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Muscle stretching protocols and health promotion strategies for public employees

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PhD CICLE XXXVIII

Academic Year 2024/2025

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ABSTRACT

The dissertation investigates strategies to promote an active lifestyle among public employees, focusing particularly on reducing work-related stress and preventing musculoskeletal disorders through physical exercise and specific stretching protocols. In modern society, characterized by fast-paced rhythms, widespread sedentariness, and increasing psychological pressures, these two phenomena represent complementary aspects of the same physiological and mental imbalance, with significant consequences for individual and public health. The overall aim of the work is to identify accessible and sustainable interventions capable of improving workers' physical and mental well-being, fostering a balance between productivity demands and personal health.

The research combines theoretical and experimental approaches to assess the effects of different stretching methodologies — static stretching (SS), proprioceptive neuromuscular facilitation (PNF), foam rolling (FR), and the newly introduced positional transversal release (PTR). The experimental studies, conducted on diverse populations, analyzed variables such as range of motion (ROM), tissue sensitivity, muscle hardness, balance, and neuromuscular performance, including the observation of remote effects mediated by myofascial chains. Results show that all techniques enhance flexibility without negatively affecting strength or stability, while PTR stands out for its quick application and effectiveness comparable to more traditional methods.

The experimental analysis was later extended to occupational settings, evaluating the impact of exercise and stretching programs on mental health, physical well-being, and cardiac variability in different groups of workers. Evidence indicates that regular, even brief, physical activity can reduce perceived stress, improve autonomic regulation, and promote greater psychological resilience. The research thus converges in proposing an integrated model for workplace health promotion, based on simple, evidence-based, and adaptable exercise protocols designed to enhance well-being and prevent the negative effects of sedentary behavior.

Chapter I outlines the theoretical framework, illustrating the physiological mechanisms of stress and the effects of sedentariness on the human body. It explains how the prolonged activation of the hypothalamic–pituitary–adrenal axis and the autonomic nervous system can impair immune, metabolic, and cognitive functions. The author discusses how modern work habits—often marked by poor posture and long periods of sitting—contribute to physical and psychological dysfunction, identifying movement as a key factor for restoring equilibrium. Within this perspective, stretching plays a dual role: it supports musculoskeletal health and acts as a regulator of stress and relaxation. The chapter reviews the main stretching techniques

and introduces innovative concepts such as non-local effects and myofascial connectivity, which expand the understanding of the body's adaptive responses to elongation stimuli.

Chapter II presents the series of experimental studies forming the empirical foundation of the thesis. These investigations explore the acute and chronic responses to various stretching and myofascial release methods, evaluating morphological, sensory, and neuromuscular parameters. Among them, the Positional Transversal Release (PTR) technique emerges as an innovative approach combining passive stretching with targeted stimulation of the myotendinous junction. Findings demonstrate significant increases in range of motion comparable to traditional protocols, but with shorter execution times. Other studies examine the influence of exercise sequencing, cross-over effects on untreated body segments, and differences between stretching and foam rolling regarding sensory perception and tissue response. A further investigation on older adults confirms that regular static stretching programs can improve flexibility, reduce muscle stiffness, and prevent falls, thus supporting functional autonomy in aging.

Chapter III translates experimental evidence into practical applications for workplace health promotion. It analyzes several studies conducted on teachers and healthcare professionals—occupational groups particularly exposed to stress and inactivity. Notably, results highlight the positive relationship between physical activity, adherence to the Mediterranean diet, and self-perceived well-being in the post-pandemic period. Another study employs portable multisensor devices to monitor heart rate variability, offering an innovative tool for real-time assessment of stress levels. In addition, a pilot intervention based on myofascial stretching shows significant reductions in musculoskeletal discomfort and perceived stress among healthcare staff. These findings lead to broader reflections on the importance of integrating brief movement sessions into daily work routines and adopting organizational policies that actively support employee well-being.

Chapter IV describes the author's scientific and professional development within the Sport and Exercise Sciences Research Unit. It outlines the methodological and experimental skills acquired throughout the doctoral journey, the international collaborations established, and the scientific outputs resulting from this work. This section highlights the evolution of a research path grounded in scientific rigor and practical application within the field of health promotion.

The thesis concludes with an extensive bibliography and appendices that include abstracts of published studies, conference presentations, and records of scientific collaborations. Overall, the work traces a coherent path that begins with the theoretical analysis of stress and sedentariness, continues with the experimental investigation of stretching mechanisms, and culminates in the implementation of evidence-based interventions for workplace health. The dissertation thus offers an original contribution to the fields

of Health Promotion and Exercise Sciences, proposing a practical and scientifically grounded model for fostering sustainable well-being.

Keywords: work-related stress, sedentariness, stretching, foam rolling, physical activity, occupational health, heart rate variability, health promotion.

ACKNOWLEDGEMENTS

LIST OF ABBREVIATIONS

ACTH: Adrenocorticotropic Hormone
ANOVA: Analysis of Variance
ANS: Autonomic Nervous System
AR: Aerobic Resistance
BF: Biceps Femoris
CB: Controlled Breathing
CC: Control Group
CG: Control Intervention
CRH: Corticotropin-Releasing Hormone
DF: Dorsiflexion
FR: Foam Roller
HF: High Frequency
HPA: Hypothalamic–Pituitary–Adrenal Axis
HR: Heart Rate
HRV: Heart Rate Variability
IPAQ: International Physical Activity Questionnaire
L-BIA: Localized Bioimpedance Analysis
LF: Low Frequency
MCS: Mental Component Summary
MFR: Myofascial Release
MG: Medial Gastrocnemius
MTJ: Myotendinous Junction
PA: Physical Activity
PCS: Physical Component Summary
PF: Plantar Flexor
PhA: Phase Angle
PIC: Persistent Inward Current
PNF: Proprioceptive Neuromuscular Facilitation
PPI: Pulse-to-Pulse Interval
PPT: Pain Pressure Threshold
PPG: Photoplethysmography

PSLR: Passive Straight Leg Raise
PTR: Positional Transversal Release
REST: Resting Condition
R: Resistance
ROM: Range of Movement
RT: Resistance Training
SB: Sedentary Behavior
SBJ Standing Broad Jump
SDNN: Standard Deviation of NN Intervals (Heart Rate Variability Index)
SAMS: Sympatho – Adrenal Medullary System
SS: Static Stretching
ST: Semitendinosus
TH: Tissue Hardness
V: Detector (Voltage)
VLF: Very Low Frequency
WHO: World Health Organization
Xc: Reactance
YBT: Y-Balance Test
Z: Bioimpedance

LIST OF TABLE AND FIGURES

CHAPTER I – INTRODUCTION

In modern society, shaped by urban growth, technology, and fast work rhythms, people are constantly exposed to physical and psychological demands that challenge the body's natural balance. Two main consequences of this lifestyle are chronic stress and sedentary behavior. Both conditions are now recognized as major public health problems and share a common origin: the body's difficulty in adapting to continuous environmental and social pressures. Stress is a psychophysiological response that helps the body face challenges and maintain internal stability. In short-term situations, stress is useful because it prepares the body to respond to danger or effort. However, when stress becomes chronic, the systems that regulate this response, especially the neuroendocrine and autonomic systems, remain overactivated. This leads to high levels of cortisol and other stress hormones, changes in metabolism, and reduced immune and cardiovascular function. At work, chronic stress often appears as work-related stress, which can cause anxiety, burnout, fatigue, and reduced productivity.

At the same time, sedentariness, spending long periods sitting or inactive, has become one of the main features of daily life in industrialized societies. Office work, technology use, and transport systems limit physical movement and increase the amount of time people spend sitting each day. Sedentary behavior is linked to musculoskeletal pain, metabolic disorders, cardiovascular disease, and even psychological problems such as depression and anxiety. Studies show that a lack of movement can also increase perceived stress and reduce heart rate variability, an indicator of the body's ability to adapt to challenges.

These two conditions often reinforce each other in a vicious cycle. Chronic stress reduces motivation to move, while physical inactivity increases stress responses and makes recovery more difficult. In this context, physical activity becomes an important preventive and therapeutic tool. Regular exercise helps regulate hormonal and emotional responses to stress, improves muscle and cardiovascular health, and supports better mental well-being. Introducing exercise and relaxation programs, especially in the workplace, can therefore reduce stress, prevent sedentary-related disorders, and promote a healthier and more productive lifestyle.

Understanding the connection between stress and sedentariness means seeing them not as separate problems but as two aspects of the same imbalance caused by modern living.

1.0 Mechanisms of stress response

Stress is a psychophysiological response to stimuli known as stressors. It enables the organism to adapt and restore internal balance. Homeostasis and allostasis both maintain physiological stability but through different mechanisms. Homeostasis regulates daily parameters, such as body temperature, while allostasis responds to unexpected events and re-establishes equilibrium following prolonged exposure to stressors. Continuous activation of the allostatic system leads to tissue deterioration, known as “allostatic overload,” which may result in pathological conditions. The organism reacts through adaptive mechanisms, showing both local and systemic responses (1).

Alterations may occur at endocrine, organic, and biological levels. When stressors are repeated, they can become pathological. In modern society, stress is often chronic and arises from occupational, relational, and social pressures. Psychological effects may include anxiety, burnout, and depression, along with sleep disturbances, poor concentration, and reduced productivity. Individual responses to stress vary, yet the physiological mechanism remains consistent: initially, the Sympatho–Adrenal Medullary System (SAMS) is activated, followed by the Hypothalamic–Pituitary–Adrenal (HPA) axis (2-4). Stress induces the hypothalamus to release corticotropin-releasing hormone (CRH), which stimulates ACTH secretion and consequently increases cortisol production (3).

These physiological processes prepare the body to face emergencies, but prolonged activation can damage both physical and mental health. Chronic stress exposure can alter brain function, producing cognitive, behavioral, and mood disturbances (5). It can reduce brain mass and weight (6), negatively affecting memory and cognition (7).

. Severe stress weakens the immune system, increasing the risk of malignant tumors (8). It also adversely affects cardiovascular function, inducing sympathetic overactivity, elevated heart rate, and increased blood pressure. Stress-related risk behaviors include myocardial ischemia progression, arrhythmogenesis, platelet aggregation, and endothelial dysfunction (9).

At the gastrointestinal level, stress alters absorption, permeability, mucus and acid secretion, ion channel function, and intestinal inflammation (10). Inflammatory conditions such as Crohn’s disease and gastric ulcers are often associated with high stress levels (11). Stress also increases colonic motility and directly influences the gut–brain axis. Because stress can exacerbate numerous pathologies, it is essential for the medical community to recognize its role as a risk factor and adopt integrated therapeutic approaches (both pharmacological and non-pharmacological), while considering individual differences (12)

1.1 Physical exercise as a preventive factor of work-related stress

In recent years, the growth in urbanization, technology and the adoption of an occidental lifestyle promoted the increase in the percentage of sedentary time. According to WHO, 3.2 million deaths a year are associated with sedentary behaviors (SB). SB, defined as time spent in sitting and/or reclining postures during waking hours, with energy expenditure less than 1.5 metabolic equivalents (METs), is often associated with the presence of musculoskeletal disorders (such as pain or discomfort in the back, neck upper and lower limbs) which are among the most prevalent employment problems (13). Back and neck pain are respectively the first and fourth causes of years lived with disability (14, 15). Sitting for a long time in non-ergonomic workstations is associated with musculoskeletal dysfunctions (16). The use of strength training programs appears to have better effects on pain than other types of exercises for the treatment of chronic musculoskeletal disorders (17). Nevertheless, exercise programs proposed in the literature for the treatment of issues such as back, neck and shoulder pain and carpal tunnel syndrome are methodologically deficient with small samples (18-23). In addition, SB was associated to adverse metabolic health, unhealthy ageing, poor bone health, premature mortality and an increased risk of some cancers. SB is present in different contexts such as home (free time), school and workplace. The domain of work is very important because it occupies most of the total daily sedentary time among desk employees (24). Working adults spend almost half of their waking time at work (between 8 and 11 hours a day) (25). In addition to musculoskeletal issues, it is also necessary to refer to the concept of long-term work-related stress, but also to depression, inflammation, obesity, diabetes, arthritis, infectious diseases and sleep disorders. In physiology, a stressful situation induces the production of stress hormones, an activation of the autonomic nervous system (ANS) with an increase in muscle activity and consequent increase in physical performance. This short-term response is a physiological survival mechanism for responding to life's dangerous situations. However, long-term activation of this stress response mechanism induces excessive production of cortisol and other stress hormones that negatively affects overall health. Work stress has been associated with reduced heart rate variability that coincides with reduced parasympathetic activation. HRV analysis can be used as a screening tool to monitor work-related stress levels (26). Absenteeism (time absent from work due to disease or disability) and presenteeism (reduced productivity at work) can result from these issues, affecting medical expenses and work productivity, as well as the health of the individual (27, 28). Although many health agencies recommend reducing time spent in SB, to date there is no clear dose-response relationship to support the proposed guidelines (29). WHO PA guidelines 2020 estimate that adults should perform at least 150-300 minutes/week of moderate intensity aerobic physical activity, or at least 75-150 minutes/week of vigorous intensity aerobic physical activity (30). However, many adults are physically inactive, with 1 in 4 adults worldwide not meeting these guidelines. For this reason, numerous studies in recent years have focused on health promotion outside and inside workplaces. A wide number of programs have been created to reduce SB and promote physical activity among sedentary workers. The use of physical activity in the workplace is a valuable tool for improving physical and mental health by reducing health care costs and absenteeism (31). The use of technology (phone applications in combination with wearables) is often used to reduce SB and increase physical activity levels (32, 33). Despite this and the many published literature reviews, it is unclear how effective exercise interventions can be in the workplace (34-36). There is conflicting evidence on the effectiveness of physical activity interventions in improving psychological well-being, with promising results but inconclusive evidence due to methodological gaps (37). In Addition, despite there are many programs on health promotion within different work environments, there is a gap in health promotion programs for tertiary education personnel (universities and colleges) (38). In a systematic review, Muir, Samuel D et al, sought to identify what factors concurs to make the uncertain results obtained from the administration of exercise protocols in sedentary workers (39). In particular, they investigated what factors determine user involvement in these programs and positive health behavior change. The results showed that older workers are more likely to engage in these exercise programs, while younger workers are more likely to improve their health-related behaviors. Then, in relation to gender, females engage more than males, but males are more likely to improve health behaviors. In addition, it was observed that workers who were already physically active before participating in a physical activity program are more likely to engage and gain benefits. Physical activity is an effective tool for reducing work stress levels by acting on both the physical and mental components. The challenge is to be able to engage the less physically active employees who need these programs a lot. New exercise programs should consider this information for greater effectiveness (39). Despite latter evidence, the results obtained did not clarify which exercise protocol may be the most appropriate to reduce stress levels (40). Short and simple exercise/fitness programs appear to provide similar benefits to more complex ones, being able to improve musculoskeletal disorders such as low back pain (41). The simplicity of a program could be a factor that could permit greater adherence to the exercise program, reducing the probability of dropout. In order to promote physical activity, it can also be achieved through goal settings, periodic positive feedback on progress, and self-monitoring (42).

The management of work-related stress requires a multidisciplinary approach that integrates various salutogenic strategies (43). The salutogenic approach promotes empowerment, prevention, and resilience. At the individual level, it focuses on physical and mental health, self-awareness, and stress reduction. It is crucial to foster a culture of well-being within workplaces by engaging both employees and employers, making healthy choices a routine practice rather than an occasional event (44).

Rest and leisure time are essential for restoring energy and mitigating occupational dysfunctions (45). Since the onset of the COVID-19 pandemic, there has been increased interest in programs and interventions aimed at enhancing the well-being of healthcare workers (46). Strategies to prevent or reduce burnout require a multidimensional framework that includes both individual and organizational interventions (47). On a personal level, workers should be encouraged to engage in self-care, stress management techniques, mindfulness, and regular physical activity. Strategies must be individualized to account for personal differences. At the organizational level, workplace policies should promote work–life balance and include programs that provide psychological support, employee wellness, and continuous professional development.

Individuals cope with difficult and stressful situations through adaptation strategies known as coping mechanisms. The most common are emotion-focused and problem-focused coping strategies. Cognitive-behavioral approaches are also effective, as they aim to modify maladaptive cognitions and strengthen active coping skills (48). Implementing these strategies requires self-awareness and consistent practice. Among the most effective individual techniques are relaxation practices, which activate the parasympathetic nervous system and lower heart rate, respiratory rate, and blood pressure (49, 50). These methods also influence the release of beta-endorphins and reduce ACTH and cortisol levels (51, 52), thereby improving awareness and resilience (53). Meditation helps workers remain focused on the present, reducing anxiety related to past or future concerns, while breathing techniques have been shown to decrease stress biomarkers, anxiety, and depression (54). Yoga has proven highly effective in preventing musculoskeletal and psychological problems, reducing stress and burnout, and improving sleep quality. Moreover, a decrease in salivary cortisol has been observed immediately following yoga practice (55, 56). Stretching reduces accumulated physical tension often caused by stress; incorporating short stretching sessions during the workday provides restorative breaks, alleviates musculoskeletal discomfort, and enhances productivity (57). All of these techniques can be implemented in the workplace through brief sessions that do not interfere with professional duties (58).

1.2 Stretching and its impact on the human body

Although stress and sedentariness influence multiple domains of human health, the scope of this thesis is deliberately focused on specific physiological aspects related to musculoskeletal function. In particular, the interventions examined in the following chapters address parameters such as range of motion (ROM), tissue mechanical properties, and their acute and chronic modulation through stretching and myofascial techniques. The aim is not to cover the full spectrum of sedentary-related consequences but to investigate in depth a clearly defined portion of physical health, allowing for precise experimental control and more robust interpretation of the observed adaptations. Focusing on this clearly defined physiological dimension represents a deliberate methodological strength of the thesis. By narrowing the scope to ROM and tissue-related parameters, the work avoids dispersive interpretations and allows for a more rigorous examination of how specific, easily applicable interventions influence measurable aspects of physical function.

Stretching represents one of the most widely used and extensively studied practices within exercise science and rehabilitation. It can be defined as a set of techniques and exercises aimed at increasing joint range of motion (ROM) and the flexibility of muscles and tendons. In recent years, growing attention has been devoted not only to its effectiveness in improving mobility, but also to its potential systemic effects on the cardiovascular, neuromuscular, and postural control systems (59-62). Although its primary purpose remains the enhancement of flexibility, stretching exerts influences that extend beyond musculoskeletal parameters, encompassing injury prevention, functional recovery, and even psychophysiological adaptation to training.

The mechanisms underlying the increase in ROM following stretching are multifactorial and involve sensory, neural, and structural components affecting both muscle and tendon tissues (63, 64). When a muscle is exposed to repeated or sustained elongation, adaptations occur that modify the perception of tension and discomfort, allowing greater stretch amplitudes before pain or resistance is perceived. This phenomenon, known as increased stretch tolerance, is considered one of the main contributors to flexibility improvements after both acute and chronic interventions (65, 66). At the neural level, passive stretching has been shown to transiently decrease α-motoneuron excitability, thereby reducing the central drive responsible for maximal force production (67, 68). This is attributed to a reduction in persistent inward currents (PIC), depolarizing currents that amplify synaptic input and sustain the high discharge frequencies required for maximal muscular output (69). Consequently, while stretching enhances movement amplitude, it may temporarily impair force and power generation if performed immediately prior to explosive or strength-demanding activities.

The most common stretching modalities include static stretching (SS), proprioceptive neuromuscular facilitation (PNF), and dynamic stretching (70). All of these techniques effectively increase ROM, yet they differ in their acute effects on performance and in the physiological adaptations they induce. The efficacy of stretching in enhancing flexibility is strongly dependent on the duration of the applied stretch, with longer bouts generally producing greater increases in ROM but also higher risks of transient decrements in neuromuscular performance (71, 72). The type of stretching applied is a key determinant of these outcomes: while static stretching, especially when prolonged beyond 60 seconds per muscle group, has often been associated with reductions in strength and power output, dynamic stretching tends to elicit opposite effects, facilitating neuromuscular activation and improving temperature-dependent tissue extensibility (73, 74).

More recent evidence, however, indicates that short-duration static stretches (below 60 seconds) may not significantly impair performance, suggesting a dose-dependent relationship between stretching duration and functional impact (75, 76).

A rapidly expanding line of research concerns the effects of stretching on balance and postural control. Findings are heterogeneous: some studies report improvements in balance following regular stretching practice, particularly among untrained individuals or those with impaired stability (77, 78), while others show temporary decrements in postural steadiness after long static stretching sessions (79, 80). These discrepancies likely stem from methodological variability, including differences in stretch typology, duration, and testing protocols—ranging from simple single-leg stance assessments to complex stabilometric analyses (81, 82).

In parallel with traditional stretching, alternative or complementary soft-tissue techniques such as myofascial release (MFR) and foam rolling (FR) have gained significant attention. MFR refers to a set of manual therapies applied to muscles and fascia with the aim of reducing soft-tissue restrictions and improving joint mobility. When compared with static stretching, MFR has shown equivalent efficacy in enhancing ROM, with the additional advantage of not requiring joint movement—an important aspect in subjects with articular limitations or during post-injury recovery phases (83, 84). The mechanisms underlying MFR-induced ROM gains appear to be largely neurophysiological, involving pressure-sensitive mechanoreceptors such as Golgi tendon organs and Ruffini endings, which modulate motoneuron excitability and reduce muscle tone, thereby facilitating movement amplitude (85, 86).

Foam rolling, on the other hand, is a self-administered myofascial technique commonly integrated into both warm-up and recovery protocols (87, 88). Studies have demonstrated that FR produces ROM increases comparable to those achieved through static stretching, though the mechanisms are distinct. Improvements following FR are attributed primarily to altered pain perception, temporary reductions in muscle stiffness, and thermoregulatory effects rather than to structural remodeling (89, 90). The increase in pressure pain threshold (PPT) frequently observed after FR suggests that sensory modulation plays a crucial role in enhancing perceived flexibility (91, 92). Interestingly, sham rolling—mimicking the movement without applying pressure—can also lead to ROM improvements, reinforcing the hypothesis that increased temperature and central nervous system activation contribute significantly to the observed effects (93-95).

An intriguing and increasingly investigated aspect of stretching and FR concerns their non-local or remote effects—physiological changes occurring in body regions different from the one directly treated. Two primary explanatory models have emerged. The first is the "crossover effect," which refers to ROM increases in the contralateral homologous limb following unilateral stretching, likely due to neural adaptations or increased stretch tolerance mediated by central mechanisms (96-98). The second is grounded in the concept of myofascial chains, according to which muscles and connective tissues form a continuous mechanical network capable of transmitting tension and force across distant segments (99, 100). Within this framework, localized mechanical input can influence distant joints along the same myofascial line, as demonstrated by studies showing improved cervical ROM following lower limb stretching interventions (101-103).

The theoretical foundation for the myofascial chain model derives from the concept of biotensegrity, which views the musculoskeletal system as an integrated structure where tension and compression are dynamically balanced. Empirical evidence supports this model, showing that manipulations applied to one region, such as the posterior chain, can induce measurable changes in other connected regions, even in the absence of local morphological alterations (100, 104). Clinically, this has significant implications, suggesting that addressing fascial continuity rather than isolated muscle groups may be an effective approach in cases of pain or limited joint mobility.

Another parameter that has received increasing attention is the sequencing of exercises within a training session. In resistance training, it is well established that exercises targeting strength should be prioritized early in the session, while exercise order has less impact on hypertrophy-oriented programs (105, 106). In the context of stretching, the influence of intra-session sequencing remains underexplored. Preliminary data indicate that performing static stretching on antagonist muscles during inter-set rest periods in strength training may enhance overall performance, whereas prolonged static stretching on agonist muscles before strength or power activities tends to impair them (107, 108). Additionally, combining stretching of agonist and antagonist muscle groups within the same session may leverage reciprocal inhibition mechanisms, promoting improved joint mobility and motor control (109-111).

In older adults, stretching acquires particular relevance for maintaining functional mobility and preventing falls. Age-related reductions in ROM contribute to diminished gait efficiency, impaired balance, and increased fall risk (112-114). Regular static stretching interventions have been shown to improve joint ROM and gait parameters, while long-term programs can decrease passive muscle stiffness and promote better movement economy (115, 116). Acute studies demonstrate that a single prolonged static stretch can reduce gastrocnemius stiffness in older adults, with magnitudes comparable to those observed in younger populations (112, 117). However, chronic adaptations of tissue stiffness in response to stretching remain underexplored, and further research is needed to elucidate the relationship between long-term ROM improvements and reductions in passive stiffness (118-121).

Although often perceived as a simple practice, stretching elicits a complex array of physiological responses. Increases in ROM are predominantly mediated by sensory and neural adaptations rather than structural changes, but contributions from viscoelastic and hydration-related factors—especially under compressive techniques such as FR and MFR—are gaining scientific recognition (122-125). Changes in local temperature, fluid dynamics, and fascia gliding behavior may collectively influence tissue viscoelasticity, thereby enhancing overall movement capacity (126, 127).

In conclusion, stretching represents a multifaceted physiological intervention capable of producing both immediate and long-term adaptations within the neuromuscular, fascial, and sensory systems. Its capacity to enhance range of motion and reduce passive stiffness is well established, although these effects are mediated through a complex interplay of neural modulation, sensory adaptation, and alterations in the viscoelastic properties of muscle-tendon units (63, 65, 89). When applied appropriately, stretching improves joint efficiency and movement economy; however, when performed excessively or at inopportune times—such as prolonged static stretching immediately before high-intensity or explosive exercise—it may transiently reduce muscle force and power output due to diminished motoneuron excitability and central drive (67, 68, 128).

Complementary modalities, including myofascial release (MFR) and foam rolling (FR), have emerged as valuable alternatives or adjuncts to traditional stretching. These techniques not only promote improvements in flexibility but also appear to enhance proprioceptive feedback and modulate pain perception without inducing performance decrements (84, 88, 90). The observed benefits are likely linked to neural desensitization mechanisms and local changes in tissue temperature and hydration, which together facilitate smoother fascial gliding and reduced mechanical resistance (122, 126, 127).

Within athletic settings, stretching continues to serve as a fundamental element of warm-up and recovery routines, provided that its parameters—such as duration, intensity, and timing—are adjusted to the specific physical demands of the sport. Dynamic stretching and shorter-duration static protocols are particularly useful before explosive or strength-based activities, as they optimize muscle readiness while avoiding the transient inhibitory effects associated with long static holds (72, 74, 75). Conversely, in clinical and rehabilitative contexts, stretching interventions contribute to restoring joint mobility, minimizing soft-tissue stiffness, and promoting neuromuscular coordination following injury or immobilization (61, 83, 129). In older adults, the inclusion of structured stretching programs holds significant preventive and functional value. With advancing age, progressive declines in ROM and increased muscle stiffness contribute to reduced mobility, balance impairments, and elevated fall risk. Regular stretching can mitigate these changes by decreasing passive muscle tension, enhancing flexibility, and improving gait performance (112, 115, 117). Evidence also suggests that the neuromechanical adaptations induced by stretching in older populations mirror those observed in younger adults, indicating that flexibility training remains an effective strategy across the lifespan (116, 118, 121).

Looking forward, further research is needed to clarify the relative contributions of morphological, neural, and perceptual mechanisms in determining the outcomes of different stretching modalities. Integrative studies employing imaging, neurophysiological, and biomechanical approaches may help elucidate how sensory tolerance, fascial mechanics, and motor control interact to produce functional improvements (101, 104, 125). Such insights would allow for more targeted application of stretching across performance, rehabilitation, and aging domains, ultimately optimizing its role as a scientifically grounded intervention for movement efficiency and human health.

In recent years, research has increasingly focused on understanding not only the flexibility-related outcomes of stretching but also its acute and chronic physiological effects on muscle, fascia, and neural systems. While the existing literature has clarified the influence of different stretching modalities—such as static stretching (SS), proprioceptive neuromuscular facilitation (PNF), and dynamic techniques—many aspects remain uncertain, particularly regarding the local versus non-local responses, the mechanisms underlying performance modulation, and the long-term adaptations associated with regular stretching practice.

To address these gaps, the experimental studies presented in Chapter II were designed to investigate specific variables that may influence the effectiveness of stretching and related interventions. These studies examine, in both acute and chronic settings, how different application techniques (including foam rolling, positional transversal release, and static stretching), administration orders, and session structures affect range of motion, sensory and morphological parameters, balance, and neuromuscular performance. Moreover, they explore whether these interventions produce local and remote (non-local) effects, potentially mediated by myofascial continuity or neurophysiological mechanisms.

The overall aim of these works is to deepen the understanding of the determinants of stretching efficacy, clarifying how factors such as duration, modality, and sequence shape both immediate and lasting adaptations. By combining physiological, sensory, and mechanical perspectives, these studies contribute to building a more comprehensive framework for interpreting stretching-related responses and for optimizing their practical application in exercise science, rehabilitation, and healthy aging.

The aim of this thesis is to evaluate the administration of exercise protocols inside and outside the workplace, attempting to define the dose-response relationship and reducing the level of work-related stress.

CHAPTER II – PRELIMINARY AND TRAINING STUDIES

2.0 Chapter II Overview

This chapter provides an overview of a series of preliminary research initiatives conducted as part of the broader investigative framework. These studies should be regarded as exploratory analyses focusing on specific variables or as methodological training exercises, with particular attention to their potential application in addressing musculoskeletal disorders and work-related stress among public administration employees. Each of the following sections presents a published research project, outlining the outcomes derived from the implementation of muscle stretching protocols.

List of publications discussed within Chapter II:

- 2.1 Thomas E, Ficarra S, Scardina A, Bellafiore M, Palma A, Maksimovic N, Drid P, Bianco A. Positional transversal release is effective as stretching on range of movement, performance and balance: a cross-over study. BMC Sports Sci Med Rehabil. 2022 Nov 30;14(1):202. doi: 10.1186/s13102-022-00599-8. PMID: 36451202; PMCID: PMC9714235.

- 2.2 Thomas E, Scardina A, Nakamura M, Bellafiore M, Bianco A. Acute effects of different administration order of stretching exercises: effects on range of motion and cross-over effect. J Sports Med Phys Fitness. 2024 Feb;64(2):95-102. doi: 10.23736/S0022-4707.23.15289-3. Epub 2023 Oct 30. PMID: 37902800.

- 2.3 Ficarra S, Scardina A, Nakamura M, Patti A, Şahin FN, Palma A, Bellafiore M, Bianco A, Thomas E. Acute effects of static stretching and proprioceptive neuromuscular facilitation on non-local range of movement. Res Sports Med. 2024 Nov-Dec;32(6):1015-1027. doi: 10.1080/15438627.2024.2326520. Epub 2024 Mar 9. PMID: 38459925.

- 2.4 Thomas E, Scardina A, Patti A, Izzicupo P, Nakamura M, Konrad A, Campa F, Bellafiore M, Bianco A. Acute local and non-local morphological, sensory and fluid responses to stretching and foam rolling in young females. Heliyon. 2024 Oct 9;10(20):e39023. doi: 10.1016/j.heliyon.2024.e39023. PMID: 39640605; PMCID: PMC11620026.

- 2.5 Thomas E, Scardina A, Pinto G, Serafini S, Nakamura M, Konrad A, Campa F, Bellafiore M, Bianco A. Determinants of acute effects of stretching vs. foam rolling: Morphological, sensory and fluid responses. J Bodyw Mov Ther. 2025 Sep;43:130-136. doi: 10.1016/j.jbmt.2025.04.026. Epub 2025 Apr 16. PMID: 40483113.

- 2.6 Pinto G, Di Bartolo L, Scardina A, Nakamura M, Warneke K, Patti A, Bellafiore M, Bianco A, Thomas E. Acute effects of foam rolling on local and remote range of motion: a myofascial perspective? Res Sports Med. 2025 Jul 18:1-14. doi: 10.1080/15438627.2025.2532525. Epub ahead of print. PMID: 40679233.

- 2.7 Nakamura M, Scardina A, Thomas E, Warneke K, Konrad A. Chronic effects of a static stretching intervention program on range of motion and tissue hardness in older adults. Front Med (Lausanne). 2024 Nov 25;11:1505775. doi: 10.3389/fmed.2024.1505775. PMID: 39655232; PMCID: PMC11625549.

2.1 "Positional transversal release is effective as stretching on range of movement, performance and balance a cross-over study"

Ewan Thomas, Salvatore Ficarra, Antonino Scardina, Marianna Bellafiore, Antonio Palma, Nemanja Maksimovic, Patrik Drid and Antonino Bianco

Introduction

In recent years, the interest in stretching exercises has grown and the possible effects on the cardiovascular and nervous system (59, 60) and its role in injury prevention and rehabilitation (61, 62), sporting enhancement (71), and balance have been investigated. Although, the main aim of stretching still remains to improve range of movement (ROM) of a joint and increase the flexibility of muscles and tendons (62, 130). The effects of stretching in increasing ROM have been attributed to four main mechanisms, involving sensory, neural and structural (muscle and tendon) adaptations (63). Differences in adaptational mechanisms may be observed after acute or chronic interventions (63, 64). Although changes in stretch tolerance are those most frequently observed regardless of intervention length (65, 66). Among the stretching methodologies, the most common and most studied are static (SS), proprioceptive neuromuscular facilitation (PNF) and dynamic stretching (70). Acute responses on ROM, indicate that stretching improves ROM in a time-dependent manner (71, 72). Effects which seem to be independent of stretch typology (71, 131). Stretching has been also deeply investigated in regard to its effects on performance as power, strength and speed (71, 128). Differently from ROM, the acute effects seem to strongly depend on stretch typology. Bouts of SS are generally observed to negatively affect strength or power performance if applied immediately before the activities (74, 75). Conversely, dynamic stretching may enhance power activities as jumping (74). There is still debate if stretch duration is crucial in performance hampering since some studies have observed decreased performance parameters after a single set of short duration stretching (76, 132) while others have observed such effects only after sets of longer duration (above 60 s) (128). The performance hampering effects observed as a consequence of stretching are attributed to reduced neural drive by a reduction of the excitability of α-motor neurons (67). This reduction in motor neuron excitability is ascribed to a reduction of persistent inward current (PIC), a depolarizing current with the property of amplifying the synaptic input–output of α-motor neurons (68) allowing higher depolarization frequencies, necessary for maximal force production (69). Therefore, reduced PIC will determine reduced synaptic output with a reduction in the force output of muscles. These effects have been only observed after passive stretching (67), which may explain the discrepancy in outcomes observed after dynamic stretching.

