p > 0.05$). However, there were no significant differences after applying post-hoc comparisons ($p > 0.05$). These results are supported by the data presented in Figure 11.

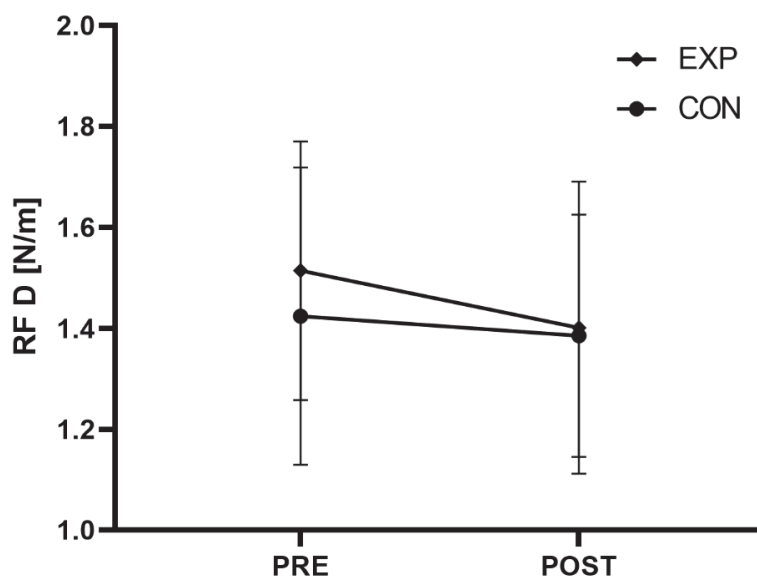


Figure 11. Intergroup comparison of Rectus femoris elasticity.

The second part of the analysis examined the correlation between the following assumptions: (1) Change in test ($\Delta(\text{Post: Pre})$ [RSI/5 m/30 m]) vs. baseline level of viscoelastic muscle properties (Myoton pre) (Figure 12), (2) Change in test ($\Delta(\text{Post: Pre})$ [RSI/5 m/30 m]) vs. susceptibility of tissue to training ($\Delta(\text{Post: Pre})$ [myoton F/S/D]) (Figure 13). (3) Baseline results: RSI/5 m/30 m vs. tissue susceptibility to ($\Delta(\text{Post: Pre})$ [myoton F/S/D]) (Figure 14). The results are presented as a correlogram with Spearman's rank correlation coefficient $r \in (-1; 1)$. The degree of correlation between the evaluations according to the grading standards [24] is shown in Table 5.

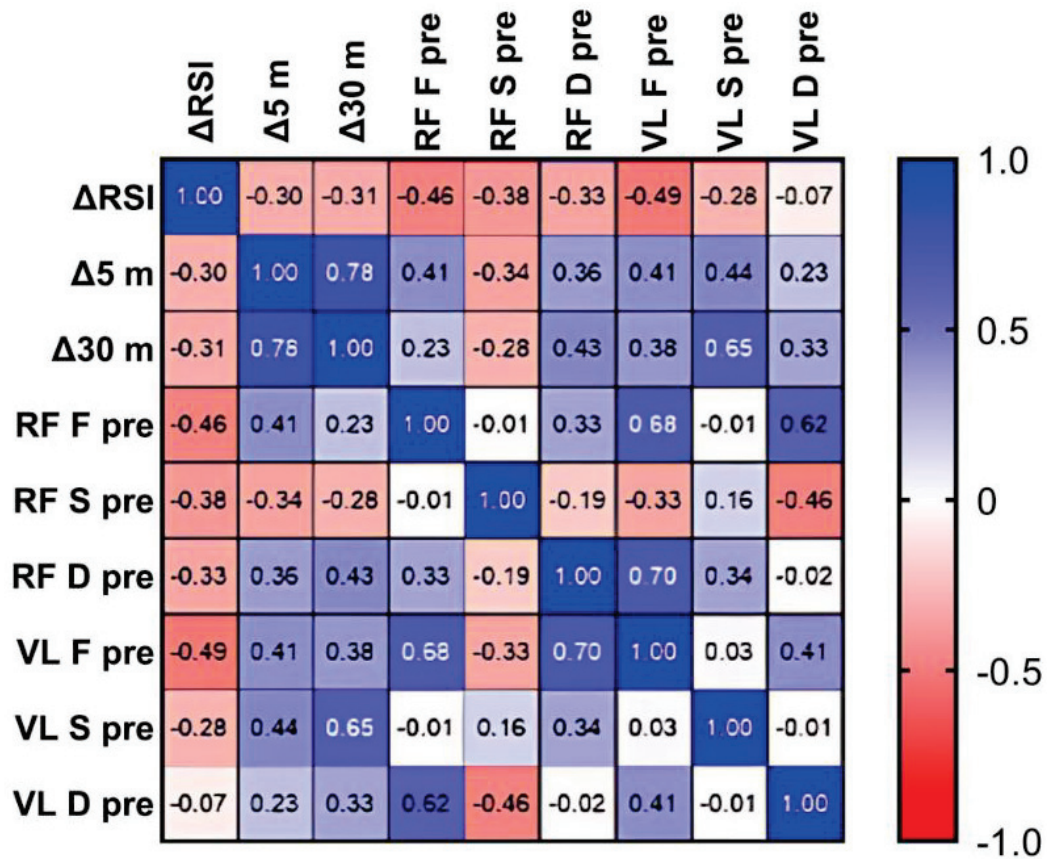


Figure 12. Spearman rank correlation matrix for the above assumptions 1.