Another investigated aspect of stretching is its effect on balance. However, univocal conclusions could not be drawn since in some cases balance increased (77, 78), in others decreased (79, 80) or no effects (81, 82) were observed regardless if measures of static or dynamic balance were assessed. Differences in outcomes were identified pertaining either to stretch typology (79) or the screened population (77). Untrained individuals or those with impaired balance may benefit from performing stretching. While longer bouts of static stretching may reduce the evaluated balance parameters. Most importantly, high heterogeneity among the evaluation tests adopted can be observed across studies (i.e. single leg stance, stabilometric platforms, swinging platforms, etc.), which could be an additional factor influencing the discrepant results.

Other approaches different from stretching have been adopted for ROM improvement. One of these is myo-fascial release (83) (MFR, a term which incorporates a wide variety of manual techniques applied to muscles and fascia (84)), usually employed by physical therapists. These techniques when compared to stretching, have shown to provide equally effective results on ROM improvement (133-136). Advantages of MFR techniques are that (1) they do not involve joint movement, allowing to be specific on soft tissues, therefore limiting joint dysfunction when present (reason by which many physical therapists adopt them) (134) and (2) it generally does not require much time to achieve a result (90–120 s) (137). Neurophysiological adaptations seem to acutely drive the increased ROM observed [29]. These are thought to involve the Golgi reflex arc mediated by Golgi tendon organs and Ruffini and Pacini receptors which are sensitive to pressure (85). The application of manual pressure to deep fascia decreases motor neuron excitability and may lead to reduced muscular tension allowing greater ROM (86). However, conclusive mechanisms explaining the increased ROM observed are still to be determined.

Effects of MFR have been also studied on performance measures of power, force development or agility and static and dynamic balance, however, these usually result unaffected by the application of the techniques (84, 138-140). Since MFR techniques have the potential to improve ROM without impairing performance or balance parameters as observed after SS, it has been speculated that MFR could lead to improved muscle efficiency (139) supporting its use in sporting and rehabilitation environments.

Within this context, a hybrid technique incorporating components of SS and MFR is here proposed. This novel technique, the Positional Transversal Release (PTR), involves a passive stretch of the targeted muscle, followed by manual stimulation of the musculotendinous junction of the stretched muscle. Therefore, with this study, we aim to examine and compare this novel approach to traditional stretching and determine the acute effects on measures of ROM, performance and balance in healthy individuals.

Methods

Design

This study aimed to examine and compare a new technique to two traditional stretching approaches and determine the acute effects on ROM of the lower limb, jumping performance and dynamic balance in healthy individuals. A crossover research model was adopted in which each participant was tested on four occasions. A 1-week wash-out period was provided between subsequent testing sessions. The participants were tested in a university laboratory environment. On each visit, each participant was tested in three occasions (described in the following paragraphs). Each visit lasted ~ 1 h.

The study was conducted in accordance with the deontological norms laid down in the Helsinki Declaration and the European Union recommendations for Good Clinical Practice and the Ethical Standards in Sport and Exercise Science Research (141). The study was approved by the University bioethical committee (protocol n°65/2021).

Participants

Thirty-two participants (mean ± SD: 25.34 ± 5.56 years; 68.77 ± 12.54 kg; 172 ± 8.83 cm) of which 13 females and 19 males participated in this study. The participants were recruited from a population of university students. All participants provided written informed consent. Each participant was informed about the procedures, risks and benefits, of participating in this research but not regarding the research hypothesis. Subjects were eligible of inclusion if healthy, while no limitations regarding sex or age were applied. Subjects were excluded if injured or complained of musculoskeletal or neurological constraints. Competitive athletes were also excluded.

Procedures

A total of thirty-five participants were initially recruited. Recruitment procedures were carried out through social media advertisement. Each participant therefore voluntarily participated. Each participant after being informed about study procedures had to complete a questionnaire regarding physical activity level (IPAQ) (142). Based on individual responses the participants were classified as moderately active. All the subjects were asked to maintain their normal physical activities during the study but to refrain from any form of stretching exercise. Each participant had to attend the laboratory on four occasions, 1 week apart from each visit. The first visit served as a control, while on the following visits each participant had to undergo a different intervention. The administration order of the interventions was randomized for each participant.

On each visit, each participant underwent a passive straight leg raise test (PSLR) for both lower limbs to assess ROM, the standing long jump (SLJ) to assess a measure of performance and the Y-balance test (YBT) for dynamic balance. Each test was assessed at baseline (T0), immediately after the stretching intervention (T1) and fifteen minutes after the end of the intervention (T2). During the first visit (control session), the participants sat on a chair for fifteen minutes between T0 and T1. Of the thirty-five participants initially recruited, three did not attend the laboratory for all planned consecutive assessments, therefore these were not included in the final analysis. Recruitment and procedures are presented in Fig. 1.1

Measures

To ensure a high scientific standard, all measures were assessed by the same investigator, which was blinded regarding the proposed interventions. T0 and subsequent T1 and T2 were implemented at the same time of the day for each participant. To avoid any bias, measurements were performed without warm-up in the following order: (1) PSLR, (2) YBT and (3) SLJ. The YBT and the SLJ were sampled three times and only the best measure was retained for investigation. The PSLR was assessed once, in order to avoid a repetition-dependent effect on ROM (143).

Range of movement Hip flexion was measured with a Gyko inertial sensor system (Microgate, Bolzano, Italy) (144). Using Bluetooth 4.0, information was streamed to a computer with dedicated software (Gyko RePower; Microgate, Bolzano, Italy). A standard procedure for the PSLR was adopted. Participants were first instructed to lie supine on a medical bed. The Gyko was strapped at the level of the distal end of the femur of the tested leg. Inelastic straps were used to fix the contralateral limb to the medical bed. The tested leg was then passively lifted in full extension to the limit of the available ROM, or the point the participants started to feel pain or discomfort (145). The procedure was repeated for both limbs. The testing order of the limbs was randomized across participants.

Y-balance test A standard procedure for the YBT was adopted using a YBT kit (Functional Movement Systems®, Chatham, USA) (146) for dynamic balance evaluation. The kit is composed of a centralized platform and three pipes which connect to the platform. Each pipe is oriented in a different direction. The pipes are marked with 1 cm increments over which a moveable reach indicator is positioned. Participants were asked to move each reach with their leg as far as possible along each pipe while standing with the other leg on the central platform. The procedure was then repeated with the contralateral leg. In order to identify a "balance index" for each leg, leg length was calculated (From the anterior superior iliac spine to the lower margin of the lateral malleolus). The balance index was calculated by summing the distances covered over the three pipes and dividing this measure by three, to obtain an absolute mean distance. The absolute mean distance was divided by leg length, and the result was finally multiplied by 100.

Standing long jump A measure of performance of the lower limbs was collected through the SLJ. Each participant was asked to stand behind a line, with the feet at shoulder width and the toes touching but not crossing the line.

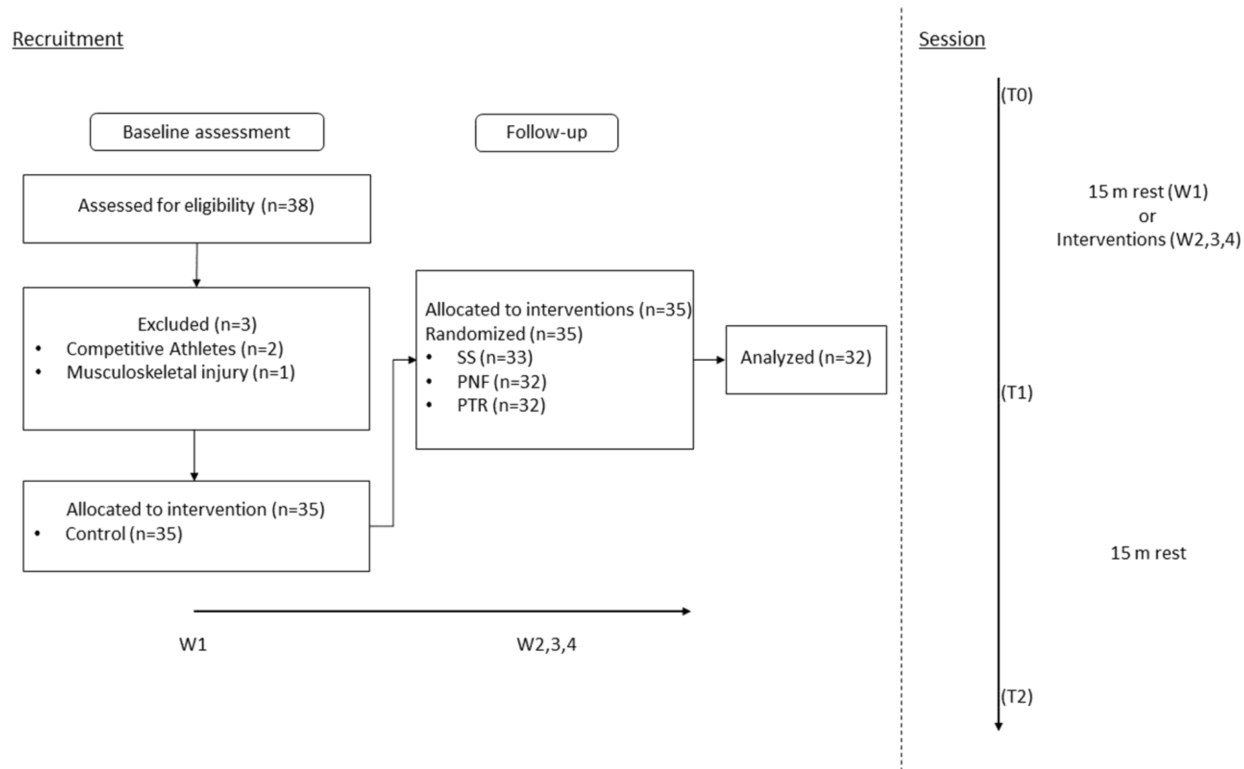


Fig. 1.1 Schematic representation of recruitment and assessment procedures.
The left panel shows the recruitment procedures, assessment for eligibility and allocation to intervention groups. The right panel shows the time course of the assessment procedures for each session. W1: Week 1 (control measurements); W2,3,4: Weeks 2, 3 and 4 (subsequent evaluations, intervention measurements); T0: baseline evaluation; T1: post intervention or post-15 m rest evaluation; T2: evaluation after 15 m-rest post T1; SS: static stretching; PNF: proprioceptive neuromuscular facilitation; PTR: positional transversal release

At the "go" of the investigator, the participants had to jump as far as possible. The participants were allowed to swing their arms during the jump. The jumping distance was measured through a tape measure, from the take-off line to the heels of the participant (147).

Interventions

All interventions were carried out by the same investigator (kinesiologist and manual therapist with ~ 5 years of experience). Each subject had to undergo all the proposed interventions and was informed about the stretching procedure prior to its administration. The stretching procedures were intended to target the hamstring muscles.

The static stretching (SS) intervention consisted of 8 sets of a 30-s passive stretch with 30-s rest between each set. Each participant was asked to sit on a mat, with the knees straight and the feet touching a wall with the feet in dorsiflexion at 90°. Without flexing the knees, each participant was instructed to flex their trunk over the hips in order to reduce the hip flexion angle. The investigator helped the participant towards the maximum tolerable trunk flexion angle and maintained the participant's position for the duration of the planned stretch by positioning his hands on the lower back of the participant. This protocol was chosen since it is frequently adopted in studies evaluating the acute effects of stretching (148).

An equated volume protocol concerning the stretch phase was adopted for the Proprioceptive Neuromuscular Facilitation (PNF) intervention. The protocol consisted of 8 sets of stretching with a 30-s rest between each set. The procedure was the same as that of the SS. Once the participant reached the maximum tolerable flexion angle, the passive stretch was maintained for 10-s, followed by an isometric contraction against the investigator's hands for 6-s, followed by a 4-s post-isometric relaxation phase. This procedure was repeated 3 times for each set.

Fig. 1.2 Representation of the Positional Transversal Release technique. The main scheme depicts the positioning of the participant and positioning of the operator. The box on the left lower aspect of the scheme depicts the positioning of the hand of the operator on the myotendinous junction of the hamstring muscle of the participant

The positional transversal release (PTR) consisted of 1 to 2 mechanical stimulations of the proximal insertion of the hamstring muscles at the level of the myotendinous junction (MTJ). This was defined as an area ~ 5 cm below the ischial tuberosity. Each participant was instructed to lie prone on the edge of a medical bed, with one limb hanging from the medical bed. The investigator with one arm passively accompanied the leg of the participant towards a sub-maximal hip flexion (stretch phase). No pain or discomfort had to be perceived by the participant. The investigator positioned his other hand, more specifically his knuckle, on the medial side of the hamstring's MTJ (149, 150) and through a rapid motion, in a transversal direction (compared to the hamstring muscle fibres), stimulated the MTJ (Fig. 1.2). During hand positioning, the investigator manually appraised the tension of the ham- string's MTJ. Subsequently to the application of the PTR technique, the investigator re-appraised the hamstrings muscle tone and if a reduction was manually perceived the procedure was repeated on the contralateral limb. Otherwise, one additional stimulation was provided. No additional stimulations were provided, regardless if the perceived tone resulted or not decreased.

Statistical analysis

A priori sample size calculation was performed through G-power (G*Power version 3.1.9.4, ES = 0.3, $1 - \beta = 0.80$, $\alpha = 0.05$) which defined as 32 the minimum number of participants required for the planned research model. Data are presented as means and standard deviations. Inferential statistics were carried out with Jamovi (The jamovi project (2021). Jamovi (Version 1.8.0.1) [Computer Software]. Retrieved from https://www.jamovi. org). A Shapiro–Wilks test was performed to identify the normality of the distribution of all parameters. Repeated Measures Analysis of Variance (ANOVA) was performed for each measure using a 3 × 4 model to identify time and group interactions. Post-hoc Bonferroni corrections were performed to identify differences between the assessments for each group. Graphs were created with GraphPad Prism8 (GraphPad Software, San Diego, CA). An alpha level of $p < 0.05$ was defined for the statistical significance of all the tests.

Results

Of the 35 participants initially enrolled, 32 completed all the evaluations (three participants did not show up for subsequent evaluations, Fig. 1.1) therefore, data from 32 participants were analysed. No significant differences were observed when comparing the T0 across the 4 weeks for all tests (No differences between the control session and interventions at T0). No significant differences were observed for the control measures across time (T0, T1 and T2). When considering the interventions, a significant time × group interaction was observed for ROM (Right leg $F = 4.62$; $p = 0.0002$, Left leg $F = 2.64$; $p = 0.017$). Post-hoc analysis revealed increases from T0 to T1 for SS, PNF and PTR. In all groups, ROM remained elevated in T2. No differences were observed for any intervention between T1 and T2. No differences were observed across interventions during the same time point evaluations (Table 1.1 and Fig. 1.3). No time × group interactions were observed for the SLJ ($F = 1.53$; $p = 0.158$) and the YBT (Right $F = 0.57$; $p = 0.678$, Left $F = 0.68$; $p = 0.605$).

Table 1.1 The effects of stretching on variables of ROM, Jumping performance and Balance

	Control T0	Control T1	Control T2	SS T0	SS T1	SS T2	PNF T0	PNF T1	PNF T2	PTR T0	PTR T1	PTR T2	ANOVA
ROM (°)													
PSLR R	87.1 ± 22.6	87.8 ± 22.5	90.3 ± 23.8	90.1 ± 22.9	96.7 ± 22.1[a]	94.0 ± 23.2[a]	89.7 ± 21.7	99.3 ± 23.8[a]	98.2 ± 24.2[a]	91.1 ± 21.7	95.8 ± 23.1[a]	96.5 ± 24.3[a]	<0.001*
PSLR L	86.7 ± 22.4	88.7 ± 22.3	89.4 ± 23.5	89.7 ± 21.1	94.6 ± 21.9[a]	95.6 ± 21.8[a]	90.8 ± 21.1	98.4 ± 24.8[a]	97.8 ± 25.2[a]	90.1 ± 22.9	95.1 ± 22.7[a]	95.6 ± 24.0[a]	0.017*
Performance (cm)													
SLJ	167.0 ± 34.5	168.7 ± 35.4	167.3 ± 36.4	171.9 ± 36.5	171.3 ± 37.7	175.0 ± 35.0	172.7 ± 35.1	167.5 ± 38.7	170.0 ± 36.0	173.8 ± 38.1	173.3 ± 37.4	174.0 ± 39.7	0.158
Balance (%)													

YBT R	93.2 ± 7.5	94.9 ± 7.7	96.0 ± 7.6	97.1 ± 6.6	97.4 ± 6.0	97.0 ± 6.7	96.9 ± 6.5	97.0 ± 7.0	98.0 ± 7.5	97.0 ± 6.7	96.8 ± 6.8	98.0 ± 6.8	0.678
YBT L	92.5 ± 9.2	94.4 ± 7.0	95.4 ± 7.8	96.5 ± 6.7	97.3 ± 6.7	97.4 ± 6.6	96.1 ± 6.9	97.0 ± 7.1	97.4 ± 6.8	97.2 ± 6.9	97.4 ± 7.1	97.4 ± 7.1	0.605

Data are presented as means ± SD; T0: baseline evaluation; T1: post-test assessment; T2: assessment 15 m post-test; PNF: proprioceptive neuromuscular facilitation; PTR: positional transversal release; ANOVA: group × time interaction; a post-hoc difference with T0; *Significant $p < 0.05$

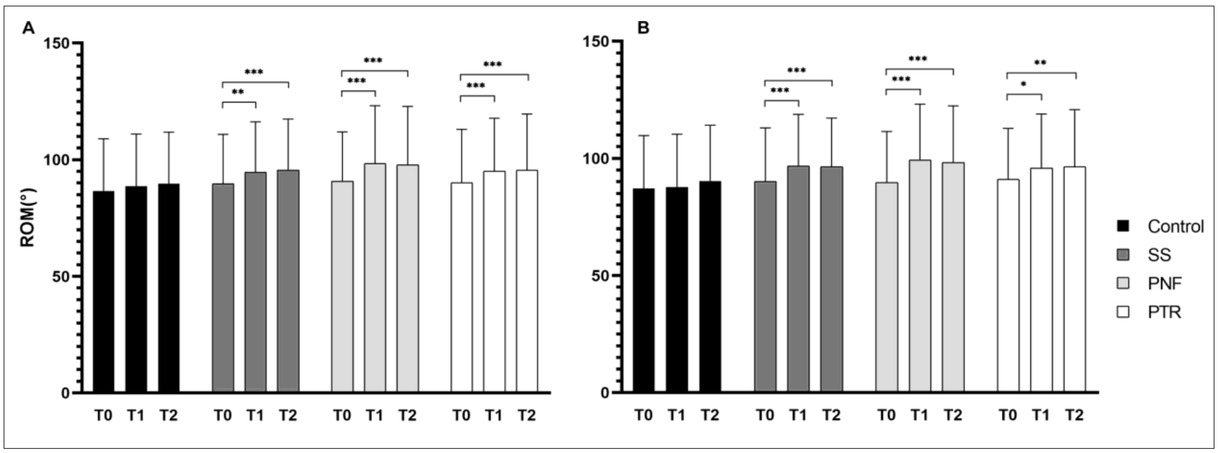


Fig. 1.3 ROM of the lower limbs. A shows measures pertaining to the left leg. B shows measures pertaining to the right leg. ROM: range of movement; SS: static stretching; PNF: proprioceptive neuromuscular facilitation; PTR: positional transversal release. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Discussion

The aim of the present investigation was to examine and compare the acute effects of a novel technique to two stretching techniques applied to the hamstring muscles on ROM of the lower limbs, jumping performance and dynamic balance. Our results indicate that all three interventions were equally effective in increasing ROM, while no acute effects were observed for jumping performance and dynamic balance. The acute ROM increases observed as a consequence of stretching are generally attributed to a reduction in sensation (63), which may either reflect a psychological alteration or the willingness of each participant to tolerate greater torque since these expect that following stretching interventions an increase in ROM should occur. The mechanisms attributed to increased ROM after MFR are usually ascribed as either Golgi tendon organs mediated or by Ruffini and Pacini mechanoreceptors reflexes (151). Despite these mechanisms had been also traditionally attributed to stretching, more recent evidence does not support these assumptions, suggesting that modulation of pain sensation occurs (152, 153). In this context, we tried to develop a technique which incorporated both aspects in order to improve the sought ROM. The first aspect of the PTR is the lengthening position, which may influence the participant's sensation in a similar-stretching man- ner. The second aspect is the mechanical and transversal stimulation of the MTJ. It is known that the MTJ is rich in mechanoreceptors, Golgi tendon organs on the muscular side and Pacini receptors on the tendinous side (86, 154). These latter, which can be also found in the dermal layer, are sensitive to rapid pressure changes and vibration. In addition, Ruffini receptors can be found in ligaments and dermal tissues which are usually subjected to stretching and are particularly responsive to tangential forces (155). Different experimental investigations have found interactions between these cutaneous receptors and proprioception (156-158) and subsequent applied research supports the inclusion of cutaneous stimuli, such as vibration, for ROM improvement (159-161). The mechanical stimulation provided tangentially to the MTJ aims to stimulate both Pacini (through the rapid movement which mimics a high-frequency vibratory stimulus) and Ruffini receptors (through the cutaneous transversal direction). In a review by Proske and Gandevia (154), the authors describe that these receptors may provide positional sense by acting as "limit detectors", which would suggest that alteration of afferent signals by these receptors may alter the proprioceptive positional limit of a joint. However, there are no studies evaluating if these specific effects may favour or limit ROM. Yet, we observed an immediate increase in ROM in both limbs after the application of the PTR.

To be noted, all the applied interventions were statistically equivalent, however the PNF stretching showed the greatest increases in absolute terms from T0 to T1 (SS > 5.75° [6.4%]; PNF > 8.6° [9.5%]; PTR > 4.85° [5.4%]), while being the only one showing a decremental trend from T1 to T2 (SS > 0.3° [0.3%]; PNF < 0.3° [− 0.35%]; PTR > 0.55° [0.6%]). Results consistent to those reported by Behm et al. in a systematic review evaluating the acute effects of different stretching typologies on ROM (71). Future studies should also consider the time course of these techniques incorporating 30 m or 60 m post- intervention assessments. Pertaining to jumping performance we expected a reduction in the observed values following the two stretching interventions (162-165). These reductions, however, were not present. A careful analysis of the available scientific literature has emphasized that vertical jumps are more frequently employed as a performance post-stretching measure. Only the study of Merino-Marban et al. (166) published in 2021, similarly to our investigation, evaluated the acute post-stretching effects of long jump performance. Regardless of the differences in the analyzed populations (being that primary school children were employed), the authors did not evince significant differences after the SS intervention. Differences in the biomechanical contribution of each joint are present between horizontal and vertical jumps (167, 168). Therefore, a difference in jumping technique could have determined differences in the observed outcomes across studies.

Another point to be considered is that our intervention intended to target the hamstring muscles. Such factor could be an additional element to consider affecting jumping performance (169), since the ham- strings, despite being crucial during the propulsion phase, are not the main muscular contributors of jumps (170). However, according to Robertson et al. and Kotsifaki et al. (167, 168) the hip and knee (which are both affected by the hamstrings) contribute for ~ 50% to 60% of the total work during horizontal jumps. Results from previous investigations on the effects of MFR on jumping performance provide disagreeing evidence, highlighting that MFR can either enhance it or be similar to dynamic warmups, but could also result in a performance reduction if applied for more than 1 min (171-173). In addition, as described in the previous paragraph, also in MFR studies vertical jumps are more frequently employed to assess lower limb performance. Only the studies of Itotani et al. (138) and Queiroga et al. (174) evaluated the effects of MFR (using two different approaches) on long jump performance. In the study of Itotani et al., the authors evaluated the immediate and post-5 days effects of continuous MFR. Despite increased jumping measures being present after 5 days, no immediate post-MFR effect was observed. Whereas Quieroga et al., applied self MFR immediately before a horizontal jump and the evaluation of different measures of ROM. Although the general increases in measures of ROM, no difference in the horizontal jump performance was observed. The results of both studies are similar to those observed in the present investigation following the PTR intervention. However, the paucity of published literature regarding both effects of stretching and MFR on long jump performance prevents us to infer definitive conclusions.

Long bouts of static stretching (greater than 45 s duration) have been observed in several investigations to acutely impair strength (80, 175), a reduction which could also affect one's ability to balance (75, 176). However, no univocal conclusions have to date been drawn. The increased stretch-induced joint position (175) and the reduced tendon-unit stiffness (177) could be fac- tors increasing balance ability through proprioceptive feedback after stretching. Conversely, the reduction in neural drive post-stretching (67) could determine reductions in balance. In addition, other factors such as the previous training experience of participants or differences in assessment procedures can possibly explain the differences observed across studies. A recent study by Coratella et al. (81) has evaluated the effects of passive stretching on dynamic balance performance and muscle efficiency. While no significant differences were observed in measures of either static and dynamic balance, the authors report decreased maximal voluntary muscle contraction and decreased muscle activation, underlining a reduction in muscle efficiency. However, we did not observe a reduction in measures of balance after the SS intervention. Since we used relatively short stretch durations (30 s) and our population comprised young healthy individuals, it is plausible that if a reduction in muscle efficiency, as suggested by Coratella et al., was present, this did not transfer to a reduction in balance, being that balance is a multifactorial ability (178). In addition, different outcomes also emerge when considering the effects of PNF on balance. The majority of the investigations carried out on healthy subjects, observe increases in both static and dynamic balance parameters (179-181). These are particularly evident pertaining to dynamic balance in the medio-lateral direction (182, 183). Results are in contrast to those observed in this investigation since neither for the PNF stretching we observed any effect on balance. However, these investigations applied the PNF stretching to multiple muscles (i.e. adductors and abductors, or hip flexors and extensors or all muscles of the lower limb). Therefore, the positive effects observed may not necessarily reflect the stretching phase of the PNF, but rather the contraction, which could have determined increased strength and therefore increased ability to balance (184). Indeed, the only study applying PNF to a single group of muscles, more specifically the hamstrings (as in the present investigation), observed no increase in bal- ance in neither the anterior–posterior nor medio-lateral directions (185). No effects on balance were also observed after the application of the PTR technique. Results that seem to be in line with other studies investigating balance after the application of MFT techniques (140, 186). However, it is important to stress that not many studies have been carried out on such a specific topic and being that a wide range of techniques may fall under the concept of MFR and a large heterogeneity of balance assessment procedures are adopted, it is very difficult to compare our results with those of other studies. In particular, most of the studies evaluating the effects of MFR tend to use foam rollers or carry out self-MFR interventions. Differently, the PTR technique implemented in the present investigation was manually applied by an investigator. In addition, studies may evaluate static rather than dynamic balance or adopt procedures different from the Y-balance included in the present investigation. The present manuscript is not without limitations. Despite statistical power being reached, this is the first intervention proposing the PTR as a method for ROM improvement. Therefore, a broader body of evidence is needed to confirm our results. In addition, we adopted a research model which only accounts for intra-individual differences.

A further aspect which warrants attention is that the number of PTR stimuli (1 or 2) was determined according to the operator's perception of the hamstrings tone. Also, the PTR differently from stretching cannot be self-administered. Therefore, coaches or therapists need to be familiar with the correct execution of the technique. However, the application of the techniques by a single operator within this study was intended to limited inter-operator differences. Despite the above discussed limitations, our results confirm the effects of PTR on ROM and are in line with other studies regarding jumping performance and balance. The results of the present study, which arise from a population of healthy active participants, highlight a potentiality for the use of the PTR, with outcomes similar to SS and PNF. It is interesting to note that both stretching protocols (8 sets of 30 s with 30 s rest) needed at least 8 min to be carried out, while the PTR only required one to two stimuli to elicit a measurable response. Future perspectives will be to evaluate the feasibility and effectiveness of the PTR in clinical settings. If our results will be confirmed, the advantage would be to have a form of therapy which could be applied quickly and limit pain which might arise during longer forms of treatment.

Conclusion

The present study indicates that the three proposed interventions are equivalent pertaining to the acute effects on range of movement, jumping performance and dynamic balance. Increased range of movement and no detrimental effects on performance and balance were observed. The positional transversal release technique is, however, less time-consuming compared to static stretching or proprioceptive neuromuscular facilitation, which could represent an advantage in acute settings. Despite these preliminary results, future interventions need to confirm these findings.

2.2 "Acute effects of different administration order of stretching exercises: effects on range of motion and cross-over effect"

Ewan Thomas, Antonino Scardina, Masatoshi Nakamura, Marianna Bellafiore, Antonino Bianco

Introduction

Effective exercise and rehabilitation programs are conceived based on specific parameters. The main investigated ones include exercise intensity, volume, density, and frequency (105). however, other important parameters which also warrant attention are exercise velocity and order of exercise administration. This latter in recent years has gained increasing attention (106, 187). Evidence concerning different forms of exercise however does not provide unequivocal conclusions.

Among resistance training programs (RT) it appears that if the main aim of the exercises is to increase strength, it is favorable to perform the exercises in which strength gains are warranted at the beginning of the intervention (106, 188). Conversely, if the aim of RT programs is hypertrophy, exercise order does not affect the intended outcomes (106). Concerning aerobic interventions (AR), to the best of our knowledge, there are no studies investigating the effects of different combinations of exercises on vascular or metabolic outcomes. However, exercise order has been investigated in concurrent AR and RT programs (187, 189, 190). Suggesting that overall, RT should precede AR if the intended aims are optimizing strength increases, (191) glucose management, (189) and arterial stiffness reduction (187). However, the intra-session exercise sequence doesn't seem to affect maximal oxygen consumption6 and post-exercise oxygen consumption (192).

Despite evidence on exercise order for RT and AR is growing, this has still not been investigated in stretching, especially for information concerning ROM. Limited information is however available regarding other aspects of stretching. a concurrent investigation of RT and stretching in which static stretching (SS) was performed in antagonists muscles during inter-set rest has noted that strength increased following such intervention (107). However, an opposite effect is usually observed when SS is performed on the agonist's muscles before RT or athletic performance which usually determines decrements in performance outcomes (108). To be noted that these impairments are usually observed following long bouts of stretching (>60 seconds) and if no warm-ups (as those performed through dynamic stretching) precede the intended performance (108, 128). Further, different investigations have reported that if stretching is first performed in a limb, a joint's range of movement (ROM) improves not only in the stretched limb but to a moderate extent also in homologous muscles of the contralateral limb (148). Such an effect is named the "crossover effect". To date, the crossover effect is the sole known effect concerning the administration order of both stretching and ROM. The increases in contralateral ROM have been suggested to be caused by increased stretch tolerance, mechanisms which are also usually attributed to increased ROM when stretches are applied to the measured limb (97), based on the unaltered non-stretched muscle strength and activation variables (98, 193). Such effects seem to be present regardless of the stretch technique, being this observed for either static or dynamic stretching (194). To the best of our knowledge, it is still unknown if the order of intra-session exercise administration within stretching programs may influence the outcomes of ROM. in order to verify if different exercise orders may affect ROM outcomes we underwent an intervention in which SS was administered to the right knee extensors and knee flexors (and combinations of these) and measured ROM in both the stretched and non-stretched muscle groups of both limbs. We administered stretching to agonist and antagonist muscle groups since it is known that during the lengthening of a muscle, a stretch reflex is generated (myotatic reflex) (109) which will then produce an inhibitory reflex to the antagonist muscle group, named reciprocal inhibition (110, 111). Therefore, our hypothesis is that unilateral stretching of muscles will determine: 1) increased ROM in the stretched muscles, 2) increased ROM in the homologous muscle groups of the non-stretched limb, 3) greater increases in ROM in those groups who will stretch both agonists and antagonists muscle groups.

Materials and methods

Study design

The study aimed to examine the effect of unilateral intra session stretching order on ROM outcomes of the lower limb. Measures of both limbs were collected to identify a crossover effect. The study was conducted in accordance with the deontological norms laid down in the Helsinki declaration and the European union recommendations for Good clinical practice and ethical Standards in Sport and exercise Science research. The study was approved by the University of Palermo bioethical committee (protocol n° 65/2021). Participants in order to participate in this study each participant had to sign a written informed consent. a total of 108 participants took part in this study (55 male and 53 female, aged 23±3.1 years; body weight 66.5±12.7 kg; height 167.3±9.6 cm). The participants were recruited between September 2022 and March 2023 from a population of university students. All participants were informed regarding the procedures, risks and benefits of participating in the research prior to their participation. participants with musculoskeletal, neurological or orthopedic constraints were excluded.

Procedure

Each participant was instructed to complete a questionnaire regarding the level of physical activity (IPAQ).20 From the screening of individual results, these were considered moderately active (h/week of physical activity 9.2±5.7). Each participant attended the laboratory once. The laboratory visit lasted approximately one hour. After completion of the IPAQ questionnaire, the anthropometric screening (height and weight) was carried out and each participant was randomly allocated, based on outcomes of an online sequence generator (Graph pad) to an intervention group. Randomization was further controlled for sex in order to balance the number of male and female participants in each group. The experiment included 4 experimental groups and a control group. The experimental groups comprised one group to whom stretching was applied to the knee extensors (KE), one group to whom stretching was applied to the knee flexors (antagonist muscles, KF), one group to whom the stretching was first applied to the KE and then to the KF and one group to whom the stretching was first applied to the KF and then to the Ke. These muscle groups were selected since they can be easily self-stretched and SS exercises are commonly applied in both sporting and rehabilitation contexts. No stretching was applied to the control group. After the allocation of participants, these were assessed before the intervention (T0), immediately after the intervention (T1) and 15 minutes after the intervention (T2). The participants allocated in the control group sat on a chair for 15 minutes between T0 and T1. All participants sat on a chair for 15 minutes between T1 and T2. a passive straight leg raise (PSLR) and passive hip extension (PHE) tests were assessed at T0, T1 and T2 for both the right and left lower limbs.

Measures

All measures were undertaken by the same investigator who was blinded to the proposed intervention. Measures included the PSLR and the PHE. These were randomized concerning the right or left lower limb and their order within each limb. All measures were assessed once in or der to avoid a repetition-dependent effect (143). All measures were assessed through a Gyko inertial sensor system (Microgate, Bolzano, Italy) (144). Information concerning ROM was Bluetooth streamed to a computer with dedicated software (Gyko Repower; Microgate, Bolzano, Italy).

Passive straight leg raise

In order to assess hip flexion the inertial sensor was firmly strapped at the anterior-distal aspect of the femur of the assessed limb. During the assessment, the participant was lying supine on a medical bed with the contralateral limb firmly strapped with inelastic straps in order to avoid compensatory movements of the leg or the pelvis. The tested leg was passively lifted (in a hip flexion motion) while in full Ke to the limit of available ROM or the maximum tolerable pain for each participant (145). During the assessment, the participants were also instructed to keep their eyes closed in order to reduce the influence of visual inputs on their perceived sensation.

Passive hip extension

In order to assess hip extension the inertial sensor was firmly strapped at the posterior-distal aspect of the femur of the assessed limb. During the assessment, the participant was lying prone on a medical bed with the contralateral limb firmly strapped with inelastic straps in order to avoid compensatory movements of the leg and the pelvis. The tested leg was passively lifted (in a hip extension motion) while the knee was kept flexed at 90°, to the limit of available ROM, or the maximum tolerable pain for each participant (145). As for the PSLR, during the assessment, the participants were instructed to keep their eyes closed.

Interventions

All interventions were carried out by the same investigator (A.S.). The SS procedures were intended to target: 1) the KE; 2) the KF; 3) first the KE and then the KF; or 4) first the KF and then the KE. A protocol consisting of eight sets of 30 seconds of SS with 30 seconds of rest between each set was applied to each muscle group. Such protocol was chosen for consistency with a previous study (195). The SS was always applied only to the right leg of each participant. In order to target the KE, each participant was asked to rest the knee of the right leg on a mat placed on the ground, keeping the femur perpendicular to the floor. The heel of the right leg was then passively brought up to the point of discomfort of KF angle by the investigator who maintained such position for the time required to perform a set of SS. The contralateral limb was kept anteriorly, with the knee at 90° so as to stabilize the trunk. The trunk was kept parallel to the anterior aspect of the right femur (figure 1.4). In order to target the KF, each participant was asked to lie in a supine position. The left leg was strapped with in elastic straps in order to avoid compensatory movements of the limb and pelvis. The right limb was then lifted by the investigator while in full extension up to the point of discomfort of hip flexion. While lifting the limb, the investigator also maintained the right ankle of the participant in a neutral position with the foot at 90° to the leg (figure 1). When the KE and KF protocols (either KE/KF or KF/KE) were combined, a 30-second rest was provided between the two stretching procedures (KE or KF above described).

Figure 1.4 Example of the KE stretching intervention in the left panel and of the KF stretching intervention in the right panel.

Statistical analysis

In order to identify an appropriate sample size G-power (G*Power v. 3.1.9.4, F-Tests, ES=0.3, 1-β=0.80, α=0.05) was adopted, which defined as the minimum number of participants required for the planned research model. Data are presented as means and standard deviations. Intraclass correlation coefficient (ICC) and the percentage of coefficient of variation (%CV) from the repeated measures of the control group were calculated to check the test-retest reliability and heterogeneity of data, respectively. Differences between groups and assessments were assessed using a repeated measure analysis of variance (ANOVA) model using two factors (accounting for time [T0 vs. T1 vs. T2] and groups [CG vs. KE vs. KF vs. KE/KF vs. KF/Ke]). effect size (ES) measures were calculated (196). ES values in the range of 0.00-0.19 were considered trivial, and values in the ranges of 0.20-0.49, 0.50-0.79, and ≥0.80 were considered small, moderate, and large, respectively. If the interaction effect was significant, a post-hoc analysis was conducted. Post-hoc Bonferroni corrections were per formed to identify individual differences between assessments for each group. All analysis were performed with Jamovi (The Jamovi Project 2021, v. 1.8.0.1). Graphs were created with Graphpad prism8 (Graphpad Software, San diego, ca, uSa). Alpha was set at 0.05 for all analysis.

Results

Table 1.2 Distribution and proportion of gender for each group

Group	Total	Male	Female
Control	22	10	12
KE	22	11	11
KF	21	11	10
KE/KF	21	12	9
KF/KE	22	11	11
Total	108	55	53

Groups were randomized but balanced for sex in order to account for intrinsic ROM differences (Table 1.2). ICC for measures of right and left PSLR and right and left PHE were 0.91, 0.86, 0.94 and 0.92, respectively.

%CV for measures of right and left PSLR and right and left PHE were 24.5, 23.8, 27.4 and 29.9, respectively. No differences were observed for the CG neither for time (T0 vs. T1 vs. T2) nor ROM measures (PSLR and PHE) for both limbs (stretched vs. non-stretched). A time x group interaction was identified only for the stretched limb for both the PSLR (p=0.0007; f=3.55; ES=0.73) and the PHE measure (p=0.04; f=1.99; ES=0.55) (Table 1.3, figure 1.4 and 1.5). Post-hoc analysis for PHE of the right leg observed an increase in measures of ROM within groups only for KE and KF/KE from T0 to T1 and for PSLR of the right limb an increase of ROM only for KF and KE/KF from T0 to T1. Only for KE/KF a prolonged effect (from T1 to T2) of the intervention was observed for the PSLR measure. No other significant differences were observed in the stretched limb. These results highlight that ROM moderately increased only for the last stretched muscles in each group. No differences for the PSLR and PHE in no group were observed in the non-stretched limb, despite a tendency for increased ROM was present (Table 1.3, figure 1.6 and figure 1.7).

Table 1.3 The effects of the stretching interventions on ROM of both limbs

Groups	PSLR R			PSLR L			PHE R			PHE L		
	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
Control	98.8 ± 24.5	99.5 ± 23.4	105.4 ± 26.5	99.0 ± 23.5	99.3 ± 22.4	104.9 ± 25.2	58.9 ± 15.7	58.9 ± 15.3	60.2 ± 17.8	58.0 ± 17.6	60.2 ± 16.9	60.7 ± 18.8
KE	99.4 ± 23.2	104.6 ± 26.0	105.0 ± 27.8	101.7 ± 30.3	107.7 ± 28.2	102.8 ± 35.1	58.5 ± 14.0	61.3 ± 15.1	60.9 ± 18.3	61.3 ± 16.9	60.8 ± 17.3	60.9 ± 18.3
KF	95.9 ± 20.5	108.5 ± 23.4	102.6 ± 23.2	98.4 ± 22.9	104.0 ± 23.7	104.2 ± 23.2	60.2 ± 8.4	61.3 ± 9.5	61.3 ± 9.7	57.5 ± 9.9	57.8 ± 9.5	58.5 ± 10.2
KE/KF	98.3 ± 24.9	112.4 ± 23.3	110.4 ± 27.5 a	99.6 ± 26.9	108.7 ± 24.6	104.7 ± 26.0	63.3 ± 13.0	66.5 ± 14.5	64.9 ± 16.5	64.8 ± 17.0	65.1 ± 16.9	65.1 ± 16.3
KF/KE	103.2 ± 23.7	110.5 ± 27.8	108.4 ± 29.6	100.3 ± 23.3	107.7 ± 24.3	107.7 ± 27.7	59.3 ± 11.0	65.5 ± 13.3 a	63.4 ± 14.5	62.1 ± 11.9	63.1 ± 13.1	63.2 ± 11.8
ANOVA	*P = 0.0007* F = 3.55 ES = 0.73			*P = 0.27* F = 1.25 ES = 0.44			*P = 0.04* F = 1.99 ES = 0.55			*P = 0.74* F = 0.64 ES = 0.31		

Data are presented as mean ± SD. T0 = baseline evaluation; T1 = post-test assessment; T2 = assessment 15 min post-test; L = left; R = right; ANOVA = time × group interaction. *Significant $P < 0.05$; a = time interaction, difference with T0.*

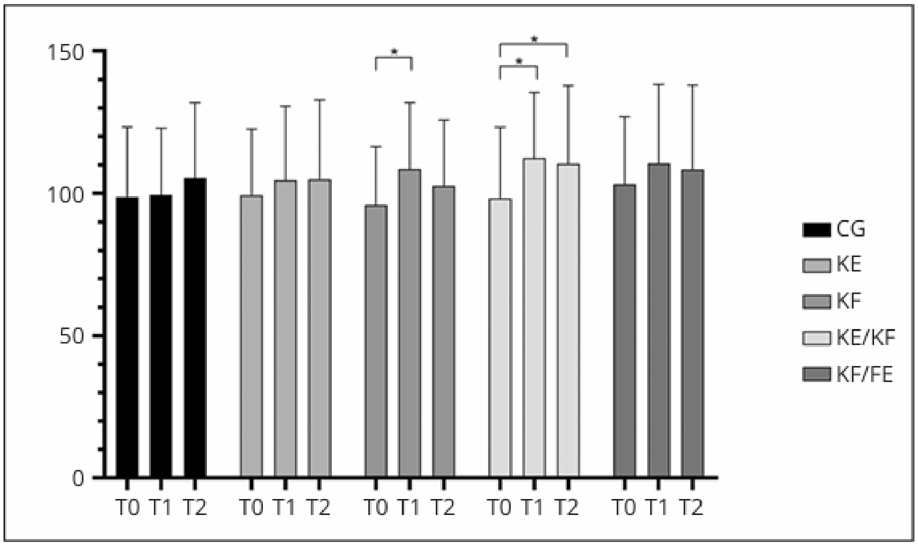


Figure 1.5 Measures of hip flexions (°) of each group across time for the right leg. CG: control group; KE: participants who stretched the knee extensors; KF: participants who stretched the knee flexors; KE/KF: participants who first stretched the knee extensors and then the knee flexors; KF/KE: participants who first stretched the knee flexors and then the knee extensors; T0: baseline evaluation; T1: post-stretching evaluation; T2: 15-min post-stretching evaluation. Significant difference ($P < 0.05$).

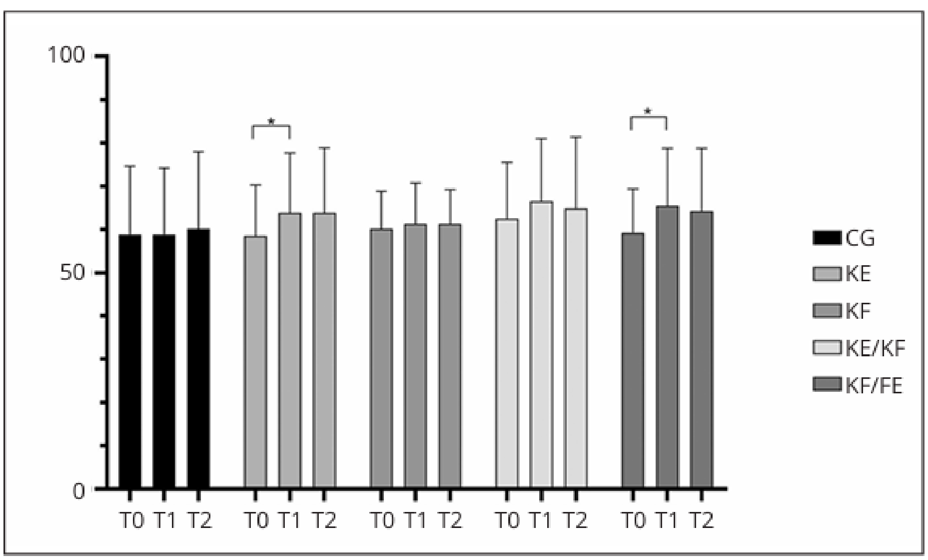


Figure 1.6 Measures of hip extension (°) of each group across time for the right leg. CG: control group; KE: participants who stretched the knee extensors; KF: participants who stretched the knee flexors; KE/KF: participants who first stretched the knee extensors and then the knee flexors; KF/KE: participants who first stretched the knee flexors and then the knee extensors; T0: baseline evaluation; T1: post-stretching evaluation; T2: 15-min post-stretching evaluation. Significant difference ($P < 0.05$).

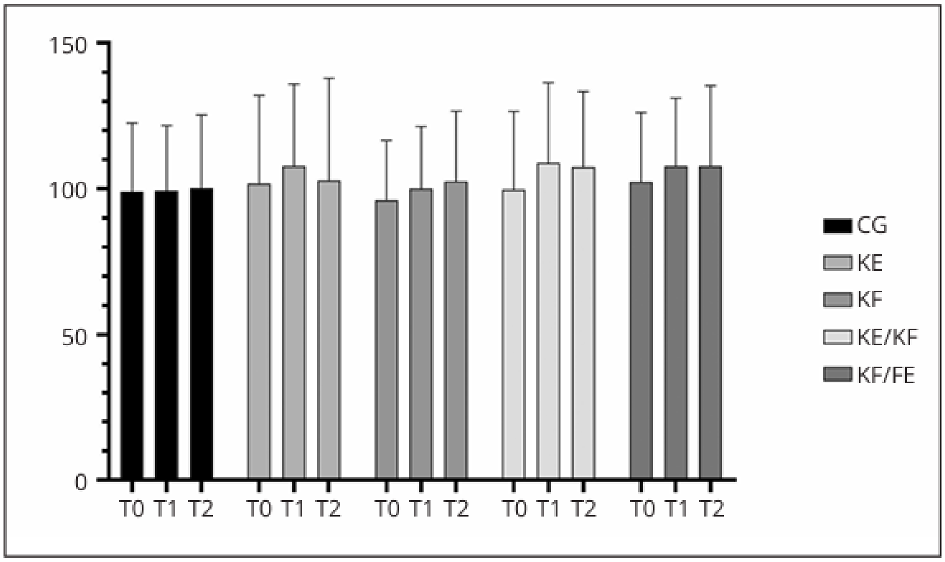


Figure 1.7 Measures of hip flexions (°) of each group across time for the left leg. CG: control group; KE: participants who stretched the knee extensors; KF: participants who stretched the knee flexors; KE/KF: participants who first stretched the knee extensors and then the knee flexors; KF/KE: participants who first stretched the knee flexors and then the knee extensors; T0: baseline evaluation; T1: post-stretching evaluation; T2: 15-min post-stretching evaluation.

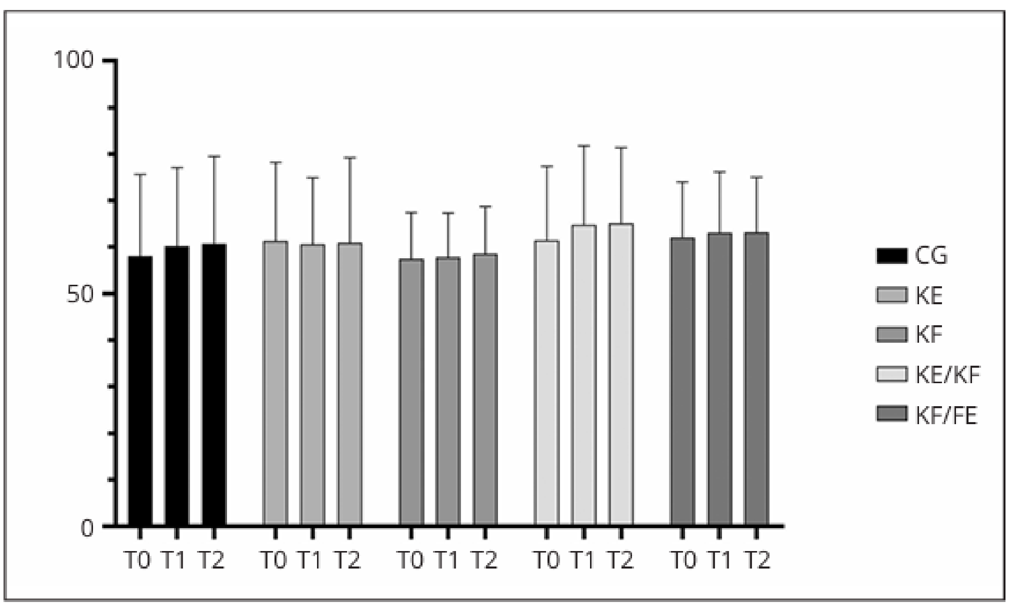


Figure 1.8 Measures of hip extension (°) of each group across time for the left leg. CG: control group; KE: participants who stretched the knee extensors; KF: participants who stretched the knee flexors; KE/KF: participants who first stretched the knee extensors and then the knee flexors; KF/KE: participants who first

stretched the knee flexors and then the knee extensors; T0: baseline evaluation; T1: post-stretching evaluation; T2: 15-min post-stretching evaluation.

Discussion

Our study aimed to determine if the order of intra-session exercise administration could influence ROM outcomes. our initial hypothesis thought to determine that unilateral stretching of muscles would 1) increased ROM in the stretched muscles, which was comprehensively confirmed; 2) increased ROM in the homologous muscle groups of the non-stretched limb, which, however, was not observed; and 3) greater increases in ROM in those groups who stretched both agonists and antagonists muscle groups, again not observed. However, an unexpected outcome was detected. consistently with the stretched limb, the last administered exercise produced significant ROM increases, regardless of the order of the assessed motion. Within our experimental design, we had four experimental groups and planned that one of the two stretching protocols was shared for three of these groups (i.e. KE was present either alone, at the beginning of the proto col [KE/KF] and at the end of the protocol [KF/KE], and vice versa for the KF), therefore, it would be plausible to suppose that the measure related to the stretched muscle, would have increased in all groups in which the stretch protocol was performed. Further, our hypothesis considering the neurological impact of reflexes (i.e. reciprocal inhibition) was that stretching a muscle would produce relaxation of its antagonist, therefore by stretching both the KF and the KF (regardless of the order), ROM gains would be larger compared to those groups in which KE or KF muscles were stretched individually. Such theoretical background is supported by human research in which "heteronymous IA connections" (projections of muscle spindle IA afferents to motorneurons of various muscles of the same limb) are present (197, 198). To this extent, the so-called "neural theory" has been investigated by other authors who measured non-local joint ROM in which increases in ROM were seen without any changes in compliance or viscoelastic properties of tissues (199). In a recent study by Masugi et al., (200) the authors aimed to confirm such theory and evaluated if stretching may alter monosynaptic reflexes of the lower limbs. Results of their study revealed that monosynaptic spinal reflexes in multiple lower limb muscles were inhibited during stretches, in both stretched and non-stretched muscles of the ipsilateral but not contralateral limb. Such inhibition was seen to be greater in high-intensity stretches compared to low-intensity ones. However, such inhibition was not observed at stretch cessation, underlining that decreased excitability returns to baseline levels when the muscle is passively shortened, similar to that of the antagonist's muscle. These findings suggest that reduced excitability is not observed at stretch cessation if variations in muscle length are obtained by passive elongation, rather than a reduction in length (of the antagonist muscle) caused by an active contraction (of the agonist muscle) (63, 201, 202).

Concerning our result of the intra-session order, we observed that the last exercise produced a significant but moderate effect pertinent to the assessed motion. Since neural mechanisms, as above discussed do not seem to have an influence on passive ROM adaptation, tissue properties or increased stretch tolerance may have a role in the observed outcomes. Concerning tissue properties, a study (203) that evaluated the effects of antagonist stretching (adductors) on agonist movement economy (abductors), observed that when ROM of the adductors increased, a decrease in energy expenditure in the abduction motion was present. The authors suggest that their results are due to a decrease in passive resistive torque. A decrease in the muscle-tendon unit stiffness of the agonist may determine less tension for the agonist to move. Such results may suggest that an increase in agonist ROM may favor antagonist ROM. However, the authors measured neither abduction ROM nor muscle activation or tissue properties. If the results of the above-mentioned study would confirm that tissue properties were altered for the agonist's muscle after antagonist muscle stretch, we would have confirmed our third hypothesis, which however, was not observed. In addition, other studies have investigated the modification of tissue properties on the contralateral limb (non-stretched) to that of the stretched limb which, again, were not observed (193, 194). Although to date there is still no data supporting such an assumption, a possible theory might include the possibility that a previously performed stretch of an antagonist's muscle might alter the proprioceptive feedback (204) (modifying the perception of the participant) in an agonist muscle, being that a common drive extends to antagonist muscles if these share a common function (204).

It is important to underline that no prolonged effects of the stretching protocols were observed at T2 (15 minutes after the stretch cessation), which might provide further insight into the observed outcomes. We also need to point out that in a previous study (205) and in other studies which applied the same stretching protocol (194) a prolonged effect was observed (at least after 10 minutes from the stretch cessation). however, in both studies, the SS intensity provided was greater (which was considered as "the maxi mum tolerable" and "that to achieve the greatest possible ROM"). It could be plausible that a relatively fast return close to baseline levels of ROM might have occurred from the cessation of the stretching procedure to the assessment of ROM, not allowing us to observe a variation in ROM, especially in the combined protocols. however, neither the protocols applying SS individually showed a prolonged effect, which further suggests that this latter was not observed for the lower intensity provided. Another aspect investigated within this study was the effect of acute stretching of the stretched limb on outcomes of ROM of the contralateral (non-stretched) limb (crossover effect). A recent meta-analysis (72) investigating the acute non-local effects of passive stretching on ROM has observed that moderate magnitude increases in ROM (ES=0.86) can be observed in the contralateral limb. Such effects have been attributed to either neural responses or increased pain tolerance (108, 128, 206). The majority of studies evaluating ROM on the non-stretched limb have observed increases in ROM (98, 193, 194). Such increases have been observed for the hamstrings and the quadriceps muscles, (72) as those targeted within this study. However, such an effect has not been observed in all studies administering unilateral stretching (207-209). The intensity of the stretches provided within this study was administered up to the point of discomfort; therefore we cannot consider these as high-intensity stretches. During intense stretches, type III and IV afferents (mechanoreceptors and nociceptors) discharge, with effects that can last between 5 to 10 minutes (169) with the potential to alter pain tolerance. Intense stretches cause greater pain and stimulation of muscles (210) and may be essential to also induce non-local effects. Within studies observing a clear crossover effect, (98, 193, 194) the stretch intensity was administered up to maximal tolerable discomfort. Conversely, in those studies which did not observe a cross-over effect the in tensity of the stretch was either considered as "controlled" or between "70% to 90%" of the maximum tolerable (207-209). Therefore, it is plausible that in order to observe a generalized increase in ROM which depends on modifications in pain tolerance, intense stretches need to be performed. Despite the lack of acute interventions evaluating the effects of different stretching intensities on the crossover effects, a study by Nakamura et al., (211) evaluated the effect of a 4-week stretch intervention by comparing low and high-intensity stretches. Results of the study revealed that a crossover effect on ROM was observed only in the high-intensity group. However, the author considered as high intensity a degree of perceived discomfort of 6-7 on a scale of 0 to 10 which may be considered as submaximal, as provided in the present investigation. Studies comparing low, moderate and high intensity stretches concerning acute crossover effects are warranted.

Limitations of the study

This study is not without limitations. Despite the included sample having a number of participants above the required statistical power, we acknowledge that each subgroup was composed of a limited number of participants (approximately 21 per group). Future studies using a cross-over design could be carried out to overcome such a limitation. In addition, all participants were young. Further, only measures for ROM were collected and neither tissue nor neuromuscular measures were assessed, preventing us to infer any possible underlining mechanism regarding the intra-session exercise order. Future studies should also consider evaluating other muscle groups and different stretching protocols (in terms of intensity and duration). However, this is the first study to have evaluated the effects of stretching order on outcomes of ROM, identifying an intra-session exercise order effect.

Conclusions

An effect concerning the order of the stretching exercise intervention appears to be present. Measures of ROM have been observed to significantly increase only for the last stretched muscle in each intervention group. No crossover effect was observed in the contralateral limb. Our results are useful for fitness enthusiasts, sports coaches and physical therapists for increasing the effectiveness of stretching exercise programs and for researchers in improving the design of studies in which the range of movement is evaluated.

2.3 "Acute effects of static stretching and proprioceptive neuromuscular facilitation on non local range of movement"

Salvatore Ficarra, Antonino Scardina, Masatoshi Nakamura, Antonino Patti, Fatma Neşe Şahin, Antonio Palma, Marianna Bellafiore, Antonino Bianco and Ewan Thomas

Introduction

Acute stretching increases the range of movement (ROM) (71), regardless of stretch typology (static stretching (SS), proprioceptive neuromuscular facilitation (PNF) or dynamic stretching) (212). However, there is a time-dependent effect (70), especially for longer stretches (>60s for muscle groups) which can maximize ROM improvements.

Researchers typically focus on assessing the effects on local ROM, for example, plantar flexor muscle's stretching may increase ankle ROM. However, there is a growing interest among therapists and researchers in the concept and alternative treatment approaches related to myofascial chains (99, 100, 213). This approach originates from the assumption and anatomical evidence that muscles and connective tissues do not function as independent units but rather behave as interconnected elements, forming a biotensegrity system (101). This interconnectedness involves the transmission of tensions along specific myofascial chains (100, 102, 214). Therefore, the application of a stretch to a given joint could have a non-local effect (104). Sought to investigate whether myofascial treatments could effectively modulate ROM in distal body segments. The meta-analysis carried out by the research group indicated that ROM could be increased in distal body segments, however, these effects were of small magnitude (104). Although of small magnitude, the conceptual framework of myofascial chains offers an explanatory avenue for those cases of painful musculoskeletal conditions with diagnostic imaging without apparent local abnormalities (215, 216).

In another recent systematic review with meta-analysis conducted by Behm et al. in (2021), the acute effects of stretching on non-local ROM in healthy individuals were examined (Behm et al., 2021). Again, the existence of non-local effects were confirmed. Compared to the interventions evaluated by (104) the effects of stretching appear to be of moderate magnitude. However, out of the eleven studies included in the meta-analysis, nine examined the impact of unilateral stretching on the contralateral homologous muscles known as the "crossover effect". This effect may result from neural or psycho-physiological mechanisms (like increased stretch/pain tolerance (217)) instead of the myofascial chain's concept (71). To this extent, Konrad et al. (218) investigated the effects of a 7-week stretching and foam roller intervention of the plantar foot sole and evaluated if the structure of a non-local area (the gastrocnemius, which is incorporated within the same myofascial chain and anatomically contiguous) changed. Notwithstanding a remote increase in ROM, no morphological adaptation was observed, therefore, confirming stretch tolerance adaptational mechanisms (218). Within the two remaining studies included in the study of Behm et al. (71), the authors evaluated non-local remote effects (71, 100) by administering stretches to the lower limbs and measuring ROM in either the upper limb (Shoulder Extension – ShE) or the cervical spine. However, different rationales are provided by Behm et al. (71) and Wilke et al. (100) for the increased non-local ROM observed, indicating augmented stretch tolerance or the existence of a strain transfer along a myofascial chain, respectively. Additional evidence supporting non-local effects was presented in another study by Wilke et al. (103), in which local stretching (cervical spine) was compared to remote stretching (gastrocnemius and hamstrings) for measures of cervical spine-ROM. The authors of this study (103) indicate no significant differences in ROM increases between groups, suggesting equal effectiveness between local and myofascial chains stretch in enhancing ROM.

Given the paucity of existing literature on non-local stretching effects, the aim of this investigation will be to analyse the effects of acute stretching interventions in young, healthy subjects to verify the presence of non-local remote effects. This will be achieved by providing a stretch to the lower limbs and measuring ROM in the upper limbs. In addition, since the previously discussed studies have either included dynamic or SS, we will also include PNF to determine specific stretch typology effects.

Materials and methods

Participants

Twenty-nine university students, recruited between November 2021 and March 2022, took part in this study (16 male and 13 female, mean age 24.1 ± 2.8 years, 171.4 ± 8.8 cm height, and 67.9 ± 12.5 kg body mass). Informed consent to participate, without disclosing research aims, was signed by participating individuals. Competitive athletes and individuals with musculoskeletal, neurological, or orthopedic constraints were excluded. A priori sample size calculation based on previous research (Behm et al., 2015) was performed using G-power (G*Power version 3.1.9.4, ES = 0.3, 1-β = 0.80, α = 0.05. F-test for repeated measures considering 3 groups [CG, SS, and PNF] and 3 time points [T0, T1, and T2] on ROM were performed). The results defined the minimum number of participants required for the planned research model as 27. The study, approved by the University bioethical committee (protocol n°65/2021), was conducted in accordance with the Helsinki Declaration ("World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects," 2001) and the European Union recommendations for Good Clinical Practice.

Procedures

According to the International Physical Activity Questionnaire-IPAQ (142) assessed for all participants, the sample consisted of moderately active participants (hours/week of physical activity 8.2 ± 6.5). All participants were asked to refrain from starting new structured exercise programs, specifically stretching, during the study period. Each participant attended the laboratory on three separate occasions, one week apart (each visit was planned keeping time of the day consistent for each participant). During each visit, the temperature was constant within the laboratory at 24°C. Participants were assessed before a stretching intervention (T0), immediately after (T1), and 15 minutes after the end of the interventions (T2). On the first visit, the participants attended a control session (CS) in which no intervention was proposed, and they were asked to sit on a chair for 15 minutes (between T0 and T1). During the subsequent visits, the participants underwent either a SS or a PNF intervention which was randomized across participants. A passive straight leg raise test (PSLR) and the Shoulder extension (ShE) tests (71) were assessed at T0, T1, and T2, on each visit for both limbs. The investigator assessing the tests was blinded to the interventions and the investigator administering the interventions was blinded to the measurement collection. However, the participants were not blinded to the proposed interventions (Figure 1.9).

Measures

All measures were conducted by the same investigator (S.F.) who was blinded to the proposed interventions. Each measure was assessed in both limbs which were randomized across participants. Lower limb dominance was assessed by asking the participants which leg they would use to kick a ball, whilst upper limb dominance was assessed by asking the participants which hand they would write with. All participants were right- handed, except for two men and one woman, and had corresponding leg and hand dominance. All ROM measures were assessed once, in order to avoid a repetition- dependent effect (143). The assessments were conducted using a Gyko inertial sensor with dedicated software (Gyko RePower; Microgate, Bolzano, Italy) (144).

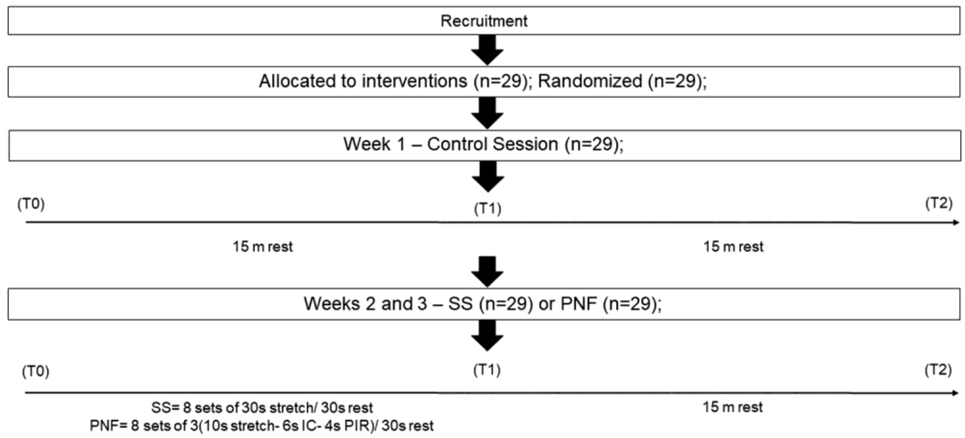


Figure 1.9 Schematic representation of the recruitment process and the administered interventions. SS: Static Stretching. PNF: Proprioceptive neuromuscular facilitation. IC: Isometric contraction: PIR: Post isometric relaxation.

PSLR and ShE ROM were assessed. During PSLR the inertial sensor was firmly strapped to the distal anterior aspect of the femur of the assessed limb. The participant lay on a medical bed with the contralateral limb firmly strapped using inelastic straps to avoid compensatory movements. The tested leg was passively lifted while in full knee extension to the limit of available ROM, or the maximum tolerable pain for each participant (145). The same limit was applied to assess the maximum ShE ROM which was performed with the participant sitting on a chair. The inertial sensor was fixed to the anterior aspect of the distal end of the humerus. Inelastic straps were used to secure the legs and torso of the participant to the chair, preventing compensatory movements of the lower limbs, pelvis, and upper body. The assessment began with the upper limb fully extended from a neutral position, relaxed next to the body.

Interventions

The interventions, which were intended to target the knee flexors through SS and PNF, were administered by the same investigator (A.S.) who was blinded to the measurement assessment. Participants who did not complete all three visits were excluded from the study.