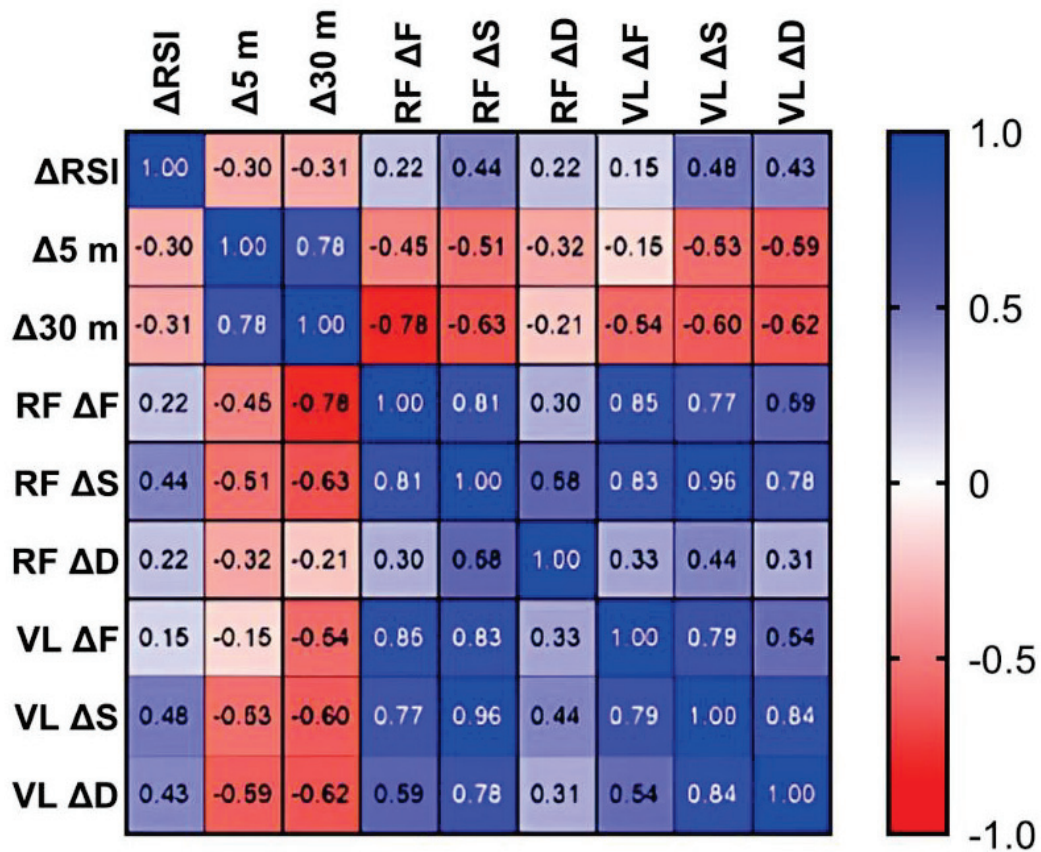


Figure 13. Spearman rank correlation matrix for the above assumptions 2.

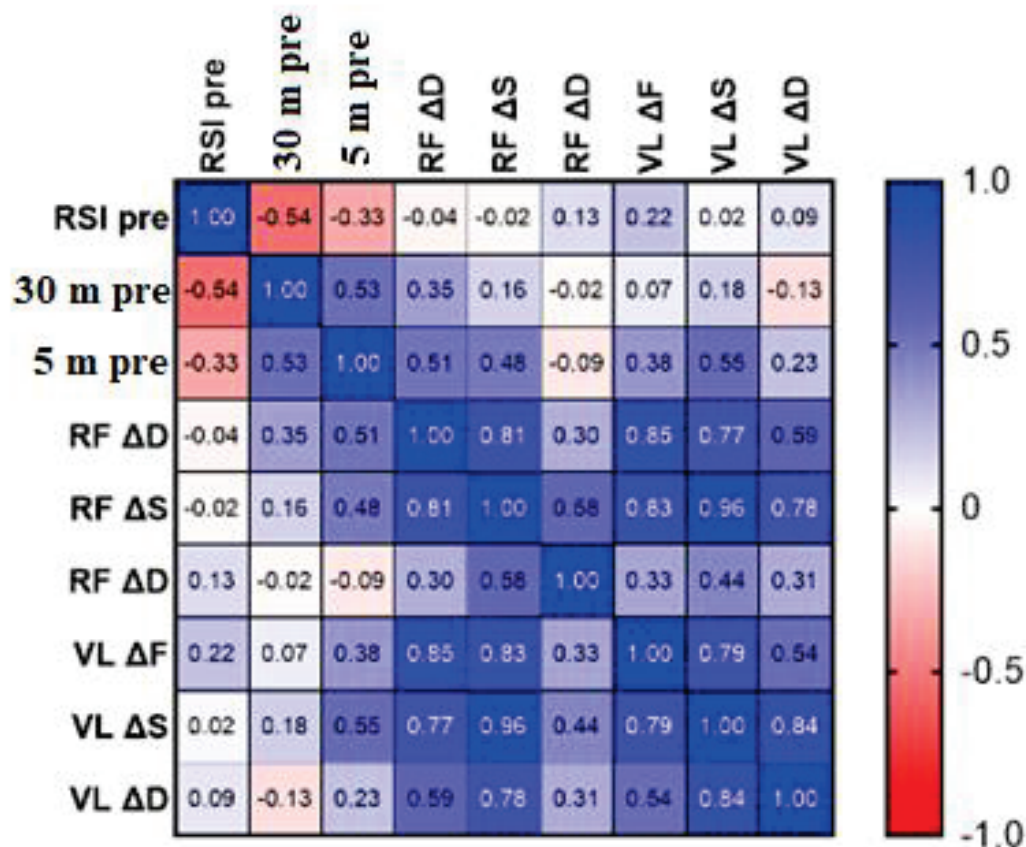


Figure 14. Spearman rank correlation matrix for the above assumption 3.

Table 5. Grading table of Spearman correlation coefficient.

Grading Standards	Correlation Degree
$r = 0$	No correlation
$0 < r \leq 0.19$	Very week
$0.20 \leq r \leq 0.39$	Weak
$0.40 \leq r \leq 0.59$	Moderate
$0.60 \leq r \leq 0.79$	Strong
$0.80 \leq r \leq 1.00$	Very strong
$r = 1.00$	Monotonic correlation

r—correlation coefficient.

Correlation analysis revealed a statistically significant moderate positive correlation between VL D pre and $\Delta 5$ m variable ($r = 0.65$; $p < 0.05$). A statistically significant, strong negative correlation exists between RF ΔD and $\Delta 5$ m variables ($r = -0.78$; $p = 0.01$).

4. Discussion

The conducted study aimed to evaluate the impact of a programmed 10-week plyometric training on two physical aspects, speed and power, as well as its effect on and changes in the biomechanical properties of the quadriceps muscles (muscle tension, flexibility, muscle stiffness) in amateur football players. The study's results indicate that the programmed additional plyometric training significantly improved sprint performance over both 5 m and 30 m in the experimental group (EG). Analysis of Bonferroni test results showed significant differences in performance before and after the intervention in the experimental

group, confirming the hypothesis that plyometric training can contribute to improving starting speed and overall sprint speed in amateur football players.

Plyometrics, as one of these methods, is considered a potentially effective tool for improving the functional performance of soccer players [25,26]. In the works of other authors, improvements in sprint results over short distances (5 m) and longer distances (40 m) have been observed, which may suggest that plyometric training translates into acceleration as well as the maximum speed achieved by the athlete [27]. A low or moderate training volume is recommended for plyometric training based on the drop jump exercise. In the 20 m sprint results, Eduardo Sáez Sáez de Villarreal and others observed the most excellent effectiveness in groups following a 7-week program based on training twice a week. Additionally, compared to a group with twice the training volume, the measured results were weaker at the end of the study (approximately 120 jumps per week vs. 240 jumps per week) [28]. In the literature, one can also encounter research protocols similar to the one proposed in our study—encompassing both forms of vertical jumps (drop jump) and horizontal jumps (multiple jumps and hurdle jumps). Such a protocol was implemented in the study by Loturco et al. [29], where improvements in sprint performance along with reactive strength were observed among professional football players, who achieved statistically significantly better results regardless of whether they performed exercises with vertical or horizontal work specificity.

Each sprint phase is characterized by a different component of the direction of the interaction forces between the foot and the ground during the acceleration phase and the phase of reaching maximum speed [30,31]. Different placements of the center of mass and the direction of force vectors from the ground in various sprint phases can represent different forms of exercise. For the acceleration phase, horizontal jumps are recommended; beyond a distance of 10 m, where the athlete gains speed, becomes more upright, and the force vector starts to become more perpendicular to the ground—vertical exercises such as drop jumps [32–34]. Such a sequence of events may suggest that it is worthwhile to implement various forms of plyometric exercises with different force orientations to maximize the physical potential of football players and develop overall fitness. In the study by Ramirez-Campillo et al. [35], it was found that regardless of the type of additional intervention—plyometric training, strength training targeted at sprinting specifics, or a combination of plyometric and strength methods—similar improvements in sprinting results were observed among male and female football players.

In contrast, the work of Zghal et al. [36] negates the effectiveness of isolated plyometric training in improving sprint performance. Only the research group implementing a mixed protocol—lower limb resistance training combined with plyometric training based on hurdle jumps—showed positive adaptations in the form of improved 5-m sprint results; no statistically significant differences were observed at 10 and 20 m. Even though explaining the neurophysiological phenomena that influence sprinting performance through plyometric training still presents many challenges for authors, the scientific literature offers numerous explanations. Adaptation within the main biomechanical components: parallel elastic tissue structures (Parallel Elastic Component, PEC) and contractile structures (Contractile Components, CC). In the muscle stretching phase, potential kinetic energy is stored in elastic tissues, which is released to generate concentric force when the muscle returns to its standard length (Rebound Force Response). Such a phenomenon accounts for 70–75% of the concentric increase in force, making plyometrics so efficient [37,38]. In the context of the rebound phase during a sprint, force generation is one of the key variables for running speed.

Plyometric training significantly improved the experimental group's reactive strength index (RSI). Significant differences in RSI values before and after training suggest that regular execution of plyometric exercises, such as drop jumps, improves muscle ability to quickly transition from the eccentric to the concentric phase, which is crucial for the dynamic movements characteristic of soccer. In many studies, it can be concluded that the most effective training protocols for building, among other things, reactive strength include 1–2 sessions of plyometric exercises per week [28]. The topic of plyometric training periodization was more thoroughly examined in a study by Guiyang Liu et al. [39], who concluded that a microcycle of plyometric training (<20 jumps/session) performed 4 times a week (using the so-called micro-dosing) with appropriate exercise selection is as effective a tool for building reactive strength as a protocol performed 2 times a week with twice the volume per training session (approximately 40 jumps/session).