The SS intervention consisted of 8 sets of 30 seconds of static stretching followed by a 30-s rest. Participants were instructed to sit on a mat with their knees fully extended and their feet touching a wall, ensuring that the ankle was in a neutral position at 90°. Each participant was directed to perform trunk flexion to their maximum tolerable extent during each set. The investigator, with his hands positioned on the back of the participant, at the level of the lumbar spine, assisted participants in maintaining the maximum tolerable stretched position (that was verbally described as an intensity ranging from 90 to 100%) for the requested time (195, 219). A volume-equated protocol was adopted for the PNF intervention (8 sets of stretching with a 30-s rest between each set). Each set of the PNF intervention involved the following steps: 1) Once the participants reached their maximum tolerable trunk flexion, the passive stretch was maintained for 10-s. 2) After the passive stretch, an isometric contraction of 6-s against the investigator's resistance was performed. 3) Following the contraction, a 4-s post-isometric relaxation phase was maintained. Such a procedure was repeated 3 times for each set (30 seconds of stretching) to equate the stretching duration of the SS (205). A schematic representation of the recruitment procedure and intervention organization has been included (Figure 1.9).

Statistical analysis

The data are presented as means ± standard deviations. The intraclass correlation coefficients (ICC) from the T0 of each condition were calculated to check the test-retest reliability. Differences were assessed using a repeated measure ANOVA model considering the three assessment time points (T0 vs. T1 vs. T2) for each limb and intervention (SS vs. PNF) (Time x treatment x limb interaction). Post-hoc Bonferroni corrections were performed to identify differences between assessments for each intervention. For each analysis, the effect size (ES) was calculated. Cohen δ's was adopted as a measure of ES. The magnitude of the ES was classified according to the following scale: 0–0.19 = trivial effect, 0.20–0.49 = small effect, 0.50–0.79 = moderate effect, and ≥ 0.80 = large effect (Fritz et al., 2012). Standard Error of Measurement (SEM) was also included for significant variables. All analyses were performed with Jamovi (The jamovi project (2021). Jamovi (Version 1.8.0.1) [Computer Software]. Retrieved from https://www.jamovi.org). Graphs were created using GraphPad Prism8 (GraphPad Software, San Diego, CA). Alpha was set at 0.05 for all analyses.

Results

No differences were detected for T0 across the three testing sessions for measures of either the lower or upper limbs, thereby excluding carryover or learning effects across interventions. The ICC for measures of dominant and non-dominant PSLR were 0.91 and 0.90, respectively. While for measures of the dominant and non-dominant ShE the ICC were 0.79 and 0.78, respectively.

A significant time x intervention interaction was observed for the PSLR for both limbs (dominant limb p = 0.0002, ES 0.70; non-dominant limb p = 0.0079, ES 0.56). Conversely, no significant differences were observed for the upper limbs neither for the dominant nor non-dominant limbs.

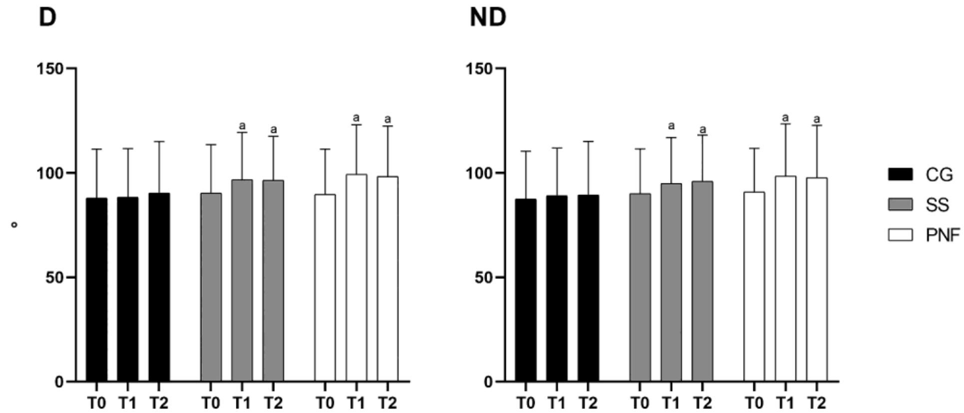


Figure 1.10 D: passive straight leg raise (PSLT) ROM of the dominant limb. ND: PSLT ROM of the non-dominant limb. a: Significantly different from T0. CG: Control Session; SS: Static Stretching; PNF: proprioceptive neuromuscular facilitation.

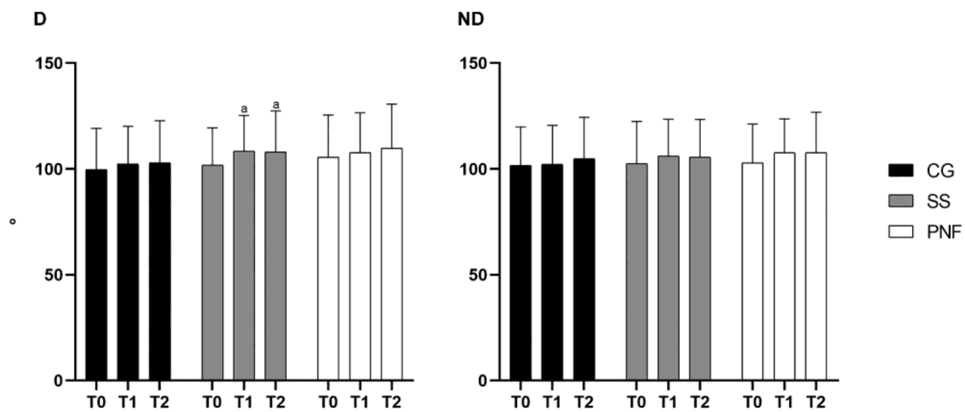


Figure 1.11 D: shoulder extension (ShE) ROM of the dominant limb. ND: ShE ROM of the non-dominant limb. a: significantly different from T0. CG: Control Session; SS: Static Stretching; PNF: Proprioceptive neuromuscular facilitation.

Post-hoc analysis revealed that following both stretching interventions, ROM measures increased from T0 to T1 for the lower limbs for both stretching interventions (SEM 1.41 for the dominant limb and 1.22 for the non-dominant limb, respectively) and remained elevated in T2 (SEM 1.45 for the dominant limb and 1.24 for the non-dominant limb for both SS and PNF, respectively) (Figure 1.9). No significant differences were observed between SS and PNF (Figure 1.9).

For the ShE of the upper limbs, a significant ROM increase was noted following SS, but not PNF, from T0 to T1 (+6.5%, SEM 1.52). This increase in ROM persisted at T2 (SEM 1.74) (Figure 3). Such an increase was observed only for the dominant limb. No significant differences were observed for the non-dominant upper limb regardless of the intervention (Figure 1.11 and Table 1.4).

Table 1.4 Effects of stretching on local and non-local ROM

	Control			SS			PNF			ANOVA (p)	ES
	T0	T1	T2	T0	T1	T2	T0	T1	T2		
PSLR D	87.9 ± 23.5	88.3 ± 23.3	90.3 ± 24.7	90.3 ± 23.3	96.7 ± 22.7[a]	96.5 ± 21.1[a]	89.7 ± 21.7	99.3 ± 23.8[a]	98.2 ± 24.2[a]	0.0002*	0.74
PSLR ND	87.5 ± 22.9	89.0 ± 22.9	89.4 ± 25.7	90.1 ± 21.4	94.9 ± 22.0[a]	96.0 ± 22.1[a]	90.8 ± 21.0	98.5 ± 25.0[a]	97.8 ± 25.0[a]	0.0079*	0.56
ShE D	99.6 ± 19.6	103.2 ± 17.8	102.9 ± 19.9	101.8 ± 17.5	104.8 ± 16.9[a]	104.8 ± 19.3[a]	105.5 ± 20.0	107.7 ± 18.8	109.8 ± 20.8	0.448	0.32
ShE ND	101.6 ± 18.3	102.1 ± 18.5	104.8 ± 19.6	102.5 ± 19.9	106.1 ± 17.4	105.6 ± 17.8	102.8 ± 18.4	107.6 ± 16.0	107.6 ± 19.2	0.551	0.30

Data are in means ± SD. T0: Baseline; T1: Post-test; T2: 15-min post-test; PNF: Proprioceptive Neuromuscular Facilitation; D: Dominant limb; ND: Non-dominant limb; ANOVA: Time × Group interaction. Significant $p < 0.05$; a: post-hoc difference with T0.

Discussion

This study sought to explore the presence of non-local effects when stretching was applied to the lower limbs and ROM was measured in the upper limbs. An acute non- local remote effect was not comprehensively present, except for ShE after SS on the dominant limb. The findings of the present investigation appear not to fully align with previous research, in which non-local remote effects on ROM were observed (71, 100, 103).

It is important to consider theoretical and methodological aspects that may contribute to the observed differences. Firstly, seven myofascial chains (divided into eleven lines) have been identified and described based on anatomical landmarks (100, 220) and related to functional movements (215). Six of these have been confirmed by anatomical evidence (100), while the superficial frontal line does not appear to be present. Two studies focused on myofascial chains (100, 103) directed their interventions towards the gastro- cnemius and hamstrings muscles, both components of the superficial back line, which extends from the plantar fascia to the epicranial fascia. The anatomical contiguity of these structures may have the potential to affect cervical-ROM. The investigation of such a chain has also been addressed in a systematic review on myofascial release interventions conducted by Burk et al. (104). In this review, all interventions reported an increase in ROM, albeit of small magnitude and based on studies with a high risk of bias. Furthermore, Behm et al. (71) conducted hip adductor and hamstring stretching and measured the ShE

ROM, evaluating the extensibility of shoulder flexors. The chosen anatomical location falls within the frontal functional line which may explain the observed increased ROM.

In the present investigation, we targeted the knee flexors and evaluated ShE- ROM to observe whether changes in ROM of a lower limb area could affect an upper limb area without targeting a specific chain. Notably, the hamstrings and gastrocnemius muscles addressed in the interventions do not exhibit anatomical continuity with muscles in the upper limb, except for the serratus anterior. The lack of anatomical proximity of the targeted muscles may have prevented the stretch- ing interventions to induce non-local ROM effects. When considering our findings alongside those of previous investigations, it may be inferred that remote non-local effects on range of motion could potentially manifest when interventions are applied to specific myofascial chains. However, significant changes in ShE ROM after SS were observed. These changes cannot be considered related to strain transfer along a specific chain or related to changes in the viscoelastic properties of muscles, since no stretching was applied to the upper limbs. Therefore, increased ROM could be attributed to neural factors. SS decreases motor neuron excitability in the stretched limb and the contralateral side (67, 201) potentially modulating autonomic nervous activity by increasing para-sympathetic and decreasing sympathetic drive (60). The auto- nomic modulation may induce a physiological relaxation state (67) which could consequently reduce resistance to stretching. While no previous investigation has evaluated autonomic activity post PNF, there is evidence for increased sympathetic activity during active SS (221) contrary to passive SS (222). In both cases, however, a tendency for a parasympathetic state is observed. Since PNF foresees a contraction phase, this latter is likely to actively stimulate motor neuron activity (223) and sympathetic drive. This factor would limit the relaxation state and thus explain the different outcomes observed between SS and PNF.

However, if SS had indeed induced a relaxation state, no differences should have been observed between the dominant and non-dominant sides. Previous studies have reported ROM differences between dominant and non-dominant sides (224). However, it is not clear whether these baseline differences may persist following stretching. Although not significant, a tendency towards increased non-local ROM (in both upper limbs) was highlighted.

Secondly, the assessment procedures should be considered. Differences between sitting and standing positions emerged when assessing ROM (225). When participants are seated and strapped, as in the present investigation, compensatory movements are limited. In addition, a seated position restricts and isolates the pelvis from the upper body, thus limiting the ROM of the upper limbs (226). This approach may have prevented the observation of differences determined by compensatory movements of the pelvis or the lower back, such as an increased lumbar angle. Nevertheless, both studies by Wilke et al., (100, 103), similar to the present study, adopted a sitting position when measuring ROM, while in the investigation of Behm et al. (71) the participants stood upright.

Based on the observed outcomes, the specificity of the intervention's application along a myofascial chain, together with the assessment procedures (sitting, standing or involving multiple trials) may be significant factors to consider when evaluating non-local remote ROM.

This study is not without limitations. A crossover design was adopted, which despite reducing interindividual differences, cannot account for intra-individual differences (differences between dominant and non-dominant limbs). Furthermore, our sample, while larger than previous investigations, is still limited to the characteristics of the recruited population (healthy young moderately active individuals). Our investigation only included measures of ROM which also limits our ability to infer precise physiolo- gical or anatomical mechanisms. Additionally, our intervention was not intended to target a myofascial chain but rather to evaluate if a generalized non-local remote effect was present. Result that we did not consistently observe. It is not possible to deter- mine if the proposed interventions applied to a myofascial chain could have yielded different outcomes.

However, to the best of our knowledge, this is the first investigation to evaluate the effects of non-local ROM while also including a PNF intervention. We recommend that future research focus on specific myofascial chains and adopt a standardized assessment methodology to evaluate the presence of non-local effects.

This study indicates that static stretching and proprioceptive neuromuscular facilitation are both effective in increasing range of motion when applied locally. However, it still remains unclear whether non-local remote effects are present. Notably, some non-local effects could be seen only in the dominant limb following static stretching but not proprioceptive neuromuscular facilitation. Assessment procedures should be standardized across studies to avoid differences in outcomes.

2.4 "Acute local and non-local morphological, sensory and fluid responses to stretching and foam rolling in young females"

Ewan Thomas, Antonino Scardina, Antonino Patti, Pascal Izzicupo, Masatoshi Nakamura, Andreas Konrad, Francesco Campa, Marianna Bellafiore and Antonino Bianco

Introduction

Static stretching (SS) and foam rolling (FR) are two techniques that are frequently applied to improve ROM of a joint, either after acute and chronic settings, or as warm-up strategies (87, 88). Concerning their acute effect on ROM, both techniques can be considered equally effective (88).

The ROM improvements observed following both techniques may be ascribed to different physiological mechanisms. These encompass morphological changes, a modification of pain sensation, increased body temperature, or a modification of the viscoelastic properties of tissues (63, 89). Regarding morphological changes, prior studies (227-230), exhibit heterogeneity, likely influenced by factors such as the duration and intensity of stretching or rolling, as well as the anatomical location of the applied intervention. More consistent results are observed regarding the modification of pain sensation (90, 210) which can be measured as an increase in pain pressure thresholds (PPT). These increases are generally observed alongside with increases in ROM. Nevertheless, most studies focus on assessing acute post-intervention effects (90, 230). However, when examining the time-course of ROM and changes in pain sensation, heterogeneity is again observed. For instance, concerning FR interventions, Kasahara et al. (91) in a first study observed that ROM improved following a FR intervention up to 30 min, while PPT remained elevated only for 10 min. Conversely, in a second intervention (230) ROM improved up to 10 min while PPT remained elevated for 15 min. Similarly to FR interventions, also in SS, the time-course measures of PPT may or may not correspond to the variations in ROM in measures other than those immediately post-exercise (92, 93). These findings indicate that both morphological variations and modifications in pain sensation may contribute, albeit not exclusively, as underlying mechanisms for the improvement in ROM. Recent evidence (94) has observed that following a FR intervention, im- provements in ROM were also observed when sham rolling was applied (i.e., just mimic the movement of FR while no pressure on the target tissue). These results, together with those of other investigations (95, 231, 232), would suggest that ROM can also improve through a warm-up effect. It is interesting to note that following both FR and SS interventions, an increase in local temperature can be observed (126, 233).

To date, only one study has evaluated the effects of a FR intervention concerning local tissue fluid hydration (using a localized bioimpedance analysis (L-BIA), also defined Electrical Impedance Myography (EIM)

(122, 123) a procedure that allows to identify raw bioelectrical parameters in specific body segments. This allows to identify Phase Angle (PhA) and vector length (Z) which are related to fluid distribution and fluid quantity (124, 125), respectively), which could be other factor influencing the viscoelastic properties of tissues., Within the study of Yoshimura et al. (126) applying FR on the plantar flexor muscle, the authors concluded that a reduction in the fluid content of the leg was observed. Such effects were more pronounced in women. It is interesting that an increase in temperature was also observed, indicating a potential relation between the warm-up effect and the variation in fluid dynamics. It is, however, still to clarify if improvements in ROM following the FR are due to the mechanical pressure of the roller over the muscle belly or an increase in local temperature. Conversely, no studies have evaluated the effects of a SS intervention on local fluid variations.

Furthermore, there has been recent interest in investigating non-local effects (when interventions are applied to an anatomical location and effects are measured elsewhere) alongside the traditional local effects (72, 148). However, the majority of these studies take into consideration the non-local effects of the homologous muscle groups in the contralateral limb, namely the “crossover effect” (72). Very few investigations, which have also provided heterogeneous results, have evaluated non-local effects in contiguous body segments (i.e. interventions carried out on the plantar fascia and effects measured in the plantar flexor muscles (218)) or segments encompassed within a myofascial chain (104, 234). In addition, despite previous research that has evaluated the effects of FR on local fluid distribution (126) observing its reorganization, there is a gap in research on the fluid dynamics of continuous body segments. Therefore, it still remains unclear whether the reorganization of these fluids may be directed towards the surrounding tissues, the bloodstream, or tissues in other body segments.

Thus, this study aimed to examine the acute effects of four sets of 30 s of SS and FR interventions carried out in the PF muscles and examine either local (where the intervention was administered) and non-local (the adjacent lower limb compartment [the thigh]) morphological, sensory, and fluid responses as potential determinants of ROM improvement. The intervention was carried out in women since these tend to have lower body water content compared to men.

Materials and methods

Participants

Fourteen healthy female participants were enrolled (the descriptive characteristics of the participants are shown in Table 1). The required sample size was calculated through G-power (G*Power version 3.1.9.4) for a repeated measure ANOVA (F-Tests effect size = 0.25, α error = 0.05, and power = 0.80) considering 3 groups (CC, SS, and FR) and 3 time-points (T0, T1, and T2) based on our previous

Table 1.5 Descrpitive characteristics of participants.

	F
N	14
Age (years)	22.3 ± 2.7
Weight (kg)	59.6 ± 8.78
Height (cm)	161.6 ± 8.77

study's ROM results (91) was more than 10 participants. Participants were recruited from a population of university students. All participants had to be free of orthopaedic, neurological, and gynaecological diseases. If participants had a previous history of injuries to the lower limbs, were excluded, as were those taking oral contraceptives. A total of three women were excluded priorly. Participants could take part in the experiment only during the follicular phase of their menstrual cycle (235, 236) in order to standardise bodily fluid balance. All participants had to refrain from using coffee or drinking water for at least 30 min before the experiments in order to further standardise bodily fluids across conditions. In order to take part in the study, all participants had to sign a written informed consent, in which the study protocol along with the risks and benefits of participating in the experiment, were disclosed. The study was approved by the bioethical committee of the University of Palermo (protocol n◦170/2023). Research was conducted in accordance with the Helsinki Declaration and the European Union recommendations for good clinical practice.

Procedure

Participants were asked to attend our laboratory on three occasions, at least 48 h apart, at the same time of the day. During each visit, the temperature of the laboratory was controlled at 24 ◦C. On each visit, participants were randomised to one of the following conditions: Control condition (CC), SS, or FR. Each participant, during each visit, was tested three times: upon arrival (T0), immediately after each intervention (T1), and after 10 min from the end of the intervention (T2). During the CC, no intervention was administered to the participants, who were instructed to sit on a chair for 10 min after the T0 assessment. Each participant between T1 and T2 was instructed to sit on a chair. At each time-point a measure of ankle ROM dorsiflexion was assessed. In addition, measures of tissue hardness (TH) and pain pressure threshold (PPT) were assessed in the medial gastrocnemius, the biceps femoris, and the semitendinosus, along with a localised bioimpedance analysis (L-BIA) in the lower leg and thigh. Since dynamic activities such as

ROM may influence TH, PPT, and L-BIA, these were assessed prior to ROM. Consequently, the order of each assessment was TH, PPT, L-BIA, and ROM. The reproducibility of this method was confirmed in a previous (91) study. All the above-mentioned measures were collected on the dominant limb, which was the right leg for all participants. Dominance was assessed by asking each participant with which leg they would kick a ball.

Outcomes

All measures were collected three times at each time point by the same investigator except for the L-BIA which was measured once.

Range of movement

ROM of the ankle was assessed in a dorsiflexion motion for the plantar flexor muscle in each participant. Each participant was placed on a medical bed in a prone position with the knee extended, with inelastic straps positioned on the pelvis and the contralateral leg in order to avoid compensatory movements. A flat, rigid surface was then strapped to the sole of the foot of each participant. A two- arm electronic goniometer (Digital Angle Ruler, resolution 0.05◦, accuracy ±0.2◦, repeatability 0.05◦) was then placed with one arm parallel to the foot sole and one arm parallel to the leg. In order to improve the reliability of each measure, the goniometer position was standardised by applying visual marks to the skin of each participant using a dermal marker. The assessment started when the foot and leg formed a 90◦ angle. The maximum tolerable pain for each participant, or the limit of available ROM (145, 205) during each assessment, was the final value retained for each assessment. Of the three collected measures for each assessment, the mean value was used for analysis.

Tissue hardness

A portable TH metre (NEUTONE TDM-N1; TRY-ALL Corp., Chiba, Japan) was used for data collection. This TH metre measures the penetration distance until a 14.71 N (1.5 kgf) pressure is reached (237). Measures of TH were collected in the medial gastrocnemius (MG) for the lower leg, the bicep femoris (BF), and the semitendinosus (ST) for the thigh. Each participant was asked to lie prone on a medical bed. Concerning the measures of the MG, a mid-point between the popliteal fossa and the proximal end of the Achilles's tendon was evaluated. For the BF and the ST, the proximal and distal reference points were the ischiatic tuberosity and the popliteal fossa, respectively. Two mid-points between these reference points were considered. A lateral point for the BF and a medial point for the ST. Each point was marked with a dermal marker to improve the reliability of the measures. During each assessment, participants were asked to relax. Of the three collected measures for each assessment at each point, the mean value was used for analysis.

Pain pressure thresholds

A portable algometer (FPX 25 Pain Tester, Wagner Instruments, CT, USA) was used to assess PPT. The participant's measurement position and evaluated points were identical to the TH assessment. The algometer was positioned perpendicular to the direction of the muscle fibers. A pressure of 1 kg per second was applied by the investigator. Each participant was instructed to say "stop" when pain, rather than just pressure, was experienced. The final value reported on the screen of the instrument, measured in kg•cm2 was considered a PPT. Of the three collected measures for each assessment at each point, the mean value was used for analysis.

Localized bioimpedance analysis

A single-frequency phase-sensitive device (BIA-101 Anniversary Sport Edition, Akern Systems, Firenze, Italy) at 50 kHz and 400 μA was used to measure phase angle (PhA) and bioimpedance (Z) [35] of the lower leg and thigh. Two pairs of electrodes (source (I) and detector (V) electrodes) for each body segment were used. For the lower leg, one pair was positioned on the upper part of the lower leg, with the lateral part of the I positioned on the popliteal crease and the V positioned distally, parallel to the I, 5 cm apart. The other pair was positioned on the lower part of the lower leg, with the lateral part of the I positioned above the tibial malleolus and the V in a proximal direction parallel to the I, 5 cm apart. For the thigh, one pair of electrodes was positioned on the upper part of the thigh with the lateral part of the I above the distal end of the ischiatic tuberosity and the V in a distal direction, 5 cm apart. The other pair was positioned with the lateral part of the I on the popliteal crease and the V positioned proximally, parallel to the I, 5 cm apart. Reference points were marked on the skin of the participants using a dermal marker to allow repeatability of the positioning of the electrodes (Fig. 1). The lower leg and thigh assessments were not performed simultaneously.

Z was standardised for each body segment according to segment length. Lower leg length was calculated in cm, from the popliteal crease to the mid-point of the tibial malleolus. Thigh length was calculated in cm, from the popliteal crease to the ischial tuberosity (Fig. 1). Prior to each test, the BIA measurements of the analyser were validated using a precision circuit with acceptance for resistance (R) measurements of 383 Ω (Ω) and reactance (Xc) values of 45 Ω. Coefficient of variation for PhA and Z were calculated on the T0 of all conditions, these being 2.88 and 1.37 for the leg and 4.17 and 3.18 for the thigh, respectively.

Interventions

A SS and a FR intervention were administered to the dominant PF of each participant. Each intervention was administered in four sets, with a 30-s rest between each set. The intensity of each intervention was controlled by asking each participant to feel the SS or the pressure of the FR at a magnitude of at least 8 out of a scale from 0 to 10. The adopted roller was a low-cost, commercially available roller (Bodymate, 30 cm in length, 15 cm in diameter in expanded polypropylene foam of medium density with a GRID surface). These characteristics were chosen following the recommendations suggested by previous research (238, 239). Familiarization for each intervention was allowed on the contralateral limb to understand either body position and intensity. Each aspect had to be familiarized separately. A maximum of 5 s of SS and a maximum of five rolls with the FR were allowed for such purposes in order to limit potential non-local contralateral effects (148, 240). For the SS, each participant was asked to stand upright and position their foot over a 35◦ inclined wedge for 30 s for each set. Each participant could adjust their body position to reach the required intensity. For the FR, the participants had to place the muscle belly of the PF over the GRID pattern of the roller. For each set, the participants had to roll the muscle belly 15 times in a proximal direction and 15 times in a distal direction over the roller. Each roll in each direction had to last 1 s, for a total time per set of 30 s. Rolling time was controlled through a metronome sound produced by a mobile device app (Metronomo: Tempo lite ver. 5.0.7, App Store, Apple). No intervention was applied to the knee flexors.

Statistical analysis

Means ± standard deviations were used to present the results. Intraclass correlation coefficients (ICC) were calculated from the T0 for all measures to check the reliability of the measures. ICC values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 indicate poor, moderate, good, and excellent reliability, respectively (241). An analysis of variance (ANOVA) for repeated measures for time and groups was used to identify differences for the main variables. Post-hoc Scheffe corrections were performed when the main variables showed a significant time-x-group interaction to identify sub-group and time differences. For each main analysis, the effect size (ES) was calculated. Partial eta squared (η^2p) was used as a measure of ES. The magnitude of the ES was classified according to the following scale: 0.01–0.059 = small effect, 0.06–0.139 = moderate effect, and ≥0.14 = large effect. All analyses were performed with Jamovi (The Jamovi Project, 2021). Jamovi (Version 2.3.12.0) [Computer Software]. Retrieved from https://www.jamovi.org. Significance was considered <0.05 for all analyses.

Results

No baseline differences (T0) were observed among interventions (CC, SS, and FR) for all investigated variables (ROM, TH, PPT, PhA, and Z) in either the lower leg or thigh.

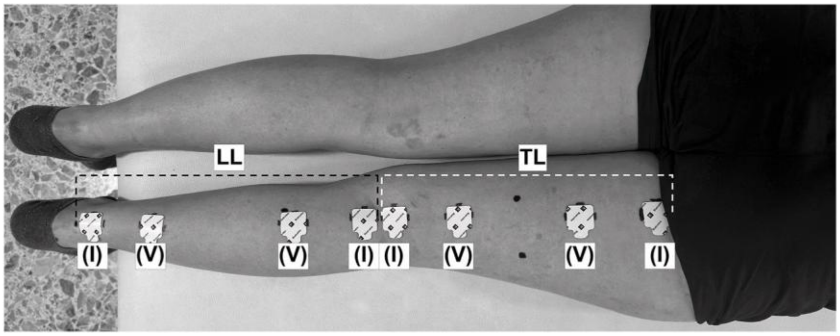


Figure 1.12 Graphical representation of electrode placement and setting for the localized bioimpedance analysis. LL: Lower leg length; I: Source electrode; TL: Thigh length; V: Detector electrode.

Local measures

ICC for ROM was 0.96. The ICCs for TH, PPT, PhA, and Z of the leg were 0.86, 0.91, 0.95, and 0.99, respectively. Concerning measures of the lower leg, a significant time × group interaction was observed for ROM, PPT, PhA, and Z (Table 2). Post-hoc Scheffe´ analysis showed differences among interventions. In particular, ROM improved for both SS and FR from T0 to T1 while returning to baseline in T2. Only for SS, the increase in ROM was accompanied by an increase in the measure of PPT from T0 to T1. Only for the FR, the increase in ROM was accompanied by an increase of both Z and PhA from T0 to T1.

Non-local measures

The ICCs for TH BF, PPT BF, TH ST, PPT ST, PhA, and Z of the thigh were 0.81, 0.83, 0.90, 0.86, 0.95, and 0.96, respectively.

Concerning measures of the thigh, no significant interaction was observed for any analyzed variable (Table 1.6).

Table 1.6 Synthesis of outcomes of ROM, TH, PPT and L-BIA for each condition and body segment.

	CC			SS			FR			ANOVA
	T0	T1	T2	T0	T1	T2	T0	T1	T2	Time x Group
Leg (local)										
ROM(°)	36.7 ± 5.62	37.1 ± 5.63	37.5 ± 6.04	36.0 ± 5.56	40.3 ± 4.98§	38.2 ± 5.04╪	35.2 ± 5.13	38.5 ± 4.63§	37.0 ± 4.61	*$p < 0.0001$; $F = 8.27$; $\eta^2p = 0.298$
TH(N)	23.9 ± 2.97	23.2 ± 3.09	23.0 ± 3.40	23.7 ± 2.22	21.9 ± 3.18	22.2 ± 3.24	23.0 ± 2.69	21.6 ± 3.79	22.2 ± 3.76	$p = 0.44$; $F = 0.94$; $\eta^2p = 0.046$
PPT(KG)	4.63 ± 1.73	4.77 ± 1.31	4.96 ± 1.66	4.87 ± 1.16	5.77 ± 1.57§	5.15 ± 1.56	4.49 ± 1.18	4.93 ± 1.20	5.17 ± 1.54	*$p = 0.03$; $F = 2.79$; $\eta^2p = 0.125$
PhA(°)	11.7 ± 1.44	11.5 ± 1.43	11.4 ± 1.58	11.6 ± 1.47	11.5 ± 1.47	11.5 ± 1.44	11.6 ± 1.17	12.1 ± 1.36§	11.9 ± 1.37	*$p = 0.0007$; $F = 5.37$; $\eta^2p = 0.216$
Z(Ω)	1.43 ± 0.22	1.41 ± 0.23	1.41 ± 0.23	1.44 ± 0.27	1.43 ± 0.26	1.42 ± 0.26	1.41 ± 0.22	1.46 ± 0.23§	1.44 ± 0.23	*$p = 0.0007$; $F = 5.40$; $\eta^2p = 0.217$
Thigh (non-local)										
TH BF (N)	17.5 ± 3.03	17.0 ± 3.32	16.1 ± 2.85	18.5 ± 2.27	17.3 ± 2.18	16.7 ± 2.95	17.9 ± 3.38	16.8 ± 3.04	17.3 ± 3.00	$p = 0.29$; $F = 1.27$; $\eta^2p = 0.061$
PPT BF (KG)	6.50 ± 1.60	6.83 ± 1.76	6.91 ± 1.97	6.61 ± 1.39	7.34 ± 2.11	7.51 ± 2.15	6.30 ± 1.36	7.14 ± 1.76	7.30 ± 2.18	$p = 0.40$; $F = 1.03$; $\eta^2p = 0.051$
TH ST (N)	15.7 ± 3.45	15.7 ± 2.77	15.1 ± 3.66	15.6 ± 3.59	15.8 ± 3.80	14.6 ± 3.66	16.0 ± 4.04	15.5 ± 4.70	16.0 ± 4.27	$p = 0.22$; $F = 1.47$; $\eta^2p = 0.070$
PPT ST (KG)	6.24 ± 1.96	6.84 ± 1.91	6.49 ± 1.54	6.13 ± 1.45	7.08 ± 2.18	7.12 ± 2.01	5.96 ± 1.60	6.58 ± 1.61	6.87 ± 1.72	$p = 0.47$; $F = 0.89$; $\eta^2p = 0.045$
PhA(°)	10.4 ± 1.88	10.0 ± 2.03	10.2 ± 2.09	10.9 ± 1.93	10.5 ± 1.73	10.5 ± 1.92	10.7 ± 1.99	10.6 ± 2.08	10.6 ± 1.79	$p = 0.44$; $F = 0.93$; $\eta^2p = 0.046$
Z(Ω)	1.11 ± 0.22	1.08 ± 0.19	1.07 ± 0.19	1.06 ± 0.19	1.04 ± 0.16	1.04 ± 0.16	1.04 ± 0.16	1.03 ± 0.15	1.03 ± 0.15	$p = 0.37$; $F = 1.08$; $\eta^2p = 0.052$

Data are presented as means ± SD; T0: Baseline Evaluation; T1: Post-test assessment; T2: Assessment 10m post-test; BF: Biceps Femoris; CC: Control Condition; FR: Foam Rolling; PhA: Phase Angle; PPT: Pain Pressure Thresholds; SS: Static Stretching; ST: Semitendinosus; TH: Tissue Hardness; Z: Impedance. ANOVA: Analysis of Variance.*:Significant $p < 0.05$, §: different from T0, ╪: T2 different from T1.

Discussion

The present manuscript aimed to understand the effects of an SS and FR intervention on morphological, sensory, and fluid responses measured locally, where the intervention was applied, and non-locally, in the adjacent body segment. Our results indicate that both interventions were able to improve local ROM. However, notwithstanding not observing differences in morphological responses, there were differences concerning sensory and fluid responses. In particular, SS was accompanied by an increase in measures of PPT, while FR was accompanied by an increase in PhA and Z. The observed effects were only present locally, without any variation, for any measured variable in the adjacent body segment. These results suggest that, following SS, decreased pain sensitivity is the main contributor to ROM improvement. While for FR, a variation in both fluid quantity (Z) and intracellular-to-extracellular fluid ratio (PhA), which may modify the viscoelastic properties of the tissue, appears to be the main contributors to ROM improvement.