In comparison with the meta-analysis by Peter A. van de Hoef et al. [40], it can be concluded that plyometric training can be an effective tool for improving RSI results among soccer players. A common denominator in many studies is the selection of exercises—countermovement jump (CMJ) is one of the most frequently chosen plyometric exercises in research protocols. In our training regimen, the role of CMJ was performed by hurdle jumps. The authors of the studies have repeatedly indicated that one of the main physiological factors behind the body's adaptive mechanisms is the stimulation of the central nervous system, which leads to increased recruitment of motor units and their synchronization, which can prove to be one of the key aspects in optimizing force production [41].

The sensitivity to speed improvement over 5 m decreases with increasing RF flexibility. Therefore, our research shows that training-induced adaptations in the biomechanical properties of RF play a key role in short-distance sprints. Additionally, higher baseline elasticity values in the VL muscle may be associated with more significant improvement in 5 m sprint performance following programmed plyometric training. This indicates that the initial mechanical properties of the VL muscle may serve as a determining factor for the ability to adapt to short-distance sprints. Despite the use of advanced measurement methods (myometry), no statistically significant changes were observed in the biomechanical properties of the quadriceps muscles, such as muscle tone, elasticity, or stiffness, in both the experimental and control groups. The lack of significant changes may be due to the limited duration of the study or the need for a more extended period of muscular adaptation at the tissue level. The impact of plyometric training on biomechanical changes at the tissue level was studied by Zubac et al. [42], who noted, among other things, a decrease in VL muscle tension after an 8-week plyometric training program. In various articles and scientific studies, it can be found that plyometric training has a marginal impact on biomechanical changes, except for improvements in flexibility.

Presumably, changes in muscle stiffness can be regulated through changes in the viscoelastic properties of intramuscular and tendinous tissues, which may require a long adaptation period. The study by Moran et al. [43] concluded that considering the number of available training methods, plyometric training is not necessarily the best choice for improving muscle stiffness; a potentially better choice could be traditional resistance training or eccentric muscle training. The measurement results of the elasticity of large-volume muscles such as the vastus lateralis and rectus femoris may be unreliable when using the MyotonPRO tool. Davidson et al. [44] reached such a conclusion in their work, where they investigated the measurement reliability of the myotonometer for selected biomechanical parameters in skeletal muscles of smaller and larger volumes. The aim of such studies is, among other things, the objectification of measurements for assessing the effectiveness of specific training protocols.

Although this study may provide valuable insights into the effectiveness of plyometric training in improving the athletic performance of soccer players, there are limitations to the study and opportunities for future research. Firstly, the study focused exclusively on measurements in a heterogeneous group of football players. Therefore, the results cannot be interpreted for other sports groups. Moreover, although the effect on running speed and muscle power was emphasized, further research is needed to explain the exact mechanisms underlying their impact on football players.

Future research should also examine individual differences in athletes' responses to plyometric training interventions, considering factors such as training experience or morphological and genetic predispositions. Moreover, comparative analyses using other forms of plyometric training with different populations of athletes and other volunteers can provide a more comprehensive understanding of the optimal approach. Gaps can be observed in the literature regarding studies on the impact of plyometrics on biomechanical changes in tissues measured in football players using the MyotonPRO device. Further research on the impact of plyometric training in football should focus on the relationships between the duration of training protocols and the recorded changes in muscle biomechanical parameters, as well as the selection of exercises, intensity, and volume. It would be worthwhile to study the response of other muscles in the context of biomechanical changes following plyometric training. The results of this study offer practical implications for optimizing training strategies that could potentially improve performance in sports. Athletes can potentially improve the performance of important motor parameters used during the game and enhance overall athletic readiness. The training program we proposed improves the motor skills of football players. However, it should not be directly implemented by coaches and players. We suggest possibly familiarizing yourself with the recommended guidelines in the context of programming such training to increase its effectiveness and success.

5. Conclusions

Our study indicated the effectiveness of implementing an additional 10-week plyometric training program for the football team in improving sprint performance and reactive strength. The program, which is performed twice a week with intervals of 36–48 h between training sessions, includes exercises such as hurdle jumps, triple jumps, and drop jumps. It is a practical training solution that enhances physical potential. No statistically significant parameter changes were observed among the experimental group regarding biomechanical measurements of muscles, tension, flexibility, and stiffness. Despite the absence of statistically significant group-level alterations in biomechanical properties, including muscle tension, stiffness, and elasticity, the findings underscore the promise of leveraging baseline muscle elasticity as a potential biomarker for predicting training adaptation. This underscores the significance of personalized training regimens in optimizing athletic performance.

Author Contributions: Conceptualization, F.M. and R.T.; methodology, F.M. and K.G.; software, R.T. and A.T.; validation, F.M., G.O., A.T. and M.W.; formal analysis, R.T. and A.T.; investigation, K.G. and G.O.; resources, F.M.; data curation, K.G. and M.W.; writing—original draft preparation, F.M.; writing—review and editing, F.M., A.T. and M.W.; visualization, K.G.; supervision, M.W.; project administration, F.M.; funding acquisition, M.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Polish Physiotherapy Association (protocol code 3/03/2024, approval date: 27 March 2024).

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

Data Availability Statement: The data supporting the reported results are available upon reasonable request from the corresponding author. No publicly archived datasets were generated or analyzed during the current study.

Acknowledgments: The authors would like to thank the staff and players of KS Polonia Łaziska-Górne for their support and participation in the study. Special thanks are extended to the Municipal Stadium in Łaziska Górne for providing access to their facilities.

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

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Nutritional Recommendations for Breath-Hold Divers

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Featured Application: The main application of this study concerns the recommendation for proper nutrition, regarding this specific population. Furthermore, if followed properly, nutritional recommendations could enhance freedivers' performance and recovery and decrease injury.

Abstract: Breath-hold diving (BHD) is defined as enduring apnea for more than 4 min. It is characterized by resistance of reactive oxygen species, reduced sensitivity to hypoxia, and low mitochondrial oxygen consumption. The energetic demands of different BHD disciplines (static, dynamic, and spearfishing) are various. Hence, the nutrition of these athletes may play an important role on their performance. The main aim of this study is to examine and propose recommendations for this specific population. Also, the aim is to divide nutrition according to main BHD disciplines (static, dynamic, and spearfishing). Mediterranean and alkaline diet could represent an appropriate BHD diet. Nutritional requirements differ between freediving (static and dynamic) and spearfishing, mainly because of the workload. Also, spearfishing includes multiple consecutive dives and hence has different recommendations. However, the proper nutrition for freedivers is based on a variety of natural foods that support acid–base balance, provide a stable source of energy, and facilitate quality recovery. By adopting healthy eating habits based on the Mediterranean and alkaline diet, along with regular hydration and adapting your diet to the specific needs of diving, divers can improve performance and ensure long-term health.

Keywords: Mediterranean diet; alkaline diet; nutrition; freediving; spearfishing

1. Introduction

Presently, breath-hold diving (BHD) is practiced in recreational, hunting, military, and competitive manners. At the elite level, freedivers can hold their breath for an impressive time of ~10 min, can reach depths > 200 m, or dive into pools longer than 300 m depending on the specific diving discipline [1]. The two main disciplines are static apnea and dynamic apnea. The periods of prolonged apnea that these athletes endure make the BHD environment extreme. Such extremity is pronounced especially in a competitive context, where the ultimate limits of the body's ability to withstand intense hypoxic and hypercapnic states are tested in hyperbaric conditions [1–6]. Frequent exposure to such physiological and physical limitations increases the risk of potential dangers to the health of freedivers [7,8]. Furthermore, it can be speculated that the specific requirements of long-term diving apnea make inadequate nutrition a greater obstacle in this sport. This speculation can be observed from nutritional recommendations in other aquatic sports [9,10]. Also, performance in synchronized swimming, defined with shorter periods of apnea, could be improved with

proper diet [11]. Hence, the nutritional recommendations for these athletes may present a potentially important factor in performance.

General Recommendations for a Healthy Daily Diet for Freedivers: Mediterranean and Alkaline Diet

Diving is a specific sport that requires exceptional physical and mental fitness [12]. Proper nutrition not only provides the energy needed for training and competitions but also supports complex biochemical mechanisms such as the regulation of acid–base balance and the management of oxidative stress, which are especially endangered during the practice of apnea diving. This is why it is necessary to develop a permanent habit of daily healthy and balanced eating. It is not only a matter of personal discipline, but also of understanding the importance of nutrition in improving physical and mental characteristics. A balanced, varied, unprocessed, and high-quality diet could be a prerequisite for achieving maximum diving abilities, safer diving, and long-term health. The combination of the Mediterranean and alkaline diet offers an optimal nutritional basis for achieving and maintaining these goals. The Mediterranean and alkaline diets share many common features, such as an emphasis on the intake of large amounts of fruits, vegetables, and minimally processed foods, but they are not completely identical [13,14]. The Mediterranean diet (MD) also includes acid-forming foods such as fish and dairy products, while the alkaline diet tends to eliminate such foods [14]. A combination of the principles of both diets can provide a balanced and healthy approach to nutrition, especially for the specific needs of freedivers.

Due to the high demands of this sport and the significant energy expenditure, freedivers are forced to carefully balance between these two nutritional approaches, depending on the training periodization. In the phase of the predominantly physical conditioning phase, when a higher protein intake is needed for recovery and muscle mass building, the Mediterranean diet provides optimal nutritional support. On the other hand, in specific apnea and/or competition phases, when it is crucial to reduce oxidative stress and optimize the body's acid–base balance, an alkaline diet can play an important role.

By combining these nutritional principles on a daily basis in accordance with the phase of the planned training process, while respecting the characteristics and intensity of operational loads and the necessary recovery during microcycles and individual training sessions, divers can ensure optimal intake of nutrients. This allows them to support endurance and performance, as well as improve diving safety and efficiency.

The literature review showed a lack of studies that examined the diet of BHD and gave full recommendations for different disciplines. Previous studies have demonstrated similar work aimed at scuba divers [15]. The authors concluded that the recommendations for recreational divers are to consume $\sim 170\text{--}210 \text{ kJ}\cdot\text{kg}^{-1}$ ($40\text{--}50 \text{ kcal}\cdot\text{kg}^{-1}$) body mass, depending on their workload underwater. Also, all divers should take special care to hydrate themselves properly. However, there are no recommendations for apnea divers. Hence, the main aim of this study is to examine and propose recommendations for this specific population. Also, the aim is to divide nutrition according to three main BHD disciplines (static, dynamic, and spearfishing). As mentioned above, an MD and alkaline diet could represent a good BHD diet.