In the present investigation, we did not appreciate any variation in TH, neither in the PF or knee flexor muscles. Concerning the PF, which were those targeted by the SS and FR interventions, our results seem in line with previous investigations (231, 240, 242). Despite a certain degree of heterogeneity among interventions in which variations in TH might be present (227), a more in-depth glance highlights that variations in TH, specifically in the PF, do not appear significant across studies. In addition, changes in TH seem to be time-dependent, with longer interventions eliciting the greatest variations. Within the present investigation, we provided for both SS and FR a volume of 120 s. Such volume, according to Takeuchi et al. (231), seems to be the one eliciting the fewest variations. Concerning the BF and ST in the thigh, again, we did not observe any variation. To date, no previous study has acutely evaluated the effects of an intervention on the TH of the thigh muscles while providing the intervention to muscles of the lower leg. However, a similar approach among continuous body segments, although considering chronic effects, has been carried out by Konrad et al. (218).

The authors applied combined SS and FR training to the plantar foot sole for 7 weeks and observed responses in the PF muscles. Despite observing a tendency of an increase in ROM, no variation in muscle structure was observed in the PFs despite the longer intervention, which, similar to the present investigation, represents the adjacent body segment.

The improvement in ROM in the present investigation was accompanied by an increase in measures of PPT only for SS. Such a concomitant increase was observed only from T0 to T1, while returning to baseline in T2 indicating a variation of local pain sensitivity. No variation in PPT was observed following the FR intervention. This result does not appear in line with previous investigations (90, 243). Within this study only female participants were included, therefore our results may vary according to the screened population. However, there is heterogeneity in findings considering sex differences in pain perception (244-246). Another possible explanation may arise from the investigations of Warneke et al. (94, 95) indicating improvements in ROM following several warm-up strategies including sham-rolling but also interventions different than FR, which would indicate that a possible warm-up effect rather than a modification of pain sensitivity could be an additional factor contributing to improving ROM following FR. Variations in PPT were not observed in the muscles of the thigh, suggesting, especially for SS, in which a variation in the PF was present, that pain perception was modulated locally rather than as a modulation of the endogenous pain inhibitory system (210).

Only following the FR intervention, we observed a variation in the parameters of the L-BIA. In particular, from T0 to T1, an increase in the PhA and Z values was observed. The action of FR appears to lead to a reduction in leg water, particularly in the extracellular compartment, as indicated by the increase in Z and PhA. Indeed, Z is inversely correlated with water content, while PhA is positively correlated with the intracellular-to-extracellular water ratio. However, while the total water in the leg decreases, no variation is observed in thigh water. Therefore, it seems that the action of FR results in the redistribution of fluids into the cells and towards vessels that would still transport water to the central areas of the body. Our results are similar to those of Brandl and Yoshimura et al. (126, 127). In the study of Yoshimura et al. (126), the author observed that following a FR intervention, local impedance increased. Similar results were observed by Brandl et al. (127) after applying instrument-assisted soft tissue mobilization to the lumbar fascia, in which again an increase in values of impedance was observed. Altogether, the results of previous investigations suggest that a FR intervention may determine local fluid variations. However, from the data of such research, it was not possible to infer more precise mechanisms. In the present investigation, we observed a concomitant increase in PhA and Z, which indicate an improvement of the intracellular-to-extracellular ratio and a decrease in the total amount of fluids, respectively (125). Extracellular water represents plasma and interstitial fluids, while intracellular water represents muscle cell mass (247). Increased intracellular water content appears to be an indicator of improved performance, muscle quality, and reduced frailty (248-250). Conversely, an increase in extracellular water is associated with inflammation, sarcopenia, muscle wasting, malnutrition, and disease (251). If we consider that we observed an increase in Z, therefore indicating a decrease in the total amount of water (252), it is plausible that the major contributor to the improved intracellular-to-extracellular ratio is a decrease in extracellular water. However, considering the viscoelastic properties of biological tissues, which also depend on tissue hydration (253, 254) a decrease in the water content would logically lead to a more rigid tissue. Therefore, we hypothesise that a concomitant decrease of extracellular fluids with an increase in intracellular fluids occurred, improving muscle efficiency (139) and therefore, ROM. The effect observed may open to possible new applications of the FR as a tool to locally reduce extracellular fluids in those conditions characterized by fluid accumulation.

We acknowledge that the present study may have some limitations. First, our measures of PPT were evaluated through a portable algometer, which was stopped by a verbal command from the participant. Such a procedure may have overestimated the measured parameter. However, the reliability of such measures for the lower leg and thigh is high (ICC = 0.91, 0.83, and 0.86). In addition, despite equalizing both interventions for intensity and volume, there is the need in future investigations to find a more physiologically oriented variable for standardization. In the present investigation we adopted a L-BIA which provides us the possibility to measures local tissue impedance, however, such approach does not allow us to estimate water quantities. Further, despite all participants declared to be active by regularly exercising, we did not screen for individual activity levels. Lastly, our analyzed sample was composed solely of women; therefore, results may differ for men. Despite such limitations, women are an underrepresented sample in the field of sports medicine (255), and have in general a lower water content and greater extracellular water quantities than men (256) (if weight is normalized). Therefore, we believe the choice of evaluating fluid response in females may simultaneously be a limit and a strength of this study. This study is the first to include a measure of L-BIA following SS and FR in the investigated and adjacent body segments.

Conclusions

SS and FR were both able to acutely improve ROM. The effects of both interventions could be appraised locally without any modification of any assessed outcome in the adjacent body segment. SS improved ROM through a modification of pain perception, while FR improved ROM through a supposed modification of the viscoelastic properties of tissues. No difference in tissue morphology was observed for any group. Only following the FR, a local increase in the intracellular-to-extracellular ratio of the leg and a decrease in total fluid content was observed. Collectively, these findings suggest that FR may serve as a potential strategy for promptly alleviating water retention in targeted body segments.

2.5 "Determinants of acute effects of Stretching vs. Foam Rolling: Morphological, sensory and fluid responses"

Ewan Thomas, Antonino Scardina, Gianmarco Pinto, Sofia Serafini, Masatoshi Nakamura, Andreas Konrad, Francesco Campa, Marianna Bellafiore, Antonino Bianco

Introduction

There are different exercise modalities which have the potential to improve flexibility and range of motion (ROM) of a joint(257). The most popular approach is stretching (257, 258). Several stretching techniques are frequently employed, which include static stretching (SS), proprioceptive neuromuscular facilitation (PNF) dynamic and ballistic stretching (258). Despite differences exist among the application of each technique, no acute differences are observed concerning their effect on improving ROM (259).

Among these techniques, SS is frequently employed not only to improve ROM, but also as a warm-up strategy (87). However, after prolonged bouts of SS (greater than 60sec), if subsequent performance is carried out, an impairment effect is frequently observed (260).

Different approaches have been employed to overcome such pitfall. One of these is the use of foam rollers (FR). A single bout of FR has been seen to improve ROM to the same extent as SS, however, without decreasing muscle strength or power (88).

FR is usually applied by compressing the muscle belly over a roller and delivering the intervention through an alternate rolling motion. However, there has been interest in alternative approaches in which FR has been applied without a rolling motion. These include static compression over the foam roller for a given time (261) and the use of a dynamic movement of the joint while the muscle belly of interest is compressed over the roller (FR-DM) (230). Both non-rolling methods were found to be as effective as the conventional rolling method.

Previous studies seeking to understand the physiological mechanisms behind ROM improvements of SS and FR, have observed heterogeneity in acute morphological changes (227, 262). However, changes in ROM are more consistently accompanied by an increase in pain pressure thresholds (PPT) (90) indicating that a modification of participants sensation is the main contributor for ROM improvement (263).

Another factor which may concur to improve ROM is fascia mobility. Fascial mobility is determined by different fascial layers sliding on each other, and in order for the sliding to occur correctly, hydration dynamics of fascia is fundamental(264). Therefore, fascia fluid dynamics(viscoelasticity) could be an important factor that contributes to ROM of a joint. The bioelectrical impedance analysis (BIA) represents a valid tool for assessing changes in body fluid distribution (252). In addition to the know conventional approach(265) a recent method has been developed to identify fluid dynamics within specific body segments, namely localized BIA (L-BIA) or electrical impedance myography (EIM) (122, 123). A recent intervention by Brandl et al. (127) applied instrument-assisted soft tissue mobilization to the lumbar fascia and observed tissue hydration through L-BIA. The results of this study highlight a reduction in the fluid content of the fascia following the intervention. However, no information on range of motion (ROM) or pain is provided. Similarly, an investigation by Yoshimura et al. (266) applied a FR intervention to the plantar flexor muscles (PF) observing that local impedance increased following such intervention, but also in the control group. Despite the interesting results reported (127, 266) these are confined to instrument-assisted soft tissue mobilization with conflicting evidence on FR. To date, there are no studies comparing the effects of SS, FR and FR-DM on changes in viscoelastic properties by analysing the level of local tissue hydration which could further assist the comprehension of physiological mechanisms involved in ROM responses. Thus, the aim of this study was to compare the acute effects of SS, FR, and FR-DM on the PF. Based on previous studies, we expect that ROM will increase mainly through a modification of pain perception rather than a morphological variation. Therefore, we will evaluate and compare the localised effects of L-BIA following SS or FR (either conventional or non-rolling methods) and hypothesise a greater effect of FR methods compared to SS due to the compression and mobilization of tissues from the roller.

Methods

Experimental design

A randomized cross-over design was adopted to compare the acute effects of SS, FR and FR with a dynamic movement (FR-DM) of the PF muscles. Each participant was instructed to visit the laboratory four times, three for the above mentioned experimental conditions and once as a control condition (CC). Each visit was scheduled with at least 48h from the subsequent visit. Each visit was scheduled at the same time of the day. During the lab visits, the temperature within the lab was set at 24°C. During each visit, each participant was tested at three time points: 1) upon arrival (T0), immediately after the intervention (T1), and after 10min from the end of the intervention (T2). During the CC participants were instructed to sit on a chair for 10min between T0 and T1. During all conditions participants were required to sit on a chair between T1 and T2. At each time point, ROM, tissue hardness (TH), PPT, Drop Jump height (DJ), and L-BIA were assessed. Since dynamic activities such as ROM and DJ may influence TH, PPT, and L-BIA, these were assessed prior to ROM and DJ. Consequently, the order of each assessment was TH, PPT, L-BIA, ROM, and DJ. The reproducibility of this method was confirmed in a previous (91) and the present study (see results section). All measures were collected on the dominant limb of the participants.

Participants

Twenty-four healthy participants were enrolled (12 male and 12 female; See table 1). Participants with neuromuscular, orthopedic and gynecological disease (or taking oral contraceptives) or those having injuries of the lower extremities were excluded from the investigation. Female participants were allowed to participate to the experiments only during the follicular phase of their menstrual cycle and always before ovulation (235) in order to standardize bodily fluid balance. In order to further standardize the hydration level of each participant, these were asked to refrain to use coffee and drink water at least for 30 minutes before each testing session. Written informed consent was provided and signed by all participants. The study was approved by the University of Palermo bioethical committee(protocol n°170/2023), conducted in accordance with the Helsinki Declaration (267) with the ClinicalTrials.gov Identifier (NCT06877832).

Table 1.7 Descriptive characteristics of participants

	M	**F**	**Tot**
N	12	12	24
Age	25.8±2.83	22.7±2.61	24.2±3.09
Weight (kg)	72.7±13.34	59.5±9.54	66.1±13.20
Height (cm)	172.0±7.57	161.2±9.45	166.6±10.3

Outcomes

All measures were collected by the same investigator. All measures were collected three times for each time point and the mean value for each test was used for analysis, except for the L-BIA, which was collected once at each time point.

Plantar flexors ROM

Each participant was placed prone on a medical bed with both feet hanging out of the bed. Inelastic straps were used to fix the pelvis and the contralateral leg of the tested participant. A flat rigid surface was fixed on the sole of the foot. A two-arm electronic goniometer (Digital Angle Ruler, resolution0.05°, accuracy ± 0.2°, repeatability0.05°) was then placed with one arm parallel to the flat surface positioned on the foot sole and the other arm following the diaphysis of the fibula. Starting position was set with the foot and the leg forming a 90°angle. The investigator passively pushed the foot into a dorsiflexion motion to the limit of available ROM, or the maximum tolerable pain for each participant (268).

Tissue Hardness

TH was assessed through a portable tissue hardness meter (NEUTONE-TDM-N1; TRY-ALL Corp., Chiba, Japan). TH was measured in the medial gastrocnemius. The participant was instructed to lie prone on a medical bed. The measurement location was set in the muscle belly at the midway of the distance between the popliteal fossa and the proximal end of the Achilles's tendon.

Pain Pressure Thresholds

PPT was assessed through a portable algometer (FPX25 Pain Tester, Wagner Instruments, CT, USA). The participant's measurement position and posture were similar to TH. The algometer was positioned perpendicular to the direction of the muscle fibers. The investigator pushed the metal rod of the algometer at a pressure of 1kg per second over the muscle belly of the participant. The participant was instructed to say "stop" when pain, rather than just pressure, was experienced.

Localised Bioimpedance Analysis

L-BIA was performed using a single frequency phase-sensitive device (BIA-101 Anniversary Sport Edition, Akern Systems, Firenze-Italy) at 50kHz and 400μA to measure PhA and vector length (127). Two pairs of electrodes (source (I) and detector (V) electrodes) were used. One pair was positioned on the upper part of the leg, with the lateral part of the I positioned on the popliteal crease (269) and the V, positioned caudally, parallel to I, 5cm apart. The other pair was positioned on the lower part of the leg with the lateral part of the I positioned above the tibial malleolus and the V, in a cranial direction parallel to I, 5cm apart. Prior to each test, the BIA measurements of the analyzer were validated using a precision circuit with acceptance for resistance (R) measurements of 383ohm (Ω) and reactance (Xc) values of 45Ω.

Drop Jump Height

A unilateral DJ was assessed through an Optojump™ system (Microgate, Bolzano, Italy) connected to a personal computer with dedicated software (OptojumpTM Next software). The system consists of two optical bars, a receiver and a transmitter, positioned 1m apart. The bars identify ground contact time(with a precision of 1ms) and then convert non-contact time into cm. Based on recommendations on optimal jump height(20 to 60cm) (270), all jumps were performed from a box with a height of 40cm. Participants had to stand upright on the box with their hands positioned on their hips. At the "go" of the investigator, the participants had to jump forward from the box into the opto-jump system avoiding stepping down from the box or hopping off of the box. Once the participant reached the ground with their dominant limb, without flexing the knee, had to jump as high as possible. A 30s rest was given between each trial.

Interventions

Each participant underwent SS, FR and FR-DM interventions. Each intervention was carried out performing four sets with a 30sec rest between each set. The intensity of the interventions was controlled by asking the participants to feel the SS or the pressure of the leg over the roller for both FR and FR-DM at a magnitude of at least 8 out of a scale from 0 to 10, where 10 was defined as maximum tolerable. The adopted roller was a low-cost commercially available roller (Bodymate, 30cm in length, 15cm in diameter in expanded polypropylene foam of medium density). A familiarization period was allowed on the non-dominant side.

Figure 1.13 Visual representation of the SS

For SS, the participants were asked to stand upright and put their dominant foot on a wedge with a 35° inclination (Figure 1). The SS intervention lasted 30sec for each set. For the FR the participants were instructed to position the muscle belly of the PF on the roller. Thirty rolls (15 proximal and 15 in a distal direction) over a period of 30 seconds were considered as one set (Figure 2A). The FR-DM consisted in positioning the belly of the PF muscles over the roller and without moving the roller, perform 15 plantar flexions and 15 dorsiflexion’s of the ankle over a period of 30 seconds while compressing the dominant leg with the contralateral (Figure 2B). For both FR and FR-DM a metronome sound produced by a mobile-phone app (Metronomo: Tempo lite ver. 5.0.7, App Store,Apple) was adopted to correctly determine either the rolling motion or the plantar and dorsiflexion motion (1 second for each rolling motion or ankle movement).

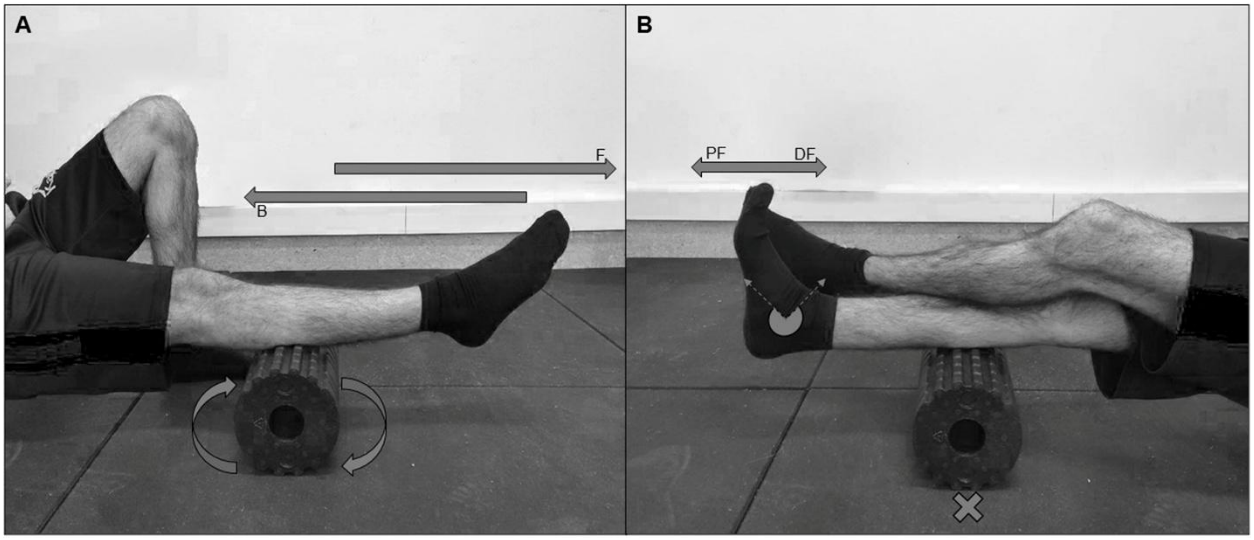


Figure 1.14 Visual representation of both the FR interventions, in which panel A shows FR and panel B, FR-DM

Statistical analysis

Results are presented as means ± standard deviations. Differences were assessed using an ANOVA for repeated measures considering time (T0vs.T1vsT2) and groups (CCvs.SSvs.FRvs.FR-DM) for main variables. Post-hoc Bonferroni corrections were performed to identify differences between assessments for each intervention. The paired, multivariate Hotelling-T2 test was performed to determine if changes were present for bioelectrical parameters (vector length and its direction defined as PhA, measured between T0 and T1, T0 and T2 and T1 vs T2). For each main analysis, the effect size (ES) was calculated. Partial eta squared ($\eta 2p$) were used as a measure of ES. All analyses were performed with Jamovi (The jamovi project (2021). Jamovi (Version 2.3.12.0) [Computer Software]. Retrieved from https://www.jamovi.org). Graphs were created using GraphPad Prism8 (GraphPad Software, San Diego, CA). Alpha was set at 0.05 for all analyses.

Results

Comparison between values at T0 among conditions

No significant differences were observed when comparing the T0 of each condition (CC, SS, FR and FR-DM), for all investigated variables (ROM, TH, PPT, DJ, vector length and PhA), indicating no baseline differences.

Comparison of timepoints for the CC

No differences were observed when comparing T0, T1 and T2 of the CC for all investigated variables, indicating no effect of time on the included variables.

Changes in plantar flexion ROM, PPT, and TH

Table 2 shows main results for all groups. ANOVA significant time-x-group interactions ($p<0.001$) were observed for ROM and PPT. Bonferroni post-hoc analysis showed that for SS, ROM increased from T0 to T1 and remained elevated in T2. These increases were not accompanied by a variation in TH while a variation in PPT was observed only from T0 to T1 with a return to baseline in T2. For FR, ROM increased from T0 in T1 while returning to baseline in T2. Such increase was not accompanied by a variation in TH while an increase in PPT was observed only from T0 to T1 with a return to baseline in T2. For FR-DM, ROM increased from T0 to T1 and remained elevated in T2. These increases were neither accompanied by a variation in TH nor PPT.

Changes in bioelectrical values

ANOVA for L-BIA showed no significant time-x-group difference for PhA and vector length. Multivariate analysis through the Hotelling T2 test including local parameters of the leg compartment of R and Xc indicates a significant variation only for the FR from T0 to T1 which remained elevated in T2 (Figure 1.15).

Table 1.8 Synthesis of ROM, TH and PPT outcomes for each condition

		ROM (°)	TH (N)	PPT (KG)
CC	T0	33.44±7.59	22.18±2.95	3.97±1.68
	T1	33.67±8.01	21.68±3.10	3.98±1.55
	T2	33.40±8.46	21.60±3.16	4.06±1.72
SS	T0	33.33±7.49	22.71±3.17	4.51±1.23
	T1	37.36±7.96[a]	21.03±3.04	5.72±1.44[a]
	T2	35.34±8.57[a]	21.51±3.22	5.10±1.69[b]
FR	T0	32.08±7.06	21.74±2.96	4.16±1.15
	T1	34.95±7.31[a]	20.64±3.60	4.81±1.31[a]
	T2	33.48±7.47[b]	20.83±3.66	4.69±1.53
FR-DM	T0	32.70±7.58	22.14±2.60	4.47±1.31
	T1	35.01±7.68[a]	21.39±2.39	4.89±1.56
	T2	34.47±7.64[a]	21.19±3.29	4.90±1.25
ANOVA	Time	*p=<.0001; F=57.4; d=1.58	*p=0.001; F=17.85; d=0.87	*p=0.0001; F=24.93; d=1.04
	Time x Group	*p=<.0001; F=6.95; d=0.95	p=0.41; F=1.02; d=0.36	*p=0.0001; F=4.79: d=0.79

Data are presented as means±SD; T0: Baseline Evaluation; T1: Post-test assessment; T2: Assessment 10m post-test; CC: Control Condition; FR-DM: Foam Rolling with Dynamic Movement; FR: Foam Rolling; SS: Static Stretching; ROM: range of motion; TH: tissue hardness; PPT: Pain Pressure Threshold; ANOVA: Analysis of Variance.*:Significant p<0.05, a: different from T0, b: T2; different from T1; d: Cohen's d.

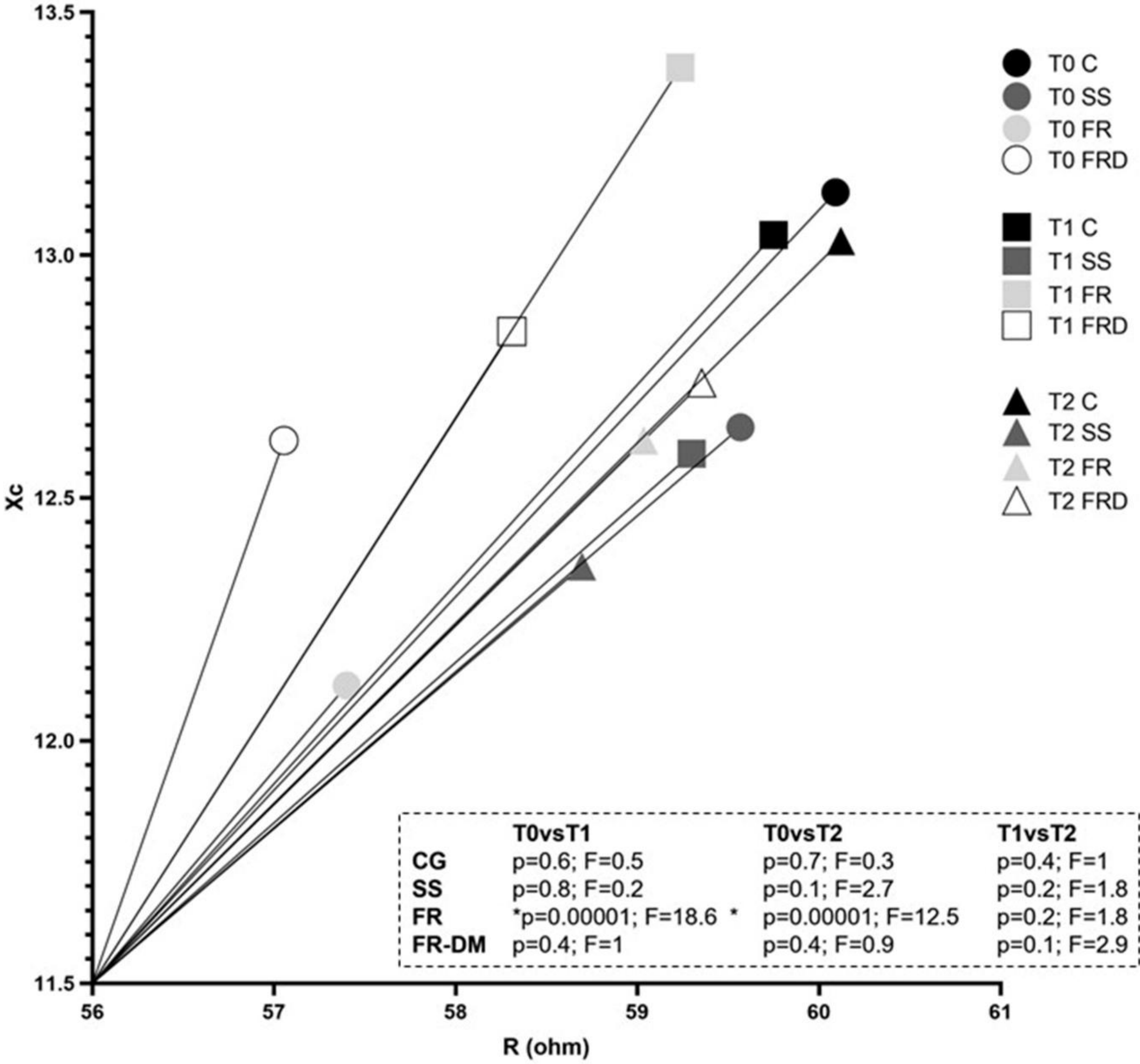
Table 1.9 Synthesis of PhA and Vector length outcomes for each condition

		PhA (°)	Vector length (Ω)
CC	**T0**	12.42±1.81	61.54±10.94
	T1	12.39±1.81	61.19±11.15
	T2	12.29±1.82	61.55±11.09
SS	**T0**	12.04±1.43	60.91±13.60
	T1	12.08±1.37	60.64±13.23
	T2	12.03±1.73	60.00±13.95
FR	**T0**	11.96±1.19	58.68±10.38
	T1	12.37±1.26	60.77±18.80
	T2	12.15±1.18	60.38±10.55
FR-DM	**T0**	12.19±1.57	58.62±15.43
	T1	12.43±1.58	59.73±11.70
	T2	12.21±1.69	60.73±12.08
ANOVA	**Time**	*p=0.01; F=4.73; η^2p=0.052	p=0.16; F=1.83; η^2p=0.019
	Time x Group	p=0.15; F=1.57 η^2p=0.052	p=0.08; F=1.88 η^2p=0.058

Data are presented as means±SD; T0: Baseline Evaluation; T1: Post-test assessment; T2: Assessment 10m post-test; CC: Control Condition; FR-DM: Foam Rolling with Dynamic Movement; FR: Foam Rolling; SS: Static Stretching; PhA: Phase Angle; ANOVA: Analysis of Variance.*:Significant p<0.05, a: different from T0, b: T2 different from T1.

Changes in performance

No differences for any group at any time point was observed.



	T0vsT1	T0vsT2	T1vsT2
CG	p=0.6; F=0.5	p=0.7; F=0.3	p=0.4; F=1
SS	p=0.8; F=0.2	p=0.1; F=2.7	p=0.2; F=1.8
FR	*p=0.00001; F=18.6	* p=0.00001; F=12.5	p=0.2; F=1.8
FR-DM	p=0.4; F=1	p=0.4; F=0.9	p=0.1; F=2.9

Figure 1.15 Resistance-Reactance graph including all groups and time points.

Discussion

With this study we aimed to understand the underlying mechanisms of flexibility enhancing methods. Our main results indicate that SS, FR either with a rolling or a non-rolling motion with dynamic movement (FR-DM) were effective in increasing ROM without impairing performance. However, we observed some differences concerning the underlying mechanisms.

Tissue hardness

In the present investigation, the acute bouts of SS, FR and FR-DM did not alter TH. Results which seem in line with previous research. In particular, studies investigating the acute effects of FR in the PF have observed that tissue hardness remains unchanged following rolling interventions (240, 242). Concerning SS, there is evidence that acute variations in TH might be present (227). However, within a recent review with meta-analysis by Takeuchi et al. (227), when considering only the PF muscles, no significant decrease of passive stiffness is observed immediately after SS intervention ($p = 0.066$). To date there are no studies evaluating the acute effects of TH on the PF muscles following FR-DM. However, the only study applying FR-DM and evaluating TH to another anatomical location (230) reports that an acute variation was present. Although, within the study of Kasahara et al (230), TH decreased also following FR with a rolling motion, which suggests anatomical differences among acute tissue adaptations.

Pain pressure threshold

Conversely, PPT showed an increase in FR and SS, indicating that a contributor for ROM improvement is decreased pain sensitivity. However, such variation in PPT was not observed for FR-DM. The increased ROM observed for all groups post-intervention was accompanied by an increase in PPT for SS and FR but not for FR-DM. Previous studies comparing the acute effects of FR and SS (88) commonly observe that PPT increase following both interventions without relevant differences between the two. In our investigation, we also followed the time course of the assessed measures up to 10min post-interventions. Despite ROM improved in T1 for all interventions, this remained elevated in T2 only for SS and FR-DM. PPT returned to baseline in T2 following SS, notwithstanding ROM remaining elevated. Two investigations by Mizuno et al (271, 272), despite not measuring PPT, have evaluated the time course of viscoelastic properties of the muscle tendon unit of the PF muscles by applying SS for 5 sets of 60s each. The authors observed that despite ROM remained elevated at least 30min post intervention, the measured passive properties returned to baseline within 10 minutes. It is plausible that additional mechanisms than those related to reduced pain sensation may also contribute to the persistent increased ROM observed in T2.

Concerning FR, the decrease in ROM observed in T2 was accompanied by a decrease in PPT. Previous investigations (90, 230) confirm that following a FR intervention, an increase in measures of PPT is observed immediately post-intervention. However, concerning the prolonged effects of FR on measures of PPT conflicting evidence was observed in previous research. Kasahara et al. (91) explored the effects of FR (3 x 60s rolls) in the knee extensors up to 30 minutes and observed that similar to the present investigation the values of PPT were increased immediately after the intervention but decreased after 10 minutes. In a second investigation by Kasahara et al. (273), in which 1 set of 30s FR was applied again to the knee extensors, PPT remained elevated up to 15min post intervention, while ROM remaining elevated only up to 10min. However, in this last investigation not only PPT but also TH were elevated up to 15 minutes. Based on previous findings, it appears plausible that a modification of pain sensation could be one of many factors which could contribute to improve ROM following FR.

Concerning FR-DM, neither a modification of TH nor PPT was observed. Therefore, improved ROM cannot be attributed neither in changes of tissue properties nor in a modification of pain sensation. Further possible suggested mechanisms for ROM improvement may either be increased blood flow and temperature or a change in the thixotropic properties of tissues (89). However, the results from TH and the L-BIA do not support a variation in fluid viscosity following FR-DM. A study by Warneke et al.(94) evaluated the effect of a conventional FR vs a Sham Rolling, identifying that no differences were present in ROM improvement among the two methodologies. These results together, suggest that the observed increase in ROM, is likely to be attributed to a warm-up effect of the ankle movement, rather than to the rolling compressing motion. Similar improvements in ROM have been also seen in other investigations performing active contractions without the use of a FR (231, 232, 274). In turn, the three investigated approaches (SS vs. FR vs. FM-DM), notwithstanding have the potential to improve ROM with no differences between interventions, may rely on different underlying mechanisms.

Drop jump

It is of particular interest that following the three interventions, no impairing effect was observed among the drop jumping performance. In the present investigation, we applied a 30sec per set SS duration which was volume equated to the two FR interventions. Previous research (260, 275) investigating the dose-response for SS on performance, have identified that impairments may be observed when the total stretching volume is longer than 60sec. Such performance impairment has been discussed to be associated to a reduction in musculoskeletal stiffness or reduction in neural drive (228). In the present investigation the total volume of the interventions was of 120s, however, contrary to previous research no significant impairment was observed for the SS condition. Despite not directly assessing stiffness, we did not observe a variation in TH, which could provide a rationale for the results observed following SS. A recent review by Wiewelhove and colleagues (263) aiming to investigate the effects of FR on performance has found trivial or even beneficial effects following such intervention. To date, only the study by Kasahara et al. (230) has evaluated the effect of a FR-DM (vs. FR and another FR technique) on performance. The authors observed that no significant interaction ($p = 0.44$) was present between the FR and FR-DM.