2. Mediterranean and Alkaline Diet

The aim of this study was to examine the previous literature and propose proper nutritional recommendations for BHD. Another aim was to examine adherence to the Mediterranean diet for those engaging in BHD. As already mentioned, the current literature focused on different diving populations, than those our research focuses on [15]. Hence, this is the first study to give full recommendations for static, dynamic, and spearfishing

athletes. In addition, the results of the MDSS questionnaire showed adherence to an MD. The results showed that only 20% of examined divers adhere to MD. Therefore, these recommendations should be considered and followed thoroughly.

2.1. Mediterranean Diet: The Foundation for Health and Diving Performance

The Mediterranean diet is rich in olive oil, fresh vegetables and fruits, legumes, nuts, and whole grains, with moderate consumption of fish, eggs, and dairy products and minimal intake of meat and animal fats [16]. This diet supports cardiovascular health, regulates body weight and reduces inflammatory processes, making it the perfect choice for maintaining health and physical performance [17]. Therefore, it could provide an optimal framework for the freediver's diet. Furthermore, the combination of omega-3 fatty acids from oily fish and nuts and omega-9 from olive oil contributes to improving circulation and lipid profile, lowering bad LDL cholesterol and raising HDL cholesterol [18]. Reduced intake of omega-6 fatty acids prevents imbalances that can cause inflammatory processes, while the anti-inflammatory effect of omega-3 and the protective role of antioxidants from vegetables and fruits, such as vitamins C and E, help to maintain health at the cellular level [19,20]. This diet not only provides optimal nutritional support but also promotes a lifestyle that combines balanced food intake with physical activity and longevity, and can be specifically tailored to the needs of freedivers or anyone who strives for health and peak aquatic performance.

2.2. Alkaline Diet: Acid–Base Balance

During an apnea, hydrogen ions accumulate, which acidify the body, and hence shorten breath-holding duration [21]. For this reason, freedivers are advised to avoid excessive intake of meat, especially processed meat, simple sugars, alcohol, caffeine, and carbonated drinks, or any foods that further create an acidic environment in the body. In contrast, the alkaline diet is based on the theory that certain foods can affect the body's pH balance, promoting alkalinity. An alkaline diet is very rich in important micronutrients and, due to this very fact, is very healthy. In general, vegetables, fruits, nuts, and seeds are considered alkaline foods that help to maintain a favorable acid–base status, which increases endurance, especially regarding the capacity of the anaerobic glycolytic metabolic system, and reduces the risk of fatigue [21]. Accordingly, recommended alkaline foods are as follows: various naturally grown vegetables such as zucchini, broccoli, and green leafy vegetables in particular (kale, spinach. . .), seasonal fruits (citrus fruits, berries, watermelon. . .), nuts and seeds (almonds, chia seeds. . .), legumes, and some whole grains (quinoa, lentils, millet. . .).

2.3. Different Nutrition Concepts Concerning the Specifics of Diving Disciplines

Freediving encompasses a wide range of disciplines that differ significantly in performance, as well as in functional–metabolic energy demands. Static apnea (maximum breath-holding on the surface at rest) is based exclusively on aerobic capacity, while dynamic disciplines such as long-distance and deep diving include pronounced anaerobic requirements [22]. On the other hand, spearfishing is characterized by the alternation of frequent shallow dives with short surface rest intervals, breath-holding at rest on the bottom (dive hold), and deep dives with longer recoveries, which requires a flexible and durable energy system with aerobic and anaerobic components [23]. Nutritional approaches to practicing these different diving disciplines must be adapted to the specifics of training and competition. While apnea training in freediving usually lasts up to an hour, spearfishing can last more than six hours, which creates different nutritional needs. Optimal nutrition for divers is based on a balanced and planned intake of calories and macro- and micronutrients, as well as adaptation of nutrition to the training and competition demands.

The three main nutrition concepts include preparation for statics, for dynamic and deep-sea disciplines, and for the specifics of spearfishing, with a common emphasis on healthy eating habits and avoiding undesirable foods before diving.

3. Static Apnea

In static apnea, divers need to maintain a lean body mass, high aerobic capacity, and lower basal metabolism [2]. Hence, the recommended diets consist of Mediterranean and alkaline diets, depending on the training periodization. All phases are characterized by eating multiple, but not large, meals, and a diet rich in vegetables, fruits, whole grains, legumes, mushrooms, cold-pressed vegetable oils, and a moderate intake of protein. Moreover, proteins should be mostly plant-based to support the optimal functioning of the body, improve the capacity to store and utilize oxygen at the cellular level, and contribute to the health of the circulatory system. In the preparatory phases, when generic physical conditioning predominates, the Mediterranean diet can provide the necessary nutrients and energy to increase aerobic capacity.

3.1. Before Competition

To achieve optimal sports results, precise nutrition for competitors is an important parameter. It is advised that 20 days before important competition nutrition should be based on an energetic, restrictive, alkaline, almost-vegetarian diet. The basis is the intake of cooked and raw vegetables which contain a small share of carbohydrates and root vegetables, to maintain an acid–base balance. Following that, various whole cereals should be consumed. In this period, the body should be exposed to fat and omega fatty acids.

About 10 days before competitions, it is recommended to gradually induce a calorie deficit. Also, good hydration is a crucial factor with mild vitamin and mineral supplementation. Carbohydrate reduction should not be to the point of inducing ketosis. Precisely, ketosis is not optimal for static apnea discipline, both due to the increase in basal metabolic rate and increased carbon dioxide production. Increased CO₂ production can lead to a faster accumulation of this gas in the body during breath-holding, which can shorten apnea time and negatively affect performance.

In the 2 days preceding a competition, moderate fasting is recommended (calorie deficit of about 40–50%), with continuation of nutrition based mainly on vegetables and olive oil, with whole cereals, mushrooms, vegetables, and fruit which is not rich in simple sugars.

3.2. Supplementation

Supplementation supports the optimal function of the organism and helps in the regulation of acid–base balance, hydration, and reduction in oxidative stress in static apneists. The recommended supplements should be introduced, including the following: Magnesium—especially in the form of magnesium citrate or magnesium malate to relax muscles, reduce stress, and support normal nervous system function [24]. Potassium—can help to maintain electrolyte balance, especially with reduced food intake and hydration [25]. Zinc and selenium—key to strengthening immunity and reducing oxidative stress [26]. Vitamin C—as an antioxidant, this helps to reduce oxidative stress and supports the immune system [27]. L-arginine or L-Citrulline—to support better circulation and better oxygen supply by increasing the production of nitric oxide which dilates blood vessels [28]. Spirulina and chlorella—rich in chlorophyll and micronutrients and can help to detoxify and maintain the body's alkaline pH [29,30]. Ashwagandha or Rhodiola rosea—adaptogens that help to regulate stress and support cortisol reduction, which can contribute to calmness, which is especially important before static apnea competition.

4. Dynamic and Depth Disciplines in Freediving

In contrast to static apnea, dynamic apnea requires a high level of anaerobic functional capacity. This is partly due to a specific physiological mechanism known as the “diving reflex”. It is activated by immersion in water during apnea, causing intense sympathetic activity and consequent peripheral vasoconstriction. As a result, blood flow to the large muscle groups of the legs and arms is significantly reduced, which causes increased reliance on anaerobic metabolism. The dominant energy metabolic background under these conditions becomes anaerobic glycolysis, with blood glucose and glycogen stored in the muscles and liver, serving as the key energy sources. Although the aerobic energy system contributes to overall endurance, anaerobic capacities dominate during high-intensity diving performances. Because of these specifics, nutrition must therefore ensure an efficient energy supply and support regeneration.

The Mediterranean diet, as a basic dietary framework for freedivers, is ideally suited to these needs. This diet is rich in complex carbohydrates, which are essential for replenishing muscle glycogen stores which is beneficial for dynamic apneists. In addition to carbohydrates, increased protein intake is essential for muscle tissue regeneration after intense exercise. More easily digestible sources of protein, such as eggs, lean meat, fish, and plant-based proteins (tofu, tempeh, legumes), are optimal for supporting muscle fiber recovery and growth. At the same time, a balanced intake of healthy fats is important, with an emphasis on omega-3 fatty acids. Following all of this, calorie intake must be aligned with the diver’s needs, especially during intense training sessions when physical conditioning is dominant, to avoid energy imbalance.

Recommendations for nutrient intake include the implementation of a Mediterranean diet with the following food groups: Carbohydrates (40–60% of total intake): focus on complex carbohydrates like quinoa, sweet potatoes, oats, and brown rice to maintain steady energy levels. Protein (20–30%): focus on muscle regeneration with high-quality protein sources (lean meat, fish, eggs, and legumes). Healthy fats (20–30%): omega-3 (flax and chia seeds, fish, avocado) and omega-9 fatty acids (olive oil). Additional focus: supplementation with creatine, beta-alanine, and magnesium to support strength and endurance. The last meal before training (3–4 h) should incorporate complex carbohydrates and easily digestible proteins (whole-grain oatmeal with almonds and banana). After training, hydration comes first and then a small meal of a combination of fast-absorbing carbohydrates (rice and fruit, especially dates and bananas) and protein (Greek yogurt, protein shake) is recommended.

Supplementation

Given the dynamic nature of these disciplines, different supplementation should be considered. The following supplements may be useful for performance enhancement: BCAA—prevents muscle catabolism and accelerates recovery [31]. Beta-alanine—increases muscular anaerobic endurance and tolerance to lactate accumulation [32]. Creatine—supports explosive power and regeneration [33]. Magnesium and electrolytes—help in muscle relaxation, maintaining fluid balance, and preventing cramps [24]. At the time of extensive conditioning and specific diving apnea training with a high volume of load (comprehensive development programs after basic preparatory phase), which significantly physically exhausts the athlete’s body and contributes to mental stress, additional supplementation is suggested with the following: B complex and L-glutamine—play an important role in maintaining optimal energy levels, reducing fatigue, and supporting recovery. B vitamins—essential for normal energy-yielding metabolism and a reduction in tiredness and fatigue [34]. L-glutamine—a non-essential amino acid involved in gluconeogenesis, the process through which glucose is produced from sources other than carbohydrates, such as lactate or glycerol [35].