L-BIA

Other main result concerns hydration of the leg compartment. Only for the FR a variation in bioelectrical impedance was observed, suggesting a change in fluid distribution. The resistance-reactance graph showed an elongation with a leftward displacement of the vector, suggesting a decrease in the absolute fluid content of the investigated segment alongside with a shift of the fluids from the extracellular to the intracellular space (Fig.3). L-BIA analysis assessed the simultaneous change in vector length and direction, highlighted a significant change from T0 to T1, which remains significant in T2 only for the FR. However, no time- x-group interaction resulted from the ANOVA, suggesting that further research is needed to deeply investigated change in fluid content and distribution. However, either vector length and PhA are near significant suggesting a decrease in absolute water content simultaneously with a shift from the extracellular to the intracellular space. The L-BIA has been traditionally developed to monitor muscle injury and disease (122, 123, 125). Findings from clinical populations indicate that when muscle injuries or alterations in muscle architecture are present a decrease in R and Xc and then in PhA, are observed (123, 276). In the present investigation, following the FR we observed an opposite trend, with a graphical increase of the length of the vector indicating a decrease in fluids. Our results are similar to those of Brandl and Yoshimura et al (127, 266). To be noted that in both studies, the authors indicate an increase in bioelectrical impedance, without however discriminating if such increase occurred in R, Xc, PhA or vector length. Despite not indicating a specific parameter, the authors conclude that their results were likely due to a decrease in the water content of the investigated tissue. Both authors (127, 266) also evaluated temperature, which resulted increased probably by an improvement of tissue microcirculation. Such hypothesis is supported by Hotfiel et al (277) which by applying 3 sets of 45 seconds each of FR over the thigh muscles, observed that the perfusion of the area increased up to 73.6% and remained elevated after 30 minutes. Therefore, if both mechanisms (improved circulation and decreased water content) are confirmed by future research, the FR could find further applications than to improve ROM or as a warm-up strategy.

Limitations of the study

This study is not without limitations. The assessment of the PPT was performed using a handheld algometer which was stopped by the investigator after verbal indication of the participant. Therefore, values could be overestimated. The results of the L-BIA suggest a reduction in water content as well as in the extracellular-to-intracellular water ratio following the FR. However, standardizing the measure of the vector for the segment length could have improved the sensitivity of L-BIA in detecting potential changes even in the water content within the leg.

Conclusion

SS, FR and FR-DM were able to acutely improve ROM without impairment of drop jump performance. SS and FR improved ROM through an increase in PPT without any variation in TH, while FR-DM improved ROM without any variation in either PPT and TH. Overall, the results indicate that ROM improved through a sensory variation or as a warm-up effect. These results indicate that SS, FR and FR-DM can be used to improve ROM or as a warm-up strategy.

Only FR was able to decrease extracellular fluids, indicating that the rolling motion combined with the pressure over the roller may be the discriminant of such effect. This result opens new scenarios on the possible applications of the FR in which a local variation in fluids needs to be achieved.

2.6 “Acute effects of foam rolling on local and remote range of motion: a myofascial perspective?”

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Introduction

Foam rolling (FR) is a commonly used technique to enhance joint range of motion (ROM) and is applied in both acute and chronic contexts, including as part of warm-up protocols (88). The ROM improvements induced by FR may be attributed to various physiological mechanisms, such as morphological adaptations, altered pain perception, increased body temperature (warm-up effect), and changes in tissue viscoelastic properties (278-281). A substantial body of literature investigated the local effects of FR, with a focus on neuromuscular, mechanical, and thermographic responses in the targeted tissue (126, 282, 283). Nevertheless, recent research also focused on the remote effects of FR, where interventions applied to one anatomical site elicit changes in distant regions. Most studies investigated these effects on contralateral homologous muscle groups, commonly referred to as the "crossover effect" (98, 284-286). Notwithstanding the evidence on the contralateral effects of FR, limited evidence exists regarding its remote effects on segments within a myofascial chain or myofascial meridian (104). The concept of myofascial chains embodies the principle of structural and functional continuity within the body, wherein tension or mechanical input in one muscular or fascial region can influence both adjacent and anatomically distant segments (100). The theoretical framework for exploring the remote effects of FR is based on Myers' conceptual work, which describes myofascial chains as continuous muscle-fascia connections capable of transmitting tension and force throughout the body (287). A systematic review by Wilke et al. provided anatomical evidence supporting the existence of the six myofascial chains proposed by Myers, suggesting that most skeletal muscles are directly interconnected via connective tissue (100). In alignment with this concept, Wilke et al. observed increased cervical ROM following lower limb stretching, reinforcing the notion of mechanical strain transmission along myofascial pathways (234). Results were confirmed by a subsequent study of the same research group comparing local stretching to myofascial chain stretching (103), further substantiating the hypothesis of interconnected myofascial pathways. Additionally, a systematic review and meta-analysis by Burk et al. examined the effects of myofascial techniques (including FR) on ROM in distant body regions, concluding that remote exercise interventions can enhance ROM, albeit with small effect sizes and potential methodological biases (104).

Despite this emerging evidence, little is known about the remote effects of traditional FR applied to the knee flexors.

The present study specifically focused on what Myers refers to as the "superficial back line", a myofascial continuity that extends along the entire posterior aspect of the body. This anatomical structure integrates and protects tissues from the plantar fascia and short toe flexors to the galea aponeurotica/epicranial fascia (287), encompassing the knee flexors and cervical region.

Another plausible explanation for the potential improvements in remote ROM following FR may involve systemic mechanisms related to increased body temperature. FR interventions may induce temperature increases not only in localized tissues but also globally (288, 289), which could potentially contribute to remote ROM variations (231).

Based on the theoretical framework of myofascial chains, which describes the body as an integrated network of muscle-fascia continuities capable of transmitting tension across distant segments, we hypothesized that FR applied to the right knee flexors could elicit acute improvements in remote cervical ROM. These effects might be mediated by either thermoregulatory or mechanical mechanisms.

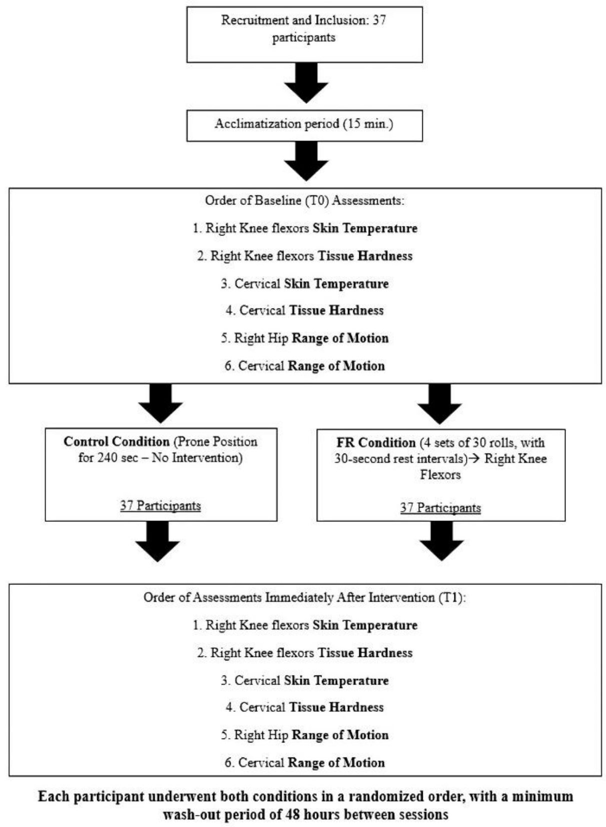
Therefore, the aim of this study was to investigate the acute effects of a FR intervention targeting the right knee flexors on both local (right hip) and remote ROM (cervical spine).

Materials and methods

Experimental design

Participants were instructed to visit the laboratory on two different occasions, with a minimum interval of 48 hours, ensuring the same time of day for both visits. The laboratory temperature was consistently maintained at 24 °C (290). All participants underwent two conditions: Control Condition (CC) or FR. These order of administration of the conditions was randomly assigned using the www.randomizer.org website. Participants, during each visit, were tested two times: upon arrival (T0) and immediately after each condition (T1). At each time-point, skin temperature (TsK) was measured in the knee flexors and cervical region. Moreover, measures of tissue hardness (TH) were assessed in the biceps femoris and semitendinosus/semimembranosus complex of the knee flexors and in the cervical region, to the right and left of the seventh cervical vertebrae (C7). Additionally, measures of right hip flexion, cervical flexion, cervical extension, right and left cervical rotation, right and left cervical inclination ROM were assessed. Since dynamic activities such as ROM may influence TH and TsK, these were assessed prior to ROM of the corresponding assessment area. Consequently, the order of each assessment was knee flexors-TsK, biceps femoris-TH, semitendinosus/semimembranosus-TH, cervical TsK, cervical TH, right hip flexion ROM, cervical ROM (Fig. 1.16). Between T0 and T1, participants, according to the randomized order, underwent either the FR intervention (FR) or a 4-minute prone control period matching the duration of the FR protocol (CC) (Fig. 1,16).

Fig. 1.16 Flow Chart Study



Participants

Thirty-seven healthy participants were enrolled in a cross-over model research design (descriptive characteristics are provided in Table 1). The required sample size, calculated through G*Power (version 3.1.9.4) for a repeated measures ANOVA (F-tests, effect size=0.25, α=0.05, power=0.80) (279), was 34. Participants, selected from a university student population, were required to be free of orthopaedic and neurological disorders. Individuals with a history of lower limb injuries were excluded. Before recruitment, each participant provided written informed consent detailing the study protocol, risks, and benefits. The research was approved by the bioethical committee of the University of Palermo (protocol n°170/2023) and adhered to the Helsinki Declaration and EU recommendations for good clinical practices.

Table 1.10 Descriptive characteristics of participants.

	M	F	Tot.
N	24	13	37
Age (years)	26 ± 4.50	24.2 ± 3.67	25.4 ± 4.28
Weight (kg)	55.5 ± 9.55	70.8 ± 10.90	65.4 ± 12.70
Height (cm)	172 ± 7.26	158 ± 7.22	167 ± 9.85

Data are expressed as means±st.deviations. F: Female; M: Male; Tot. Total.

Outcomes

All assessments were performed by the same investigator. Each measure was collected three times for time point (T0 and T1), with the mean value used for analysis, except for TsK, which was recorded once for each time point.

Skin temperature

Infrared images of the knee flexors and cervical region were captured using the FLIR ONE® Edge infrared camera (FLIR Systems) connected to an iOS smartphone via the FLIR ONE mobile application (v 6.3.1). Equipment and the laboratory environment were stabilized 30 minutes before testing. To acclimate to the standardized laboratory temperature of 24°C, participants lay prone on a medical bed with relaxed neck posture for 15 minutes, ensuring that the target skin areas were exposed and not in contact with any surface. Previous research suggests a minimum optimal acclimatization period of 10 minutes (291). The thermal images were analysed using the FLIR Tools mobile application (FLIR Tools v 2.10.0, FLIR Systems, App Store, Apple), which enabled the detection of the mean temperature of the knee flexors and the cervical region in standardized regions of interest (ROI). Specifically, TsK of the right knee flexors was assessed using the FLIR Tools App by applying a box over the region of the right posterior thigh, spanning from the horizontal level of the gluteal fold to that of the fibular head (292). Cervical TsK was evaluated by placing two boxes, one to the right and one to the left of the C7 spinous process, extending vertically from the hairline to the anatomical junction between the trapezius muscle outline and the lateral contour of the neck (292). The FLIR Tools App was used to retrieve the average TsK within the defined ROIs. The emissivity of the skin was set at 0.98, corresponding to the emissivity of clean human skin (293). To ensure measurement consistency, the thermal imaging camera was mounted on a tripod at a fixed location for all participants, maintaining a consistent distance of less than 1 meter from the ROI, as a short distance is recommended for accurate assessment of fixed body areas (290). During image acquisition, participants lay prone on a medical bed, while the camera, secured on the tripod and angled downward, targeted the designated areas. Thermal images of the knee flexors and cervical region were captured at T0 (following the 15-minute acclimatization period) and T1.

Tissue hardness

The TH measurements were obtained using a portable TH meter (NEUTONE TDM-N1; TRY-ALL Corp., Chiba, Japan), capable of measuring penetration distance under a pressure of 14.71 N (1.5 kgf) (237). A spring mechanism integrated into the upper section of the device, above the display, enables the hemispherical indenter at the lower end to retract into the body upon contact with the tissue. This design ensures that TH is assessed at a standardized compressive force of 14.71 N (1.5 kgf), corresponding to the point at which the reaction force exerted by the tissue counterbalances the preset internal resistance (237). For the assessment of right knee flexors TH, the ischial tuberosity was used as the proximal anatomical reference, while the popliteal fossa served as the distal reference. Measurements were obtained at two specific sites on the proximal third of the posterior right thigh: a lateral site corresponding to the biceps femoris and a medial site corresponding to the semitendinosus/semimembranosus complex (279). In the cervical region, TH measurements were performed using the spinous process of the seventh cervical vertebra (C7) as the anatomical reference. Specifically, data were collected at two locations, positioned 2 cm to the right and left of the C7 spinous process (294). For both knee flexors and cervical TH assessments, participants were positioned in a prone lying position on a medical bed and instructed to remain fully relaxed throughout the procedure. To enhance measurement reliability, each assessment site was precisely marked with a dermal marker before data collection. At each site, three consecutive measurements were obtained, and the mean value was calculated for subsequent analysis.

Hip flexion and cervical ROM

For hip flexion ROM, participants lay supine with the knee extended, and inelastic straps secured the contralateral leg to prevent compensatory movement. ROM was assessed using the active straight leg raise (ASLR) test, with ankle dorsiflexed and leg fully extended to evaluate hamstring extensibility (295). Right hip flexion ROM in degrees was recorded using the GYKO inertial system (Microgate, Bolzano, Italy) (296), placed just above the knee.

Cervical ROM was assessed with the participants seated, using inelastic straps attached to the chair at chest height to prevent compensatory movements. The GYKO inertial system (Microgate, Bolzano, Italy) (296)

was employed to measure active cervical ROM (flexion, extension, right/left rotation, right/left inclination), with the accelerometer positioned through a strap at the center of each participant's forehead.
For both hip and cervical ROM measurements, the maximum active ROM reached during each assessment was retained as the final value for that assessment (279, 297). Of the three measurements collected for each assessment, the average value was used for analysis. Participants were also asked to keep their eyes closed during the tests to minimize the influence of visual input on their perceived sensations. Data were transmitted via Bluetooth to a computer equipped with dedicated analysis software (Gyko RePower; Microgate, Bolzano, Italy).

Intervention

The FR intervention targeted only the right knee flexors and consisted of four 30-second sets (total intervention time: 2 minutes), with 30 seconds of rest between each set.
Participants positioned the muscle belly of the right knee flexors on the roller and executed 30 rhythmic rolls per set (15 proximal, 15 distal) from the popliteal fossa to the ischial tuberosity, maintaining a frequency of 1 roll per second, guided by a metronome application (Tempo Lite ver. 5.0.7, Apple App Store).
Participants were instructed to subjectively rate the perceived pressure exerted by the foam roller as at least 8 out of a scale from 0 to 10, The pressure applied to the right knee flexors was controlled using Numeric Rating Scale (NRS) self-reports where 0 indicated 'no pain' and 10 represented 'the worst pain imaginable,' consistent with protocols employed in prior research (280).
The rolling was performed using a commercially available, cost-effective foam roller (Bodymate; 30 cm length, 15 cm diameter; medium-density expanded polypropylene with a GRID-patterned surface), consistent with previous protocols. (238, 239). A short familiarization trial was allowed on the left (non-intervention) limb as elsewhere described.
In the CC, participants lay prone on a medical bed for 4 minutes, in order to equate with the duration as the FR protocol, between the T0 and T1 assessments, without receiving any intervention.

Statistical analysis

Means ± standard deviations were used to present the results. The Shapiro-Wilk test was performed to assess the data distribution. Coefficient of variation (%CV) were included for reliability of measures (298). A paired t-test was performed to compare baseline measurements between the two conditions (CC and FR). For variables exhibiting a normal data distribution, an analysis of variance (ANOVA) for repeated measures

for time and groups (2x2) was used to identify differences for the main variables. Post-hoc Scheffé corrections were performed when the main variables showed a significant time-x-group interaction to identify sub-group and time differences. For each main analysis, the effect size (ES) was calculated. Partial eta squared ($\eta 2p$) was used as a measure of ES. The magnitude of the ES was classified according to the following scale: 0.01–0.059=small effect, 0.06–0.139=moderate effect, and ≥0.14=large effect (299).
For outcomes exhibiting a non-normal data distribution, a Friedman test were used to analyze the variables. When the Friedman test indicated a significant difference, post hoc comparisons with Durbin-Conover correction were conducted to identify within-group differences over time.
All analyses were performed with Jamovi (The Jamovi Project, 2021). Jamovi (Version 2.6.13.0) [Computer Software]. Retrieved from https://www.jamovi.org. Significance was considered <0.05 for all analyses.

Results

Reliability of the measures

The %CV for the knee flexors TsK was 2.75. The %CV for biceps femoris and semitendinosus/semimembranosus TH were 5.19 and 5.37, respectively. The %CV for the right and left cervical regions TsK were 3.11 and 3.05, respectively. The %CV for the right and left cervical regions TH were 4.81 and 5.18, respectively. The %CV for hip flexion ROM was 3.91. The %CV for cervical flexion and extension, right and left rotation, right and left inclination ROM were 5.78, 5.53, 3.67, 4.1, 4.46 and 4.51, respectively.

Comparison between values at T0 between conditions

The paired t-test observed no significant differences at T0 between conditions (CC and FR) for any of the investigated variables (TsK, TH and ROM), confirming the absence of baseline differences or carryover effects.

Comparison of time points for the CC

A significant interaction was observed in knee flexors-TsK from T0 to T1 (p <.001), indicating a decrease in temperature. No significant differences were found between T0 and T1 in the CC for TH and ROM, indicating no effect of time on these variables.

Changes in local measures

Table 2 shows the main results for all groups. No significant changes in knee flexors-TsK were observed ($p = .054$). A significant time × group interaction was observed in the ANOVA for hip flexion ($p < .001$) and TH in the biceps femoris ($p < .001$). Additionally, a significant difference was also observed for TH in the semitendinosus/semimembranosus complex ($p < .001$). Exclusively for the FR group, post-hoc analysis indicated that hip flexion ROM significantly increased from T0 to T1 ($p < .001$), accompanied by a reduction in knee flexors-TH ($p < .001$) (Table 2).

Changes in remote measures

No significant changes in cervical TsK were observed. A significant difference was observed for the cervical region TH, but exclusively to the right of C7 ($p = .024$). A significant time × group interaction was observed in the ANOVA for cervical flexion, extension, rotation, right inclination ($p < .001$), and left inclination ROM ($p = .001$) (Table 2). Exclusively in the FR condition, post-hoc analysis revealed a significant reduction in TH only to the right of C7 ($p = .021$), accompanied by an increase in cervical flexion ($p = .006$), extension ($p < .001$), right and left rotation ($p < .001$), right ($p = .023$) and left ($p = .006$) inclination ROM (Table 2).

Table 1.11 Synthesis of outcomes of TsK, TH, and ROM for each condition and body segment.

Analyzed variable	CC		FR		*ANOVA*
	T0	T1	T0	T1	***Time x group***
Knee flexors					
TsK (ºC)	31.5 ± 1.17	30.9 ± 1.32*	31.6 ± 1.38	32.2 ± 0.95	$p < .001$; $F = 19.564$; $\eta^2 p = 0.214$
TH BF (N)	19.9 ± 3.49	19.9 ± 3.26	20.1 ± 2.95	18.1 ± 2.60*	$p < .001$; $F = 16.4$; $\eta^2 p = 0.185$
TH ST/SM (N)	17.5 ± 3.16	17.5 ± 3.13	17.6 ± 3.12	16.2 ± 3.53*	$p < .001^f$; $F = 7.49$; $\eta^2 p = 0.094$
Hip flexion ROM (º)	92.5 ± 15.4	90.3 ± 15.4	92.0 ± 14.4	99.6 ± 14.4*	$p < .001$; $F = 64.1$; $\eta^2 p = 0.471$
Cervical spine					
Right cervical TsK (ºC)	34.7 ± 1.38	34.8 ± 1.34	34.7 ± 1.18	34.7 ± 1.15	$p = .819$; $F = 0.0530$; $\eta^2 p = 0.001$
Left cervical TsK (ºC)	34.8 ± 1.33	34.8 ± 1.34	34.7 ± 1.14	34.8 ± 1.16	$p = .862^f$; $F = 0.0280$; $\eta^2 p = 0.000$
Right cervical TH (N)	17.8 ± 4.45	17.7 ± 4.50	17.6 ± 4.17	16.2 ± 3.84*	$p = .024^f$; $F = 7.05$; $\eta^2 p = 0.089$
Left cervical TH (N)	18.3 ± 4.34	18.2 ± 4.32	17.6 ± 4.06	16.9 ± 4.07	$p = .273^f$; $F = 0.759$; $\eta^2 p = 0.010$
Flexion ROM (º)	63.8 ± 12.8	60.6 ± 12.1	63.4 ± 13.5	67.6 ± 12.3*	$p < .001$; $F = 21.141$; $\eta^2 p = 0.227$
Extension ROM (º)	72.3 ± 12.4	70.7 ± 13.2	71.1 ± 13.4	75.0 ± 11.4*	$p < .001$; $F = 19.27$; $\eta^2 p = 0.211$
Right rotation ROM (º)	75.7 ± 7.95	75.0 ± 7.87	75.1 ± 7.29	78.5 ± 7.61*	$p < .001$; $F = 22.0$; $\eta^2 p = 0.234$
Left rotation ROM (º)	75.9 ± 7.23	74.6 ± 7.67	76.0 ± 7.14	79.6 ± 7.13*	$p < .001$; $F = 23.25$; $\eta^2 p = 0.244$
Right inclination ROM (º)	50.2 ± 7.90	48.9 ± 8.07	49.4 ± 7.23	51.2 ± 7.63*	$p < .001$; $F = 15.853$; $\eta^2 p = 0.180$
Left inclination ROM (º)	50.5 ± 8.95	49.8 ± 9.26	50.3 ± 8.60	53.0 ± 8.66*	$p = .001$; $F = 11.04$; $\eta^2 p = 0.133$

Data are presented as means ± SD; T0: Baseline Evaluation; T1: Post-test assessment; ROM: Range of Motion; BF: Biceps Femoris; CC: Control Condition; FR: Foam Rolling; ST/SM: Semitendinosus/Semimembranosus; TH: Tissue Hardness; TsK: Skin Temperature. ANOVA Analysis of Variance. *: significant difference compared to T0. ^{f}: Non-Parametric Repeated Measures Analysis (Friedman Test) for Variables with Non-Normally Distributed Data.

Discussion

This study aimed to investigate the acute effects of FR applied to the knee flexors on local (right hip) and remote (cervical region) ROM. Our findings indicate that FR not only enhanced ROM at the site of application but also elicited significant remote improvements in cervical mobility. Notably, ROM improvements were accompanied by a reduction in TH in both the knee flexors and the cervical region to the right of C7, without significant changes in TsK. This parallel change in ROM and TH locally but also in the remote region may indicate a potential relation between the two measures, in line with previous investigations (300), suggesting a potential strain transfer along a myofascial chain .. Specifically, FR may transmit mechanical forces along the superficial back line, a myofascial chain that spans from the plantar fascia to the epicranial fascia (220). Since no variation in TsK was observed a mechanical rather than a thermal mechanism is suggested. However, this cannot be considered a definitive conclusion, as the observed upward trend in local TsK could also suggest a systemic warm-up effect (288) which may warrant further investigation. Further, a warm-up effect cannot be fully excluded since our measures take into account TsK, which appears to be a weak predictor of intramuscular temperature (301).

Our findings on the remote cervical ROM-enhancing effects of FR are corroborated by the systematic review and meta-analysis conducted by Burk et al., which suggested that remote myofascial techniques may effectively increase ROM in distant body segments (104). Findings also supported by Do et al. and Hyong et al., both reporting increased ROM in distand body segments following either self-myofascial release and stretching exercises (104, 302, 303). Specifically, Do et al. reported that self-myofascial release using a foam roller applied to the plantar fascia resulted in immediate improvements in the flexibility of the superficial back line, evidenced by a reduction in fingertip-to-platform distance in the Toe Touch Test and an increase in hip ROM (+ ~8.5°) (302). Similarly, Hyong et al. observed remote cervical ROM increases (+ 5.07° flexion, + 5.4° extension) after passive hamstring stretching, highlighting the influence of posterior chain interventions (303). Other findings by Jung et al. who observed improvements in sit and reach test performance, as well as in both active and passive hamstring ROM, even when self-myofascial release techniques were applied to the suboccipital and plantar regions, suggesting the potential effectiveness of miofascia-based self release applied indirectly for improving muscle flexibility (304). These studies interpreted their ROM improvements by proposing mechanisms related to fascial continuity, myofascial chains, and the transmission of mechanical forces along these pathways. Their findings support our observed increases in cervical ROM following FR applied to the right knee flexors, reinforcing the hypothesis of a potential involvement of force transmission through interconnected myofascial structures. Moreover, Habscheid et al. observed that FR may produce remote hypoalgesic effects (305), underscoring the importance of evaluating pain sensitivity variables in future research as potential mechanisms underlying both local and remote ROM enhancements. Although direct evidence on the specific effects of FR on fascial parameters remains limited, our findings, alongside previous research on the remote effects of stretching and myofascial release, support further exploration of myofascial chains as potential pathways for tension and force transmission. Nevertheless, further investigations are clearly necessary, considering that some studies have not observed significant remote effects of exercise on the ROM of distant body segments. For example, Ficarra et al. found no clear effects on remote ROM (shoulders) in response to static stretching and proprioceptive neuromuscular facilitation applied to the knee flexors, likely because the intervention was intended not to target a specific myofascial chain but rather to evaluate the presence of a generalized, rather than a localized, remote effect (298). Moreover, FR may have contributed to improve ROM by modifying viscoelastic properties (thixotropic effect), potentially accompanied by reduced TH, as suggested by Thomas et al., who observed FR-induced decreases in local extracellular fluid. (279).

Interestingly, the reduction in TH observed at the cervical level (right of C7) following FR applied to the right knee flexors may suggest a potential relation between TH changes and remote ROM improvements, possibly mediated by mechanical strain transfer along myofascial chains. Nevertheless, this study did not assess the direct parameters of the myofascial chain. Therefore, future research should consider alternative hypotheses, as remote ROM variations may arise from different mechanisms, and FR could potentially induce widespread changes also through global warm-up effects (288).
However, our findings showed no significant changes in TsK in response to FR, either locally or remotely, with only a slight upward trend observed in knee flexors. Similarly, Yoshimura et al. reported a comparable trend without significant TsK increases post-FR (126). Yenil et al. also found no added TsK benefits when FR preceded proprioceptive neuromuscular facilitation stretching (306). In contrast, Keraútret et al. reported significant TsK increases after FR (280). Altogether these results highlight heterogeneity across research regarding the acute effects of FR on TsK. Lastly, our results demonstrated a significant reduction in TsK of the knee flexors in the CC. We hypothesize that this decrease is due to the susceptibility of TsK to environmental factors (301) and the participants' acclimatization to the laboratory temperature, which was lower than average human body temperature. As participants were exposed to this environment without any intervention, their TsK may have adjusted accordingly. In the study by Yoshimura et al., a similar TsK trend was observed, with TsK gradually decreng at each time point (126). Notwithstanding TsK was examined in multiple studies investigating the effects of stretching and FR (126, 307), its relationship with muscle temperature appears to be weak (301, 308). Therefore, deeper thermometric assessments, such as those employed by Yoshida et al., using a surface-type deep tissue thermometer, may offer more reliable insights into muscle warming (309).
Collectively, within a context with participants with limited flexibility who are not able to directly apply an intervention to a specific location (i.e. injury or immobilization), these may indirectly enhance it by FR to a distant not affected location. However, future studies should directly assess fascial properties and include neurophysiological variables to further clarify the mechanisms driving both local and remote ROM gains.

Limitations of the study

This study is not without limitations. The main limit of this study is that it did not evaluate pain sensitivity, one of the factors that could have provided additional insights into the mechanisms underlying the observed effects (305). In addition, despite TsK was evaluated, providing insight into mechanisms related to warm-up, a measure of muscle temperature was not assessed, limiting the comprehension of a possible global warm-up induced by the intervention. Lastly, notwithstanding participants self-reported being physically active, individual physical activity levels were not formally assessed or controlled for, which may have influenced the outcomes.

Conclusions

Foam rolling applied to the right knee flexors acutely improved both local (hip flexion) and remote (cervical) range of motion, potentially mediated by reductions in tissue hardness.Collectively, these results suggest that foam rolling is a potential effective strategy for enhancing flexibility both locally and remotely. Although strain transfer across distant anatomical regions may be a possible explenation, insights into the underlyining mechanisms warrants further attention. This may support the potential application of foam rolling to improve mobility in distant body segments, particularly when direct intervention is challenging or unfeasible. Future studies should further explore the physiological and structural mechanisms underlying these effects, with specific focus on myofascial connectivity.

2.7 "Chronic effects of a static stretching intervention program on range of motion and tissue hardness in older adults"

Masatoshi Nakamura, Antonino Scardina, Ewan Thomas, Konstantin Warneke and Andreas Konrad

Introduction

Previous studies have shown that static stretching (SS) intervention programs can increase range of motion (ROM) (112, 118, 310) and improve gait function (118). ROM is generally known to decline in older populations (112-114). This age-related decline in ROM leads to decreased mobility and balance function (311) and an increased risk of falls (312). Thus, ROM-enhancing methods such as SS interventions could likely be beneficial for increasing ROM and improving health in older populations. Several studies have suggested that resistance training can increase joint ROM (257, 313, 314). Recently, a systematic review and meta-analysis by Alizadeh et al. (315) reported that long- term resistance training increases joint ROM, and this increase has the same effect size as stretch training. In particular, resistance training emphasizing eccentric contraction results in a large increase in ROM (316). According to this evidence, resistance training and SS training programs can be useful intervention methods when the goal is to increase the ROM of a joint. Although very high-volume SS training programs (e.g., 1 h per day) can increase muscle strength and hypertrophy (129, 317), resistance training can be considered the most time-efficient method if the goal is to increase muscle strength and muscle hypertrophy. On the other hand, one of the advantages of an SS intervention program is the reduction of passive muscle stiffness (115, 116). Interestingly, an increase in antagonist passive muscle stiffness inhibits the primary active muscle movement, resulting in high energy and metabolic costs (318, 319). Therefore, decreasing passive muscle stiffness following an SS intervention program for several weeks could be essential for maintaining physical function in older adults. In a previous study examining the acute effects of SS in older adults, Nakamura et al. (112) showed that a single 300-s bout of an SS intervention can decrease the stiffness of the medial gastrocnemius (MG) and lateral gastrocnemius muscles. In addition, a systematic review and meta-analysis by Nakamura et al. showed that a single bout of an SS intervention can decrease passive muscle stiffness in older adults to a small magnitude, and the effects are comparable between older and young adults (117). Clinically, knowing whether an SS intervention program conducted for several weeks can reduce passive muscle stiffness is important. Still, to the best of our knowledge, only a few previous studies have evaluated the chronic effects of an SS intervention program in older adults (118, 119). Although SS intervention programs appear to be effective in increasing ROM in the older adult population (120, 121), no evidence is available for potential changes in muscle stiffness. Therefore, there is a need to revisit the chronic effects of SS intervention programs on passive muscle stiffness in older adults and the potential relationship between ROM changes and muscle stiffness changes. Thus, this study aimed to investigate the effects of a 10-week SS intervention program on ankle dorsiflexion (DF) ROM and tissue hardness in community-dwelling older adults. In previous studies, it was found that an SS intervention program can effectively reduce passive muscle stiffness in young adults (116), and a single bout of an SS intervention can effectively reduce passive muscle stiffness in older adults to a similar extent as in young adults (117). Thus, we hypothesized that the SS intervention program would effectively increase ROM and reduce tissue hardness (an index of passive muscle stiffness) in older adults. Moreover, we also hypothesized that the changes in ROM would be correlated with the changes in muscle stiffness.

Methods

Experimental design

In this study, participants visited the laboratory 12 times, for two measurement sessions and 10 SS intervention sessions (Figure 1). DF ROM and tissue hardness measurements in both the right and left sides were taken at the first visit (PRE measurement). The next 10 visits were made every week, and stretching of the ankle plantar flexor muscle group was performed under the supervision of the same instructor for 4 × 30-s repetitions. The SS intervention program was first performed on the right side, followed by SS on the left leg. In addition, the SS intervention program was performed more than twice per week in the home for 10 weeks. Adherence throughout the intervention program was monitored through weekly written journals, and the journal of each participant was checked every week. The last visit (12th visit) to the laboratory was conducted 48 h after the last SS intervention. Because all the participants in this study performed the SS intervention program, due to ethical considerations, there was no control group. The study was approved by the Ethics Committee of Nishi Kyushu University (R5-08) and performed in accordance with the Declaration of Helsinki.

Participants

The DF ROM and tissue hardness of the MG in both legs were measured in 29 older adults (male, n = 7; female, n = 22). Using flyers, we recruited older adults who were participating in health classes for older adults held by the university. The inclusion criteria were age > 60 years, living in the community, and being able to walk independently. Exclusion criteria were cognitive impairment, severe cardiac or musculoskeletal disorders, previous diagnosis of pulmonary disease, and hearing impairment. Five participants dropped out because of personal reasons (n = 3) and diseases not related to this study (n = 2). Thus, the total number of participants included in the analyses of the present study was 29, and their ages ranged from 62 to 85 years (average ± SD; age: 73.8 ± 5.1 years; height: 156.0 ± 6.8 cm; body mass: 52.7

± 8.0 kg). In this study, we used G* power 3.1 software (Heinrich Heine University, Düsseldorf, Germany) for the sample size calculation for a paired t-test (effect size = 0.80 [large], α error = 0.05, and power = 0.95), based on the result of a previous study on the change in DF ROM after an SS intervention program (3). Thus, more than 23 participants were needed in this study. All the participants were fully informed of the purpose and procedure of the study. All participants gave written informed consent prior to participation.

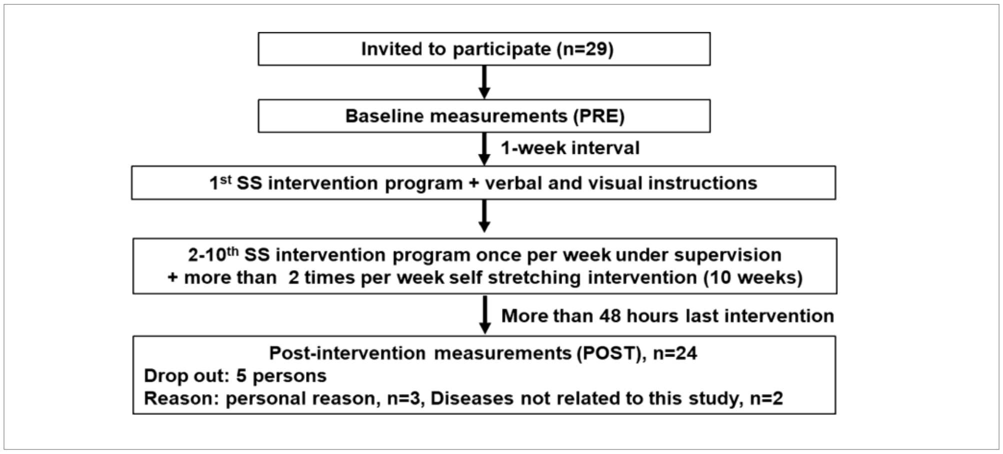


Fig. 1.17 Flow Chart Study

Outcome assessment

Assessment of DF ROM

The subject was supine with the knee joint in full extension (i.e., anatomical position). We measured the ankle DF ROM twice in 1° increments using a plastic goniometer, and the mean value at each measurement period was used for further analysis. The DF ROM was defined as the ankle joint angle just before the participant started to feel discomfort or pain (320-322). Using PRE data, we calculated the intraclass correlation coefficient (ICC) to check the test–retest reliability. The ICC of the DF ROM was 0.925 (95% confidence interval (CI): 0.82–0.968).

Assessment of MG tissue hardness

MG tissue hardness was measured using a portable tissue hardness meter (NEUTONE TDM-N1; TRY-ALL Corp., Chiba, Japan). The participant was instructed to lie supine on the treatment table. The hip and knee joints were set at 0°, and the participant was instructed to relax the ankle joint in a fully plantar flexed position. Tissue hardness was measured at the proximal 30% of the lower leg length (from the popliteal crease to the lateral malleolus) (323, 324). The tissue hardness meter measured the pushing force until a 14.71 N (1.5 kgf) pressure was reached (237, 325, 326). The participant was instructed to relax while the tissue hardness was measured three times at each measurement, and the mean value at each measurement period was used for further analysis. The ICC of the tissue hardness was 0.981 (95% CI: 0.962–0.991).

SS intervention program

Four repetitions of a 30-s self-administered ankle dorsiflexion SS intervention were performed once per week in a laboratory under direct supervision and more than twice per week in the home for 10 weeks. SS was performed with a straight knee joint to target gastrocnemius stretching. The participant was instructed to place the stretching leg as far posteriorly as possible while pushing the heel down to the ground, with the forefoot pointing forward until the point of discomfort. The SS intervention was first performed on the right leg, followed by the left leg.

Statistical analysis

The statistical analysis was performed using SPSS (version 28.0; SPSS Japan Inc., Tokyo, Japan). The Shapiro–Wilk test was used to test the normality of the data. The PRE and POST values for each variable followed normality. Therefore, we compared the PRE and POST values using the paired t-tests. Effect sizes are reported with Cohen's d and categorized as either a small effect ($d < 0.5$), medium effect ($d = 0.5–0.8$), or large effect ($d > 0.8$) (299). In addition, changes from PRE to POST values were calculated, and the Shapiro–Wilk test showed that these data were normally distributed. Thus, the relationship between the changes in DF ROM and the change in tissue hardness was examined using the Pearson product–moment correlation coefficient. A p-value of < 0.05 indicates statistical significance.

Results

Table 1 lists the results for the DF ROM and tissue hardness before and after the intervention. DF ROM showed a significant increase ($p < 0.01$, $d = 1.37$, 95% CI = −11.5 to −6.77), and tissue hardness showed a significant decrease ($p = 0.042$, $d = −0.27$, 95% CI = 0.036 to 1.81). However, there was no significant correlation between the change in DF ROM and tissue hardness (Figure 2, $r = 0.086$, $p = 0.561$).

Discussion

In this study, we investigated the chronic effects of a 10-week SS intervention program on DF ROM and tissue hardness of the MG in older adults. The results showed that the 10-week SS intervention program increased DF ROM and decreased tissue hardness, but the change in tissue hardness was small (Cohen's $d = −0.27$), while DF ROM showed a large change (Cohen's $d = 1.37$). Since there was no significant correlation between the change in DF ROM and tissue hardness (Figure 1.18), the increase in ROM in the SS intervention program was not related to a change in muscle stiffness but to a change in sensation, i.e., stretch tolerance. Our results showed that a 10-week SS intervention program can increase DF ROM in older adults, which was consistent with the findings of previous studies (118, 119). In addition, a 10-week SS training program can also decrease the tissue hardness of the MG in older adults. However, the mechanism of the decrease in tissue hardness after a 10-week SS intervention program is unclear. Nevertheless, previous studies have pointed out that changes in the flexibility of the connective tissue surrounding muscle fibers can be considered to influence decreased muscle stiffness (327, 328). Furthermore, a recent systematic review and meta-analysis concluded that SS intervention programs can increase fascicle length at rest and during stretching in healthy young populations (329). Thus, it will be necessary to investigate the mechanism of the change in muscle stiffness during an SS intervention program in older adults by conducting a detailed study that includes measurement of changes in fascicle length. Mechanical and sensory theories have been proposed as mechanisms for the ROM increase after SS intervention programs (63). As described above, the change in tissue hardness was small (Cohen's d = −0.27), while DF ROM showed a large change (Cohen's d = 1.37). Furthermore, there was no significant correlation between the change in DF ROM and tissue hardness (Figure 1.18). These results suggest that sensory theory is associated with the increased ROM seen in this SS intervention program in older adults. Previous studies have also suggested that sensory theory is related to the increase in ROM in SS intervention programs for young adults (66). These results indicate that ROM increases after SS intervention programs in young and older adults and that this increase is related to changes in stretch tolerance, i.e., sensory modification, rather than changes in muscle stiffness. Interestingly, it has been reported that there is a time gap between ROM changes and muscle stiffness changes during an SS intervention program. That is, changes in ROM have been reported to occur earlier than decreases in muscle stiffness (330). Therefore, the decrease in muscle stiffness may occur later than the change in ROM in an SS intervention program in older adults. Therefore, when an SS intervention program is designed to reduce muscle stiffness, it is necessary to measure not only the change in ROM but also muscle stiffness to monitor whether or not there is a decrease in muscle stiffness.

Table 1.12 Changes in dorsiflexion range of motion (DF ROM) and tissue hardness before (PRE) and after the 10-week SS intervention program (POST).

	PRE	POST	p-value	Effect size	95% CI
DF ROM (°)	14.6 ± 6.1	23.8 ± 7.2	$p < 0.01$	d = 1.37	−11.5 to −0.68
Tissue hardness	19.2 ± 3.5	18.3 ± 3.3	$p = 0.042$	d = −0.27	0.04 to 1.81

In this study, we focused on a specific SS training protocol to ensure consistency and observe specific effects on flexibility and ROM in a controlled manner. However, research suggests that other techniques, such as dynamic stretching or proprioceptive neuromuscular facilitation (PNF), can also increase ROM. A systematic review with meta-analyses that investigated the chronic effect of different stretching techniques on ROM showed a significant difference between the stretching techniques, indicating that SS and PNF stretching intervention programs produced greater enhanced ROM than ballistic/dynamic stretching intervention programs (121). However, a previous study comparing the effects of a single bout of SS exercise and PNF stretching exercise on older adults reported that while the increase in ROM was similar, only SS exercise effectively decreased muscle stiffness (331). Therefore, SS and PNF stretching intervention programs are effective when the goal is to increase ROM in older adults. Still, SS intervention program might be adopted when the goal is to reduce muscle stiffness. In addition, the previous study has shown that the effect of increasing ROM in a stretching intervention program is not affected by the difference in the stretched muscles (121, 327). Therefore, although this study targeted the plantar flexor muscles of the ankle joint, it is possible to expand the results to other muscles. In the future, conducting a detailed study of the effects of different stretching techniques on ROM and muscle stiffness in various muscles will be necessary.

There are several limitations to this study. Firstly, there was no control group in this study. Therefore, it is unclear whether the changes seen in this study originated from the SS intervention program. However, this study also showed high measurement reproducibility (66). Therefore, the changes in ROM and tissue hardness seen in this study were likely due to the SS intervention program. Second, we did not measure stretch tolerance or neurophysiological indices in this study. Interestingly, previous studies have reported that SS intervention programs can decrease arterial stiffness (332) and blood glucose . Therefore, it is necessary to comprehensively examine the effects of SS intervention programs on older adults. Third, the sample size calculation in this study was based on the effect size of dorsiflexion ROM improvements observed in the previous study. Consequently, this study might lack sufficient statistical power to detect significant effects in tissue hardness measurements or perform robust correlation analyses. Future studies with a larger sample size are recommended to confirm the findings related to tissue hardness and correlation analyses more conclusively. Fourth, the method of measuring the DF ROM of the ankle joint in this study was performed in the non-weight-bearing position. Previous studies reported the usefulness of measuring DF ROM of the ankle joint in the knee extension and flexion in weight- bearing positions (333, 334). In addition, SS intervention was conducted in a weight-bearing position in this study. Therefore, in future

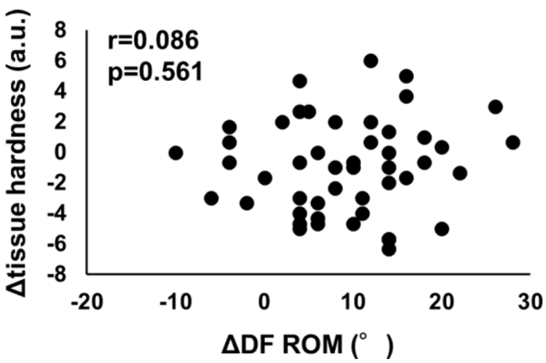


Figure 1.18
Relationship between change in dorsiflexion range of motion (DF ROM) and change in tissue hardness before and after the 10-week SS intervention program.

studies, it is necessary to conduct DF ROM measurements in weight-bearing and non-weight-bearing positions and investigate the relationship with the SS intervention program method.

Conclusion

Our results showed that a 10-week SS intervention program can increase DF ROM and decrease tissue hardness. Our results also suggested that the increase in DF ROM after an SS intervention program can be attributed to the change in stretch tolerance, which supports the sensory theory

CHAPTER III - PROMOTIONAL STRATEGIES FOR ACTIVE LIFESTYLE IN PUBLIC EMPLOYEES

3.0 Chapter III Overview

Chapter III explores strategies to promote active lifestyles among public employees, focusing on teachers and healthcare professionals. The studies presented in this chapter share the common objective of improving physical and mental well-being in workplace contexts through integrated, evidence-based interventions.
Among teachers, post-lockdown data indicate generally good levels of physical activity and moderate adherence to the Mediterranean Diet. More active teachers reported better perceived physical health, while overweight and obesity were associated with lower mental well-being. Primary school teachers showed slightly higher activity levels and physical health scores than nursery teachers, and adherence to the Mediterranean Diet increased with greater physical activity.

In healthcare workers, a structured wellness program including controlled breathing and lifestyle education improved autonomic regulation markers, suggesting reduced stress and enhanced parasympathetic efficiency. Complementing these findings, a pilot intervention based on myofascial chain stretching performed in the workplace demonstrated positive trends in flexibility and overall physical comfort, highlighting the potential of short, feasible exercise sessions to reduce work-related stress and musculoskeletal discomfort among healthcare professionals.

Overall, the chapter underscores the importance of a multidimensional approach integrating physical activity, healthy nutrition, and stress management strategies as key components of workplace health promotion. The combined evidence from these studies suggests that programs grounded in physiological regulation—supported by objective monitoring tools such as heart rate variability—can effectively enhance the health, resilience, and productivity of public sector employees.

List of publications discussed within Chapter II:

- **3.1** Scardina A, Tabacchi G, Thomas E, Navarra GA, Petrigna L, Caramazza G, Palma A, Bellafiore M. Relationship between Lifestyle Determinants and Perceived Mental and Physical Health in Italian Nursery and Primary School Teachers after the COVID-19 Lockdown. J Funct Morphol Kinesiol. 2024 Feb 17;9(1):33. doi: 10.3390/jfmk9010033. PMID: 38390933; PMCID: PMC10885123.

- **3.2** Iovino M, Antonacci Y, Genova G, Oddo F, Scognamiglio TF, Salviato N, Scardina A, Bellafiore M, Busacca A, Pernice R. Evaluation of Work-Related Stress on Healthcare Operators using Cardiovascular

Variability Indices from Commercial and Portable Multisensor Systems. Front Netw Physiol. Manuscript submitted for publication, 2025.

- 3.3 Scardina A., Cottone R., Cammarata I.G., Salviato N., Boca S., Thomas E., Bellafiore M. Effects of a Myofascial Chain Stretching Program on Work-Related Stress and Musculoskeletal Disorders in Healthcare Workers: Preliminary Results. Int. J. Environ. Res. Public Health (MDPI), Manuscript submitted for publication, 2025.

3.1 "Relationship between lifestyle determinants and perceived mental and physical health in Italian nursery and primary school teachers after the Covid-19 Lockdown"

Introduction

The COVID-19 pandemic and the consequent closing of schools with the inclusion of distance learning have made epochal changes in the lifestyles of children, affecting their physical and mental health. It has been demonstrated that in children aged 1 to 10 years, an increased use of digital technologies linked to distance learning has led to negative changes in sleep, emotions and behavior (335). Such changes were also recorded among the teachers' population, adversely affecting their lifestyle and physical and mental health (336-340). Before the lockdown, schoolteachers already represented a category of workers characterized by chronic fatigue, physical inactivity, the presence of musculoskeletal disorders, voice disorders and common mental disorders (341, 342). The lockdown has affected, from a mental point of view, the perception of well-being concerning professional practice, suggesting the need to develop interventions to improve professional well-being (337, 338), while from a physical point of view, the teacher population showed sedentary behaviors and high levels of overweight and obesity (339, 343, 344). Eating habits have also undergone changes, with an increase in the consumption of unhealthy foods (sweets, soft drinks, sausages, frozen foods and salty foods) (344, 345). Physical exercise performed at home during the lockdown appears to have had a positive effect on physical well-being, but there are still conflicting opinions on the effects on mental well-being (343, 346). Given that a worker's productivity is associated with their own level of physical and mental health, which, in turn, depends on multiple lifestyle determinants, including demographic and socioeconomic factors, education level, weight status, physical activity and workload (347, 348), the health of teachers may affect the quality of schoolchildren's education. An unhealthy lifestyle characterized by physical inactivity and incorrect eating habits is among the main risk factors for the development of chronic noncommunicable diseases (such as cardiovascular disease, cancer, chronic respiratory disease and diabetes) (349). Specifically, sedentary behaviors (SBs) can show up at different times of daily life, during free time or at work (350). SBs are defined as the time spent in sitting and/or reclining position during waking hours, with energy expenditures less than 1.5 metabolic equivalents (METs) (351). Absenteeism (time away from work due to illness or disability) and presenteeism (reduced productivity at work) can result from consequences associated with SBs, impacting medical expenses and work productivity as well as the health of the individual (27, 352). The majority of daily sedentary time is spent in the work setting, where working adults spend almost half of their waking time (between 8 and 11 h per day) (25). For example, office workers spend at least two-thirds of their working hours sitting (353), while workers such as teachers are considered "non-sedentary" during working hours because it has been estimated that they remain in an orthostatic position for 95 percent of working time (354, 355). However, this does not preclude this category of workers from performing sedentary activities during their free time, such as watching television and using computers or cell phones. In fact, some scientific evidence suggests low levels of physical activity (PA) and high levels of obesity in this category of workers (356, 357). Apparently, age has an influence; indeed, teachers with high sedentary behavior exhibit significantly lower age than others, and this could be explained by greater time spent in front of digital media, which are known as the set of means of communication that focus on digitized technologies such as computers and networks (358). Young adults spend more time in front of screens because they are more likely to use new technologies (354). In addition to age, gender can also be a determinant; indeed, female workers tend to be more active than their male counterparts because of the double commitment between work and household tasks (359). Sedentary lifestyles have also been correlated with unhealthy eating habits in teachers, a trend that tends to improve when breaks during working hours increase, emphasizing that breaks during working hours permit this category of workers easier access to healthy foods (350). Some evidence suggests that physically active teachers are more likely to consume more fruits, vegetables, legumes, grains and olive oil, while less active teachers with a higher BMI consume fewer of these foods (360). These foods are those typical of the Mediterranean area that are part of a healthy eating pattern known worldwide as the Mediterranean Diet (MD) (361). Adherence to this type of diet provides significant health benefits, improving emotional and cognitive functioning and physical health (361). Investigating this aspect is important as teachers are primarily responsible for health promotion among students and serve as role models for the acquisition of healthy lifestyles among students (362). Lifestyle behaviors such as the practice of physical activity or eating habits and psychological aspects such as physical and mental well-being and their correlations have already been studied in populations similar to ours in other countries (363, 364), while in Italy, there was a greater focus on teachers from the university sector before and during lockdown (365, 366), or studies focused on individual aspects such as mental (367-369) and physical well-being (370). Thus, little is known about the lifestyle of Italian school teachers. Therefore, the aim of this observational study is to investigate whether there are differences in nursery and primary school teachers' lifestyles after a COVID-19 lockdown period, and the relationship between perceived physical and mental health and demographics, weight status and lifestyles, such as adherence to the Mediterranean diet (MD) and physical activity level (PAL), in order to identify possible predictors of physical and mental well-being.

Materials and Methods

Participants and Study Design

A cross-sectional study was conducted with a group of 265 Italian teachers (women 96.2% and men 3.8%) voluntarily recruited from nursery schools (NSs) and primary schools (PSs) during a training course entitled "Natural Moving", organized by Regional School Office of Sicily (USR), Palermo, Italy, performed in the extracurricular hours from September to November 2021. A total of 382 teachers adhered to data collection, and 265 of them completed all the administered tests. These teachers were divided into NS (43.4%) and PS groups (56.6%). Only teachers with permanent contracts who have been practicing the profession for at least 5 years are included in this study. All participants signed informed consent, voluntarily participating in this study. The investigation was approved by the local ethics committee from the University of Palermo (protocol n° 2/2018) and complies with the criteria on the use of people in research, as stipulated in the Declaration of Helsinki.

An online survey was conducted to collect data, including age, gender, weight, height, school teaching degree (NS and PS), education level (high school and graduation degree). Three questionnaires were administered to analyze the lifestyle determinants, such as physical activity level, adherence to MD and perceived physical and mental health. These questionnaires were compiled as Google forms during video calls using the online platform "Google Meet", under the supervision of the physical activity (PA) experts. Participants were given as much time as necessary to complete the questionnaires and were asked to keep the video camera on during all the administration.

Tools for Assessing Selected Aspects of Lifestyle and Health

The PAL of the participants was assessed with the short form of the International Physical Activity Questionnaire (IPAQ-SF) converted to a digital Google form format for online administration (371). This questionnaire is indicated for adults aged 15–69 years (356, 372, 373). Its items explore the duration and frequency of exercise, including walking, moderate and vigorous PA and time spent in sedentary activities, referring to the previous week. The total IPAQ score is calculated as a continuous variable (average minutes/MET per week) or as categories (inactive, sufficiently active and active, highly active levels). An inactive PAL is obtained when an adequate PAL is not attained to fall within the other two levels. A sufficiently active PAL is attained by performing vigorous PA for ≥20 min/day for ≥3 days/week, or through moderate exercise or walking performed for ≥30 min/day, for ≥5 days/week, or again through the combination of moderate or vigorous exercise for ≥5 days/week accumulating at least 600 MET min/week. Active or highly active PA is characterized by ≥3 days per week, with an accumulation of at least 1.500 MET min/week, or by cumulative PA for seven days per week of any combination of walking, moderate or vigorous exercise for at least 3.000 MET min/week (356). To calculate the data in our study, we used the automatic IPAQ report created by Di Blasio and colleagues (371). Dietary patterns were analyzed with the Mediterranean Diet Adherence Screener (MEDAS) questionnaire (374), which assesses the adherence to the MD through 14 items, including the consumption frequency of specific foods typical of the MD, such as fruits, vegetables, olive oil, nuts, fish and wine. All foods that deviate from the MD, such as red meat, carbonated drinks, butter and sweets, were also considered in the questionnaire. Each question was given a score of 0 to 1, so the final score will vary from 0 to 14. According to the literature, we categorized the adherence to MD in this way: weak adherence, ≤5; moderate to fair adherence, 6–9; good or very good adherence, ≥10 (375).

To investigate levels of perceived health, we administered the SF-12 questionnaire (short form of the original SF-36 Health Survey) (376) converted to a digital Google form for online administration. This is a psychometric tool that with two indices, concerning the Physical Component Summary (PCS) and Mental Component Summary (MCS) assessing 8 items on physical functioning, functional limitations due to physical and emotional health problems, freedom from bodily pain, perception of general health, vitality, social functioning and mental health, the physical and mental state of participants. In other terms, it is a tool that allows us to assess the health status and health-related quality of life of particular populations. For the calculation of indices, we used a Microsoft Excel spreadsheet made by Ottoboni et al. (377), while for the evaluation of these parameters, we referred to the normative values divided by age and gender given by Schupp et al. (378).

Statistical Analysis

To identify an appropriate sample size for recruitment, the G-power software (G*Power version 3.1.9.4, using Power 1-β err prob ≥ 0.80, Effect Size = 0.3, α err prob = 0.05) was used, which defines the minimum number of participants needed for the intended research model.

An exploratory analysis was initially carried out, and participant's characteristics were expressed as means, standard deviations and percentage values. The quantitative variables present in the analysis were age, weight, height and body mass index (BMI), and the two components of the SF-12 questionnaire (PCS and MCS) were total MET min/week and MD adherence score. The qualitative variables were educational levels (secondary school and graduation degree), weight status (normal, overweight and obese), PAL (inactive, sufficiently active and active or highly active). These variables were dichotomized before the analysis.

The normal distribution of the data was verified with the Kolmogorov–Smirnov test. In the inferential statistical analysis, when variables were normally distributed, we used a t-test or One-Way ANOVA test to assess the differences between the means of the examined variables. When they were not normally distributed or qualitative, we used a non-parametric Mann–Whitney U test or Kruskal–Wallis test to assess the differences. For a more detailed analysis, we performed a stratification by PALs (inactive, sufficiently active and active/very active), weight status (normal, over and obese) and school teaching grade (NS and PS). A correlation analysis between the qualitative binary variables was performed using a non-parametric correlation test (Sperman's rho). Afterwards, a multinomial logistic regression analysis reporting Odds Ratios (ORs) and 95% Confidence Intervals (CIs) was conducted among the qualitative variables in order to evaluate associations between the PCS and MCS (which were considered the dependent variables) and the independent variables (school teaching grade, education, age, weight status, MD adherence, PAL).
The *p*-value significance level was set at 0.05.
All analyses were performed with Jamovi software (The Jamovi project (2021) Version 1.8.0.1 (Computer Software), Sydney, Australia, retrieved fromhttps://www.jamovi.org) accessed on 8 December 2023.

Results

A descriptive analysis of the quantitative variables of the sample in their totality (TS) and in the two groups of NS and PS teachers is shown in Table 1.13. The total MET score derived from the IPAQ questionnaire was high in both groups, with PS teachers having a significantly higher total MET compared to the NS group ($p = 0.046$). This difference was also present when the corresponding categorical classes (PAL) were considered (Table 1.14). Furthermore, the MD adherence was from moderate to fair in both groups, and it was not significantly different between them. The results of the SF-12 questionnaire showed that PS teachers had a higher PCS score ($p = 0.030$) compared to NS teachers, and this difference persists when dichotomized variables are considered (Table 1.14); on the contrary, both groups exhibited an MCS score above average, comparing these data with normative references to different age classes (378), but showed no between-groups differences.

Table 1.13 Descriptive statistics of the quantitative considered variables in the total sample (n = 265) and in the nursery (n = 115, 43.4%) and primary (n = 150, 56.6%) school groups.

	TS [a]	**NS [b]**	**PS [c]**
Age (years)	49.22 ± 6.95	49.0 ± 6.65	49.4 ± 7.19
Weight (Kg)	64.11 ± 10.28	63.5 ± 9.60	64.6 ± 10.8
Height (cm)	164.22 ± 6.7	163 ± 6.06 *	165 ± 7.07 *
BMI [d] (Kg/m^2)	23.77 ± 3.54	23.8 ± 3.47	23.7 ± 3.60
Total MET [e] (score)	2888.0 ± 2604.0	2678.0 ± 2736.0	3049.0 ± 2495.0 *
MD [f] adherence (score)	8.86 ± 1.90	9.05 ± 1.95	8.71 ± 1.87
PCS [g] (score)	49.65 ± 7.50	48.51 ± 7.77	50.53 ± 7.18 *
MCS [h] (score)	51.64 ± 8.05	52.11 ± 7.61	51.28 ± 8.38

Data are presented as means ± SD; a, TS, Total Sample; b, NS, Nursery School; c, PS, Primary School; d, BMI, body mass index; e, Metabolic Equivalent of Task; f, Mediterranean Diet; g, Physical Component Summary; h, Mental Component Summary; * $p < 0.05$; NS vs. PS.

Table 1.14 shows descriptive statistics for qualitative binary variables. The results indicated a significantly higher percentage of teachers with an educational secondary level in the PS ($p < 0.001$) compared to the NS and a significantly higher percentage of teachers with a high graduation degree level in the NS than the PS ($p < 0.001$). Stratifying the sample according to educational levels (see Table 1.14) in relation to PALs, no statistical differences were found.

Regarding the weight status, both groups showed a higher percentage of normal-weight teachers compared to their overweight and obese counterparts, and no difference between groups was found.

The total sample of teachers with an active or highly active level had a significantly bigger score that ranged from fair to moderate in MD adherence and PCS variables in comparison with sufficiently active ($p = 0.023$) and inactive ($p < 0.001$) teachers (see Table 1.14). Conversely, no significant difference was found in the MSC score according to the level of physical activity. In detail, the NS group with an active/highly active level exhibited a significant increase in MD adherence compared with sufficiently active ($p = 0.030$) and inactive ($p < 0.001$) teachers, while no significant difference was observed among the PS teachers with different levels of physical activity, despite their level of MD adherence being from fair to moderate. Comparing NS and PS teachers, we noted that NS teachers with an active or highly active level had an MD adherence score significantly higher than PS teachers ($p = 0.015$).

Table 1.14. Descriptive statistics of the categorized binary variables in the total sample (N = 265) and in the nursery (n = 115, 43.4%) and primary (n = 150, 56.6%) school groups.

		TS [a]		**NS** [b]		**PS** [c]	
		N	**%**	**N**	**%**	**N**	**%**
Age classes	Younger (<50 years)	140	52.8	65	46.4	75	53.6
	Older (≥50 years)	125	47.2	50	40	75	60
Weight status	Normal	190	71.7	79	41.6	111	58.4
	Overweight/obese	75	28.3	36	48	39	52
Educational level	Secondary	132	49.8	61	46.2	71	53.8 ***
	Graduate	133	50.2	71	53.4 ***	62	46.6
PAL [d]	Inactive	44	16.6	23	52.3	21	47.7 *
	Active/very active	221	83.4	92	41.6	129	58.4
MD [e] adherence	Non adherent	170	64.2	69	40.6	101	59.4
	Adherent	95	35.8	46	48.4	49	51.6
PCS [f]	Low	107	40.4	54	50.5	53	49.5 *
	High	158	59.6	61	38.6	97	61.4
MCS [g]	Low	80	30.2	34	42.5	46	57.5
	High	185	69.8	81	43.8	104	56.2

Data are presented as numeric and percentage frequencies; a, TS, Total Sample; b, NS, Nursery School; c, PS, Primary School; d, PAL, Physical Activity Level; e, MD, Mediterranean Diet; f, PCS, Physical Component Summary; g, MCS, Mental Component Summary; * $p < 0.05$, *** $p < 0.001$, NS vs. PS.

NS teachers with an active or highly active level displayed a significantly higher PCS score than their inactive counterparts ($p = 0.016$) and a sufficient level of physical health, whereas the MCS score was the same among the NS teachers, independently by the level of physical activity. The PCS score of the PS teachers did not significantly change according to the physical activity level.

Stratifying the sample according to weight status (see Table S1, Supplementary Materials), the only statistically significant difference was found to be for the PCS score in the NS teachers between normal weight and obese groups ($p = 0.041$), whereas, when stratifying the sample according to educational levels (see Table S2), only the MCS score in the NS showed a significant difference between elementary and middle/high school levels, with $p = 0.008$. Tables S1 and S2 are included among the Supplementary Materials due to the few significant observed. Stratification by gender (F/M) was not taken into consideration since men were only 3.8%.

Table 1.15. Mediterranean Diet adherence, perceived physical health and mental health by physical activity level and education teaching grade classes.

	Inactive			Sufficiently Active			Active or Highly Active		
Scores	**TS [a]**	**NS [b]**	**PS [c]**	**TS [a]**	**NS [b]**	**PS [c]**	**TS [a]**	**NS [b]**	**PS [c]**
MD [d] adherence	8.1 ± 1.7 **	8.0 ± 1.5 **	8.1 ± 1.9	8.6 ± 2.0 °	8.8 ± 2.1 °	8.4 ± 1.9	9.3 ± 1.8 **,°	9.9 ± 1.7 **,°,#	9.0 ± 1.7 #
PCS [e]	46.3 ± 8.5 **	44.3 ± 8.4 *	48.5 ± 8.1	49.12 ± 7.8 °	49.1 ± 7.5	51.2 ± 8.0	51.3 ± 6.4 **,°	50.24 ± 7.02 *	51.9 ± 5.9
MCS [f]	49.9 ± 9.3	50.6 ± 8.6	49.0 ± 10.2	51.4 ± 8.5	49.2 ± 8.2	51.5 ± 9.0	52.4 ± 7.1	53.6 ± 6.5	51.7 ± 7.4

Data are presented as means ± SD; [a], TS, Total Sample; [b], NS, Nursery School; [c], PS, Primary School; [d], Mediterranean Diet; [e], PCS, Physical Component Summary; [f], MCS, Mental Component Summary; * $p < 0.05$; ** $p < 0.001$ ***, in Total sample, NS and PS, Inactive vs. Active/Highly Active, while ° $p < 0.05$; Sufficiently Active vs. Active/Highly Active; # $p < 0.05$;NS vs. PS for Active/Highly Active.

The correlation analysis between binary variables is shown in Table 1.16. Even though low values of Spearman correlation coefficients were found, PCS was significantly correlated to the following: school teaching grade, with primary teachers showing high perceived physical health ($p < 0.05$); weight status, with overweight or obese teachers perceiving lower PCS ($p < 0.05$); MD adherence, with those adherents to MD having higher physical well-being ($p < 0.05$); PAL, with active teachers showing higher PCS ($p < 0.01$). For mental health, only weight status was found to be correlated to MCS, with overweight/obese teachers showing lower mental well-being ($p < 0.05$).

Table 1.16 Spearman's correlation coefficients between perceived physical/mental health and the considered study variables in the sample of schoolteachers (N = 265).

	PCS [a] (Low–High)	**MCS [b] (Low–High)**
	Spearman's Rho	
School teaching grade (nursery-primary)	0.117 *	−0.012
Educational Level (secondary-graduate)	−0.038	0.031
Age (younger-older)	0.069	−0.004
Weight status (normal-overweight/obese)	−0.132 *	−0.139 *
MD [c] adherence (non adherent-adherent)	0.118 *	0.029
PAL [d] (inactive-active/very active)	0.170 **	0.104

[a], PCS, Physical Component Summary; [b], MCS, Mental Component Summary; [c], MD, Mediterranean Diet; d, PAL, Physical Activity Level; * $p < 0.05$; ** $p < 0.01$.

The logistic regression analysis results for PCS and MCS are shown in Table 1.17.
Active teachers are more likely to perceive good physical health (OR 2.10, 95%CI 1.05–4.2, $p = 0.035$). Even though not statistically significant, a trend exists ($p < 0.10$) for school teaching grade and weight status: primary schoolteachers have a higher probability of physical well-being (OR 0.48, 95%CI 0.33–0.78 $p = 0.020$) compared to nursery school teachers; overweight and obese teachers have almost double the risk of perceiving low physical health (OR 0.59, 95%CI 0.33–1.03, $p = 0.062$) compared to normal-weight teachers.
With regard to mental health, the only predictor of MCS was confirmed to be weight status, with overweight and obese teachers showing a double probability of perceiving low mental health (OR 2.18, 95%CI 1.13–4.18, $p = 0.020$). A trend is shown for active teachers who have almost two-times the odds of perceiving good mental health (OR 1.89, 95%CI 0.93–3.83, $p = 0.077$).