5. Spearfishing

Spearfishing requires exceptional psychophysical endurance, as the exhausting activity can last from a minimum of two to more than six hours [23]. The metabolic energy background of spearfishing includes both aerobic and anaerobic ergogenesis, and situational performance includes a combination of fin swimming, dynamic diving for distance and depth, and static diving at rest (fish searching and ambushing). All of these activities require extremely complex physical fitness preparation conditioning, as well as specific apnea training. During intensive spearfishing with prolonged surface swimming, divers expend a significant amount of energy. This energy expenditure is estimated at around 800 kcal per hour, which can reach up to 4800 kcal after six hours [36]. Given that the average daily energy requirement is around 2500 kcal, the total daily intake for spearfishermen can reach up to 7300 kcal. Repeated exposure to hyperbaric conditions during about a six-hour diving session stimulates the secretion of atrial natriuretic peptide (ANP), a hormone that increases diuresis and lipolysis. As a result, in addition to glycogen depletion, there is a significant loss of water and fat stores [37]. It is estimated that approximately 50% of the energy required for spearfishing diving comes from fat. Spearfishermen require supplementation that covers their specific needs for long-term, exhausting activity that combines elements of static and dynamic diving, with a large calorie deficit after fishing. Their supplementation should support the optimal function of the organism, reduce oxidative stress, speed up recovery, and enable the replacement of the key nutrients lost during activity. Similar as it is in dynamics, meal before training (3–4 h) should incorporate complex carbohydrates and easily digestible proteins, whereas, after training, hydration and then a small meal of a combination of fast-absorbing carbohydrates.

Supplementation

Supplementation for spearfisherman is supposed to be similar to that of dynamic apnea. Hence, it is good to take BCAA to prevent muscle catabolism and maintain energy levels during activity, beta-alanine, which helps in the tolerance of lactate accumulation and increases anaerobic endurance, and L-citrulline or L-arginine, which increase the production of nitric oxide, improve circulation, and thus supply muscles with oxygen [28]. Furthermore, due to the frequent horizontal body position, water temperature, hydrostatic pressure, and the resulting diuresis caused by ANP sweating in the diving suit and increased breathing, increased water loss occurs [38]. Dehydration of 2% of body weight can reduce aerobic capacity by 10%. Therefore, it is important to maintain hydration during diving with the possible addition of essential electrolytes. A good choice for this is isotonic drinks that contain the following: Sodium—maintains fluid balance and prevents dehydration. Potassium—key for healthy muscle function and the prevention of cramps [39]. Magnesium—supports the work of muscles and the nervous system [40]. Calcium—important for muscle contraction [41]. Optionally, isotonic drinks can also contain carbohydrates, usually in the form of glucose, fructose, maltodextrin, or sucrose, which provide a quick source of energy and help replenish depleted glycogen reserves, as well as vitamin C as an antioxidant and B-group vitamins to support energy metabolism [42].

After spearfishing, it is necessary to replace lost fluids rich in minerals as soon as possible, for example with isotonic drinks. For rapid regeneration, it is important to first consume a carbohydrate complex, such as maltodextrin or isomaltose, or, as a healthier and more natural option, a few dates and a ripe banana to quickly restore depleted glycogen reserves. In addition, a protein shake, such as whey protein, enriched with L-glutamine is recommended for accelerated muscle regeneration, protein replacement, and reduced fatigue [35]. The post-spearfishing meal can be supplemented with Omega-3 fatty acids from fish or flaxseed oil to reduce inflammatory processes, while vitamins C and E act as

powerful antioxidants that neutralize oxidative stress caused by apnea and exhausting physical activity. B vitamins additionally support energy metabolism and reduce the feeling of fatigue, while minerals such as zinc and selenium strengthen the immune system and contribute to recovery [27].

Such supplementation enables spearfishermen to maintain a high energy capacity during spearfishing, quickly replenish glycogen reserves, protect muscles, and reduce the negative effects of oxidative stress, thereby ensuring optimal preparation for spearfishing activity and accelerated recovery.

6. Conclusions

Proper nutrition for freedivers is based on a variety of natural foods that support an acid–base balance, provide a stable source of energy, and facilitate quality recovery. By adopting healthy eating habits based on the Mediterranean and alkaline diet, along with regular hydration and adapting the diet to the specific demands of diving, divers can improve their performance and ensure long-term health. The recommendations presented in this paper can be useful for freedivers in better planning their nutrition and training, as well as in creating pre-competition and training routines.

Future studies should include an examination of BHD eating habits. Also, they should be directed towards the examination of the influences of different diets on performance parameters. However, this is the first study proposing and recommending a diet for the three main BHD disciplines.

Author Contributions: Conceptualization, I.D. and Ž.D.; methodology, N.F.; software, D.V.; validation, D.V., I.D., and N.F.; formal analysis, D.V.; investigation, D.V.; resources, Ž.D.; data curation, D.V.; writing—original draft preparation, I.D. and D.V.; writing—review and editing, D.V. and Ž.D.; visualization, D.V.; supervision, N.F.; project administration, N.F.; funding acquisition, Ž.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical review and approval were waived for this study due to the fact that this study included only review of current literature, hence no human or animal participants were included.

Data Availability Statement: All data are included in the manuscript.

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

Abbreviations

The following abbreviations are used in this manuscript:

BHD	Breath-hold diving
MDSS	Mediterranean diet service score
ANP	Atrial natriuretic peptide
MD	Mediterranean diet
BCAAs	Branch-chain amino acids

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ISBN 978-3-7258-9046-0