Table 1.17 Logistic regression results on PCS and MCS in relation to all qualitative variables.

	PCS [a] (Base Outcome Low vs. High)			**MCS [b] (Base Outcome Low vs. High)**		
	OR[c]	**95% CI [d]**	***p*-Value**	**OR[c]**	**95% CI [d]**	***p*-Value**
School teaching grade						
base outcome nursery vs. primary	1.59	0.93–2.72	0.092	0.98	0.55–1.74	0.948
Education						
base outcome primary/secondary vs. graduate	1.06	0.60–1.85	0.848	1.09	0.61–1.95	0.763
Age						
base outcome younger vs. older	1.03	0.99–1.07	0.124	0.92	0.54–1.60	0.783
Weight status						
base outcome normal vs. overweight/obese	0.59	0.33–1.03	0.062	0.48 *	0.33–0.78	0.020
MD [e] adherence						
base outcome non adherent vs. adherent	1.47	0.85–2.56	0.170	1.10	0.62–1.96	0.735
PAL [f]						
base outcome inactive vs. active/very active	2.10 *	1.05–4.20	0.035	1.89	0.93–3.83	0.077

[a], PCS, Physical Component Summary; [b], MCS, Mental Component Summary; [c], OR, Odds Ration; [d], CI, Confidence Interval; [e], MD, Mediterranean Diet; [f], PAL, Physical Activity Level; * $p < 0.05$.

Discussion

In this study, we aimed to investigate the differences in the lifestyle habits of nursery and primary school teachers, focusing on their perceived physical and mental health in relation to sociodemographic aspects, weight status, PALs and MD adherence in the months following the COVID-19 lockdown. Knowing these aspects is important to be able to identify whether or not the risk factors for the onset of chronic noncommunicable diseases associated with lifestyle determinants have changed as a result of the lockdown. The aim of acquiring these data is to improve the quality of life of this category of workers by identifying appropriate intervention strategies. Therefore, we carried out this cross-sectional study to investigate these aspects that contribute to determining the lifestyle of this class of workers, as previously shown in the literature (360). According to Gomes et al. (379), teachers' positive or negative experiences with physical activity can influence their propensity to create an educational climate that promotes PA and active lifestyles in schools.

In our study, 83.4% of teachers showed a level of physical activity ranging from sufficiently active to active or very active. This high percentage is in contrast to those reported in several studies conducted in other countries on samples of public-school teachers before (354, 356, 380) and after lockdown (364). In fact, these studies emphasize the wide prevalence of sedentary behaviors in this category of workers and show that inadequate levels of physical activity (356) predispose one to an increase in cardiovascular risk factors (354) and the appearance of musculoskeletal disorders with the onset of chronic pain (380). These findings suggest the need to promote PA both during and outside working hours. In particular, we found a significantly higher PAL in primary school than nursery teachers. These results could be explained by the kind of work activity performed. According to some evidence in the literature, this category of worker may be among those engaged in non-sedentary work activities, considering that 95% of work activities are conducted in the orthostatic position, thus maintaining a prolonged isometric contraction to oppose the force of gravity (354, 381). This may explain the high levels of physical activity of the teachers in our study. Moreover, our results might be explained by the high percentage of women (96.2%) present in our sample, who are considered to be more active compared with men as they are also employed in carrying out domestic activities (359). Our sample can be considered representative of the reference professional category; indeed, in Sicily, among 10.861 nursery schoolteachers, only 142 are men, and among 25.949 primary school teachers, only 1.154 are men. This datum indicates that there is a social propensity of women and men to prefer or not prefer this professional career (382).

The prevalence of women in this occupational field is a peculiar feature and is attributed to the tendency, since the 20th century, for women to enter the workforce, during a historical period when teaching was regarded as an extension of domestic activities (359). Furthermore, higher levels of physical activity, especially in women, were associated with a higher work capacity (383), so in this regard, our results are encouraging. Nevertheless, it should also be considered that, in our study, the teachers, by voluntary joining a training course on the promotion of physical activity, actually expressed a personal interest in this educational content that might have influenced our results. However, all teachers included were from educational backgrounds, different in degrees of exercise and sports sciences.

A recent study reported a negative relationship between BMI and food intake in schoolteachers. In particular, less physically active teachers had unhealthy food habits (360). The COVID-19 pandemic seems to have influenced this population of workers by favoring or hindering the intake of unhealthy foods (336). In our study, in both groups (NS and PS), almost 30% of teachers were overweight and obese, and BMI was negatively correlated with moderate physical activity (MA). These results are confirmed by other studies (354, 357, 383).
Regarding the relationship between PALs and MD adherence, in our study, teachers with a level of active or highly active PA had moderate to fair adherence to the Mediterranean Diet (score ≥ 9) compared to those who were sufficiently active or inactive in all groups with a lower MD adherence group (score < 9) (Table 3). This is in agreement with the results of other studies, in which teachers who adopted sedentary behaviors had unhealthy eating patterns compared to their more active colleagues (336, 350, 382). It should be emphasized that the results of adherence to the Mediterranean Diet achieved by our sample were sufficient, and these results could explain the high percentage of overweight and obese individuals. Indeed, the normal-weight teachers tended to have better adherence to the Mediterranean Diet compared with their overweight and obese counterparts. These findings are confirmed by recent studies on this population suggest increasing levels of physical activity, being careful with eating habits and proposing strategies for reducing levels of work-related stress (342, 384).
A recent study evaluated the effect of teachers' prejudice on diet and exercise and how teachers' opinions can influence children's behaviors. Nevertheless, these results might be hindered by the health level of teachers, which greatly affects the quality of teaching and, thus, the academic performance of students and their learning (385).
Comparing the two groups of nursery and primary teachers, statistically significant differences were found for the variables Total MET, PCS and educational levels. In particular, PS teachers presented higher levels in energy expenditure, perceptions of their own physical health and education compared to NS. Due to the lack of papers in the literature on the comparison of these two groups, it is difficult to provide argued comments on this issue. We have found just one study targeted on nursery school teachers assessing PA level by gender (386), where higher levels of PA in male workers have been demonstrated, suggesting that these could positively affect the PA of children. Thus, to our best knowledge, our study might be the first to assess the possible differences between these two groups of workers and, therefore, provide a new contribution to the scientific literature.
Moreover, primary school teachers showed a correlation with perceived physical health, and although this relationship was not significant in the logistic regression, a trend remained. We hypothesize that the difference concerning Total MET and PCS between the groups might be due to the higher school activity

level of PS teachers than NS counterparts during the lockdown period, as online lessons were envisaged for the primary school sector and not for the nursery.

In our study, the primary school teachers had higher levels of education than the nursery school group; however, no statistically significant difference emerged when stratifying the sample by levels of education (secondary and degree) in relation to the PALs. Pirzadeh et al. also found no correlations between education levels and PA levels, suggesting that this result could be explained by a lack of specific knowledge on the topic (387). Not having specific knowledge about a healthy lifestyle might actually limit its dissemination by teachers and, at the same time, influence one's lifestyle significantly (348). Considering that our population of teachers was formed in other educational fields and not exercise and sport sciences, the lack of specific knowledge might have influenced the results of the IPAQ and SF-12 questionnaires, leading to an overestimation of physical activity levels as well as physical and mental health. In addition, regarding the education level, a recent study (388) on preschool teachers found that the level of education possessed by teachers could influence the PA level of teachers, emphasizing that teachers could be role models for children.

The analysis of possible factors correlated to perceived physical and mental health revealed that weight status was significantly correlated to both PCS and MCS, with overweight/obese teachers perceiving lower physical and mental health; when these variables were included in a logistic regression model, the association was not confirmed for PCS, even though a kind of trend exists, while it was confirmed for MCS, suggesting that maintaining or reaching an optimal weight status could significantly influence the psychological health status and, in particular, mental well-being.

PCS was significantly correlated to MD adherence in the correlation analysis, with those who were not adherent to MD having lower physical well-being, but this association was not confirmed in the regression model.

PCS was finally correlated to PAL, and this relation was highly significant in the regression model, with active teachers having a double probability of perceiving their higher physical health. Even though the association between MCS and PAL was not evidenced, a trend exists. This result could be explained, in contrast with the previous literature, by considering teachers' mental well-being as the result not only of physical activity levels and eating habits but also as the result of factors such as the quality of the work environment (389). PA appears to be able to positively influence teachers' perceptions on physical and mental well-being, and, conversely, teachers with healthy eating habits tended to carry out a greater amount of weekly PA and perceive greater physical health (390).

The results obtained from the logistic regression analysis confirm that more active teachers tend to perceive greater physical and mental well-being, regardless of their BMI. Our results are, in part, in line with other studies, where factors, such as teachers' age, PALs and diet, are associated with perceived levels of physical and mental well-being (391). In several studies, the physical and mental well-being of teachers is rated lower than that of the general population due to the levels of physical and mental stress associated with the work activity conducted (391, 392). Higher PALs are associated with better perceptions of their own well-being, but in the case of female teachers, the contrary is also true. Indeed, the presence of dual commitments, work and domestic duties negatively affects the perception of overall health, predisposing to a higher level of stress and increased absenteeism compared to male colleagues (390). In addition, increased levels of stress, anxiety, depression and sleep disorders among teachers also appear to have occurred as a result of the COVID-19 pandemic (337, 343).

These results together confirm the need for increasing physical activity interventions targeted to teachers and promoting physical activity practice as a well-being means to achieve optimal psychological health status.

A limitation of this study is that the use of a self-reported questionnaire to collect information on the amount of physical activity performed by teachers has objective limitations that could be overcome by using inertial accelerometers. Also, weight and height that were self-reported and not directly measured contribute to limiting the study results, since the BMI and weight status categories could be biased. Moreover, another limit is that the sample was almost entirely composed of women. In addition, the sample size should be increased by extending the study to the whole national territory.

Conclusions

In consideration of the importance of the institutional role occupied by preschool and primary school teachers, assessing all those aspects that may affect the welfare of this category of workers is notable. Psychological factors may influence the implementation of sedentary behaviors, and the food choices of teachers should be considered in the intervention planning to motivate and promote the acquisition or maintenance of healthy lifestyles. Weight status seems to improve physical and mental well-being. Increasing knowledge and awareness about exercise and nutrition in this school population might contribute to a change from unhealthy lifestyles. A conspicuous body of literature has focused on health promotion interventions for students, but these interventions do not specifically include teachers. The results of the present study give hope because they show a category of workers with acceptable levels of physical and mental health, despite the difficult historical period experienced. It would be advisable to provide workplace health promotion interventions that would include both students and teachers. Integrating teachers into health promotion interventions in schools could lay the foundation for creating an educational climate that promotes healthier lifestyles.

3.2 "Evaluation of Work-Related Stress on Healthcare Operators using Cardiovascular Variability Indices from Commercial and Portable Multisensor Systems"

Introduction

Stress is a complex, multifaceted physiological response that enables the body to cope with environmental or internal challenges. While acute stress responses are essential for survival, sustained or chronic exposure to psychological and social stressors, such as those encountered in high-demand professions, can lead to adverse physiological outcomes and long-term health deterioration (393, 394). The autonomic nervous system (ANS) is strongly affected by stress, and a chronic dysregulation can produce a cascade of effects, including cardiovascular dysfunction, metabolic disease, immune suppression, and increased susceptibility to mental health disorders (395, 396).

Healthcare professionals, particularly nurses working in rotating shifts, are highly vulnerable to this kind of cumulative stress (397, 398) since continuous exposure to irregular schedules, long shifts, and emotionally intense environments often translates into heightened allostatic load and disrupted homeostasis (399), which have been associated with increased risk of obesity, sarcopenia, and systemic inflammation (400, 401). Understanding and monitoring the physiological impact of stress in such populations is crucial for early detection, prevention, and intervention (398).

In recent years, non-invasive wearable technologies have enabled the continuous monitoring of stress-related biosignals. Heart Rate Variability (HRV), assessed from electrocardiographic R-R intervals (RRI), has emerged as a robust biomarker for autonomic balance and stress-induced physiological changes (395, 402). HRV indices, spanning time, frequency, and information- theoretic domains, reflect the interplay between sympathetic and parasympathetic nervous activity, and offer insights into an individual's adaptive capacity under both resting and stress conditions (402-404). The growing use of photoplethysmography (PPG) in wearable sensors has made Pulse Rate Variability (PRV) a viable alternative to HRV. While PRV and HRV share conceptual overlap, they differ in signal origin and sensitivity to artefacts, necessitating validation when used interchangeably for stress monitoring (402, 404, 405). The integration of PPG-derived indices combined with other metrics extracted from different biosignals can allow for assessing stress-related changes across multiple physiological systems, within a network physiology framework (406, 407).

This study aims to investigate the autonomic nervous system response to stress, exploiting cardiovascular variability and signal complexity measures. Focusing on a population of healthcare workers, particularly vulnerable to chronic occupational stress, we evaluate how stress modulation strategies affect autonomic function across different physiological conditions. By combining traditional HRV indices with entropy-based measures rooted in information theory, we focus on capturing both the magnitude and the structural nuances of autonomic regulation under stress, allowing for a dynamic assessment of physiological adaptation over time. This integrative approach aligns with the principles of Network Physiology, offering a multidimensional perspective on how internal system interactions evolve in response to psychological and physiological challenges (406). In detail, the aim of the study is threefold: (a) to comprehensively characterize autonomic nervous system responses to a structured wellness intervention for work related stress by analyzing cardiovascular variability through HRV and PRV indices across time, frequency, and information domains; (b) to assess the reliability and applicability of a custom-developed multi- parametric acquisition system in operational environment against a commercial clinical-grade device to characterize the autonomic nervous system responses to controlled breathing; (c) to investigate whether HRV-derived measures in time, frequency and information-domain could serve as robust markers for detecting stress-related modulation in autonomic function. By focusing on a high-risk occupational population and leveraging recent advances in wearable technology and cardiovascular signals analysis, this study holds potential implications for improving the early detection of work-related stress responses through portable and wearable devices directly in workplace settings.

Materials and Methods

Population and Data Collection

This observational study enrolled 60 shift-working male and female nurses employed at the A.R.N.A.S. Ospedali Civico Di Cristina Benfratelli Hospital in Palermo, Italy. Participants were selected based on their demanding work schedules, which are known to elevate the risk of stress-related and metabolic disorders. Inclusion criteria required participants to be between 40 and 65 years of age, actively employed in shift work, and free from any acute or chronic medical conditions at the time of enrolment. All participants provided written informed consent after receiving comprehensive information about the study's objectives, procedures, and data protection measures. The study protocol was approved by the Ethics Committee of the A.R.N.A.S. Civico Hospital (prot. 59/2023) and conducted in accordance with the Declaration of Helsinki.

To evaluate the impact of lifestyle interventions, the entire cohort (ALL) was divided into two groups of 30 individuals. The intervention group (INT) participated in a structured program comprising educational seminars, nutritional counselling, and guided physical activity, whereas the control group (CONT) received only general theoretical information without practical support. Data collection was performed at baseline (PRE) and after approximately a six-month follow-up (POST), allowing for longitudinal assessment of physiological parameters and body composition changes.

Participants completed two validated questionnaires to assess perceived health and sleep quality at baseline: the SF-12 Health Survey and the Pittsburgh Sleep Quality Index (PSQI). The SF-12, a short version of the SF-36 Health Survey (408) provides two summary indices, the Physical Component Summary (PCS12) and the Mental Component Summary (MCS12), calculated according to Ottoboni et al. (377) and interpreted using normative data from Andersen et al. (409). The PSQI (410) evaluates sleep quality through seven components (COM 1–7), whose sum yields a global score from 0 to 21, with higher scores indicating poorer sleep quality.

Descriptive characteristics and results of questionnaires (i.e., PCS12, MCS12 and PSQI scores) for both groups at PRE are reported in Table 1. With regard to PCS12 and MCS12, an independent samples t-test was performed to assess baseline differences between CONT and INT group, after verifying normality of data distribution using the Shapiro–Wilk test. The analysis did not reveal any statistically significant differences between groups at the start of the intervention. Both groups exhibited a physical health comparable to general population according to (409), but a mental health score lower than general population, compatible with a condition of work-related stress.

Two biomedical acquisition systems were employed during the study. The first system, herein referred as the Multi Parametric Sensor Acquisition System (MPSAS), described in detail in Section 2.1.1, was developed at the University of Palermo, and enables high-resolution, synchronous acquisition of electrocardiographic (ECG), photoplethysmography (PPG), and respiratory (RESP) signals. Using this system, the PPG signal was acquired on the left middle finger.

Table 1.18. Descriptive characteristics of groups

	CONT	INT
Age (years)	51.13±5.96	54.42±5.98
Weight (Kg)	68.58±17.73	68.92±11.32
Height (cm)	164±0.09	165±0.06
BMI (Kg/m²)	25.18±5.04	25.33±3.84
PCS12	49.39±7.77	51.64±8.21
MCS12	42.74±10.17	43.52±10.16
Global PSQI	10.21±3.51	8.73±1.73
Component 1	1.16±0.76	1.00±0.89
Component 2	1.12±1.11	1.03±0.95
Component 3	1.37±0.82	1.23±0.81
Component 4	3.00±0.00	3.00±0.00
Component 5	1.54±0.77	1.23±0.51
Component 6	1.29±0.69	0.84±0.46
Component 7	0.70±0.90	0.38±0.63

Data are present ad means ± std.dv; CONT: Control group; INT: Intervention group; BMI: body mass index; PCS12: Physical Component Summary; MCS12: Mental Component Summery; PSQI: Pittsburgh sleep quality index questionnaire; Component 1: Subjective sleep quality; Component 2: Sleep latency; Component 3: Sleep duration; Component 4: Sleep efficiency; Component 5: Sleep disturbance; Component 6: Use of sleep medication; Component 7: Daytime dysfunction.

The second system was the PPG Stress Flow, a non-invasive medical device developed by BioTekna (Venice, Italy), employing multichannel photoplethysmography technology applied to the distal extremities (left and right index fingers) for enabling an in-depth analysis of autonomic nervous system (ANS) activity and HRV.

The acquisition protocol required only a few minutes of recording per subject and aimed at monitoring the autonomic regulation to provide valuable insights for the differential diagnosis of stress-related disorders. Each assessment session included two 5-minute recordings with participants seated: (i) the first during spontaneous breathing (REST), (ii) the second during a guided breathing exercise (CB), encompassing a 5-second inhalation, a 2-second pause, and a 5-second exhalation (i.e. 5 respiratory cycles per minutes), able to induce an effective improvement in mood and stress (411).

Description of the Multi Parametric Sensor Acquisition System

The developed system is based on the solutions described in several articles (412, 413) and comprises three primary sub-parts: an STM32H743 microcontroller unit (MCU) mounted on the Nucleo-144 development board, a fully customized pin-to-pin compatible expansion printed circuit board (PCB) coupled with the Nucleo, for handling signal conditioning and acquisition, power management, measurement connectors, and, lastly, the probes used for bio signal acquisition. The first probe consists of a 10-wire ECG cable with a DB15M connector compliant with CEE 93/42, class 1, utilizing only the RA (Right Arm), LA (Left Arm), LL (Left Leg), and RL (Right Leg) derivations to acquire a standard three-lead ECG. The second probe type, illustrated in Figure 1b, is a customized PPG probe designed to fit on the index or middle finger, ensuring optimal performance for PPG data collection and transmission. Various configurations were tested to determine the most effective arrangement of the LED diode and the photodetector. The finalized probe features a single LED diode operating at 940 nm, controlled by a dedicated digital driver. This design allows measurements on the fingers in transmission mode. The LED emits continuous light with adjustable intensity, controlled via a dedicated graphical user interface that also allows system initial setup and real-time signal monitoring. The light detector, an OPT101 module from

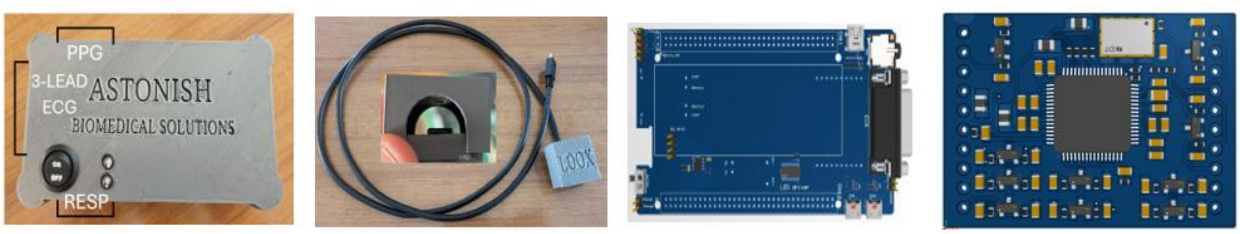


Figure 1.19 Photographic and schematic overview of the acquisition system. (a) Portable acquisition case enclosing the MPSAS system. (b) PPG finger probe used for peripheral pulse detection. (c) 3D rendering of the custom shield board PCB. (d) 3D rendering of the custom acquisition PCB integrating the ADS1298 analog front-end and its supporting circuitry.

Texas Instruments features a low-noise, single-supply monolithic photodiode with a 5.2mm2 active area and sensitivity of 0.5µV/µW at 940nm. It is also capable of signal conditioning using a transimpedance amplifier with an embedded 1M Ω feedback resistor and an 11pF capacitor. The probe connects to the main board via a shielded micro-B to USB-A cable.

To protect the ESD-sensitive circuits, each probe connector is properly equipped against ESD, in compliance with IEC 61000-4-2. The custom expansion board is designed to match the dimensions of the Nucleo-144, enabling the entire system to fit into a compact 3D-printed PLA case measuring 140.00mm *

72.00mm * 52.00mm 1a. Figure 1c illustrates the top view of the developed board: the longer edges of the PCB are dedicated to the Nucleo-144 standard morpho connectors, which provide access to all MCU used pins. The connectors for external charging and the main power switch are on the left, while connectors for various probes are on the opposite side. These include two micro-B shielded USB ports for PPG data, a mini-B USB port for the respiratory signal, a 3.5mm mini-jack reserved for future development, and a D-Sub 15-pin connector for the ECG probe. The central area of the PCB is reserved for a 3.7V, 3700mAh lithium-ion battery, powering the device for approximately six hours of continuous operation. To minimise EMI, the placement of noise-sensitive components was prioritized during the design phase by positioning them on the PCB underside, which also incorporates separate analog and digital ground planes as inner layers. Analog traces were routed on the bottom layer for optimal shielding.

The system uses the ADS1298 IC, a 24-bit, low-power analog front-end from Texas Instruments, serving as the primary integrated circuit for biopotential measurements (ADS1298, 2015). This IC is mounted on a dedicated custom PCB 1d, where each channel is further protected against overvoltage and ESD in accordance with JESD22-A114 HBM-2B (414) and equipped with a dual-stage low-pass passive filter with a 100Hz cutoff frequency. This configuration ensures readiness for future expansions. The PCB design followed IPC-2221 guidelines for layout, ensuring compliance with trace width, spacing, and other design criteria. The final board was verified for compatibility with IPC-A-600 standards, covering aspects such as thickness, hole size, and soldering quality.

Signal processing, time series and indices extraction

ECG and PPG signals were synchronously recorded using the MPSAS system at a sampling rate of 1 kHz. The ECG signal was filtered using a zero-phase 4th-order Butterworth bandpass filter with cut-off frequencies of 0.1 Hz and 20 Hz, allowing for the removal of baseline drift and high-frequency noise while preserving QRS morphology. R peaks were detected using a simplified version of the Pan-Tompkins

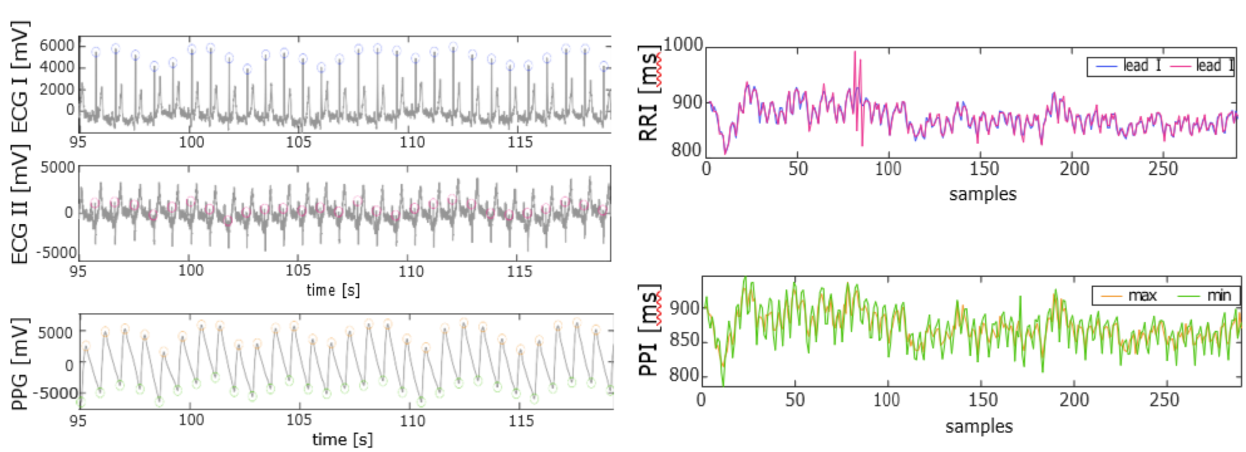


Figure 1.20 Example of signals and time series extraction. Left panel (a) shows representative ECG traces recorded from lead I and lead II, with detected R peaks marked with circles, and the PPG waveform, where the maxima and minima are identified respectively as orange and green circles. From these peak positions, beat-to-beat interval series were derived: R-R intervals (RRI) from the ECG and pulse-to-pulse intervals (PPI) from the PPG. The right panel (b) shows the resulting time series, which were used for HRV and PRV indices computation.

algorithm (415), and R-R intervals (RRI) were calculated as the temporal difference between successive R peaks, for both ECG leads, as shown respectively in blue and pink in 2a and 2b. The PPG signal was filtered using a zero-phase 4^{th}-order passband Butterworth filter with cut-off frequencies of 0.1 Hz and 15 Hz, to suppress motion artefacts and enhance the pulsatile component. Pulse-to-pulse intervals (PPI) were extracted by detecting local maxima and minima of the PPG waveform, as shown respectively in orange and green in Figs. 2a and 2b. Since the distributions of R-R interval time series extracted from the two ECG leads and between the P-P interval time series computed from minima and maxima of PPG signal did not show any statistically significant differences according to t-test, only the ECG lead I and the maxima PPI time series were retained for the subsequent analyses.

For each physiological condition (i.e. REST, CB), 300-point-long time series (5 min of the original signal) were extracted, and from these time-, frequency- and information-domain cardiovascular variability indices were computed.

To define the adopted measures, let us first denote X_n as the random variable obtained by sampling the stochastic process X at the present time n. Under the assumptions of stationarity and ergodicity, each finite-length time series can be regarded as a realization of the underlying process, so that statistical properties can be consistently estimated from a single realization of X. Moreover, in order to approximate the infinite past history of the process, it is customary to assume that X behaves as an Markov process of order m, whereby the relevant dependence of the present state X_n on its history can be captured by the finite-dimensional vector $X^m_N = [X_{n-1}, X_{n-2}, \dots, X_{n-m}]$ describing its past m states.

Time-domain indices included the mean inter-beat interval (MEAN), mean heart rate (Mean HR), the standard deviation of normal-to-normal intervals (SDNN), and the root mean square of successive differences (RMSSD), which are defined respectively as follows (Shaffer and Ginsberg, 2017):

$$MEAN = \frac{1}{N}\sum_{n=1}^{N} X_n, \tag{1}$$

being X_n the n-th RR sample and N the total number of heartbeats analyzed,

$$MeanHR = \frac{60}{MEAN}, \tag{2}$$

$$SDNN = \sqrt{\frac{1}{N-1}\sum_{n=1}^{N}(X_n - MEAN)^2}, \tag{3}$$

$$RMSSD = \sqrt{\frac{1}{N-1}\sum_{n=1}^{N-1}(X_{n+1} - X_n)^2}. \tag{4}$$

Time-domain indices quantify different aspects of cardiac variability: the MEAN represents the average heartbeat duration (while the Mean HR is its inverse), providing a direct measure of cardiac activity. The SDNN reflects overall heart period variability, capturing both sympathetic and parasympathetic influences, whereas the RMSSD specifically quantifies short-term variability primarily linked to parasympathetic modulation (395).

Prior to the computation of frequency- and information-domain indices, all-time series underwent a preprocessing stage to enhance stationarity. Slow baseline fluctuations were attenuated using a zero-phase high-pass IIR filter with a cutoff frequency of 0.015 Hz. The series were then demeaned by subtracting their average value. To ensure stationarity, each segment was examined by verifying weak stationarity conditions and by checking that mean and variance remained stable across shorter sub-windows. Subsequently, spectral analysis was performed by fitting the preprocessed data with an autoregressive (AR) model of the form (404):

$$X_n = \sum_{m=1}^{p} a_m X_{n-m} + W_n \tag{5}$$

where the coefficients α_m describe the linear dependence of the present observation of the time series, Xn, on its previous samples X_{n-m}, with delays m = 1, . . . , p. The parameter p denotes the model order, while W_n represents a Gaussian white noise process with zero mean and variance σ^2 . The model parameters were estimated using the ordinary least squares approach, fixing the maximum model order to p = 10, according to previous studies (404). The power spectral density of the autoregressive process was then derived in the frequency domain from the estimated AR coefficients as:

$$P(\Omega) = \frac{\sigma_W^2}{|A(\Omega)|^2} \tag{6}$$

where $A(\Omega) = 1 - \sum_{m=1}^{p} \quad a_m z^{-m}$is the polynomial representation in $z = e^{j2\pi f T_s}$domain, being $T_s$the sampling period, $\Omega = 2\pi f T_s$the normalized sampling frequency and $j = \sqrt{-1}$. Power spectral density was computed by integrating the power over standard frequency bands: very low frequency (VLF: 0.003–0.04 Hz), low frequency (LF: 0.04–0.15 Hz, primarily reflecting sympathetic activity), and high frequency (HF: 0.15–0.4Hz, associated with parasympathetic activity and respiration). In addition to the absolute power values, normalized values (VLF%, LF%, HF%) were calculated by dividing the absolute power within the band by the sum of the absolute power values in the three bands, to assess the relative contribution of each band to total power. The LF/HF ratio was also computed to provide a measure of autonomic modulation, even if its use as an indicator of sympathovagal balance has been recently challenged (395).

To characterize the dynamical complexity of cardiovascular and respiratory signals, two information-theoretic indices were computed: entropy (H) and conditional entropy (CE). Entropy-based measures, such as CE, have been shown to be sensitive to varying levels of sympathetic activity during postural stress, effectively capturing changes in cardiac system dynamics associated with different physiological and clinical conditions (416, 417).

The Shannon entropy quantifies the information carried by the system at the current state and is defined as follows:

$$H = H(X_n) = -\mathbb{E}[\log p(x_n)], \tag{7}$$

where E[·] represents the expectation operator, p(·) is the probability density function, and x_n is a realization of X_n. To have insight into the dynamical evolution of the system, it is possible to quantify the new information carried by the present state X_n which cannot predicted from its past state X^m_n, through the Conditional Entropy (CE):

$$CE = H(X_n \mid X_n^m) = H(X_n, X_n^m) - H(X_n^m) = -\mathbb{E}\left[\log \frac{p(x_n, x_n^m)}{p(x_n^m)}\right] \tag{8}$$

Several approaches can be formulated for estimating the probability distributions $p(x_n, x_n^m)$ and $p(x_n^m)$ which, once obtained, can be plugged into Eq. (8) to retrieve an estimate for CE. In particular, in this work each measure was estimated using both a linear model and a non-parametric estimator. The linear estimator represents a model-based approach relying on the assumption that the analyzed variables follow a Gaussian distribution. Given a generic d-dimensional zero-mean Gaussian variable X_n^d, its probability density function is:

$$p(x_n^d) = \frac{1}{\sqrt{(2\pi)^d |\Sigma_{X^d}|}} e^{\left(-\frac{1}{2} x_n^{d\top} \Sigma_{X^d}^{-1} x_n^d\right)}, \tag{9}$$

where $\Sigma_x d = E[X_n^d X_n^{dT}]$ is the covariance matrix of X_n^d. For a stationary zero-mean Gaussian process X_n, the linear parametric representation is given by an AR model of order m as in Eq. 5. The entropy of the past vector X_n^m and of $X_{n+1}{}^m{}_{+1} = [X_n, X_n^m]$ can be computed as:

$$H_{\text{lin}} = \frac{1}{2} \ln(2\pi e |\hat{\Sigma}_{X^m}|), \tag{10}$$

where $\Sigma_x m$ is the estimated covariance matrix. Then, the conditional entropy is expressed in terms of the variance of the AR prediction error:

$$CE_{\text{lin}} = \frac{1}{2} \ln(2\pi e |\hat{\Sigma}_{X^{m+1}}|) - \frac{1}{2} \ln(2\pi e |\hat{\Sigma}_{X^m}|) = \frac{1}{2} \ln(2\pi e\, \hat{\sigma}_W^2), \tag{11}$$

where σ^2_W is the prediction error variance obtained after identifying the regression coefficients of the AR model via the least squares method, coinciding with the partial covariance of Σ_{xn} given Σ_{xn+1}, being and Σ_{xm+1} the estimated covariance matrix.

A widely used model-free approach for entropy estimation is based on nearest-neighbour (kNN). The underlying idea is that the probability density around a given sample can be approximated from the distance to its nearest neighbours.

Consider N − d + 1 observations of a d-dimensional continuous random variable X_n^d. For a fixed number k of neighbour samples, the local probability density around a point x_n^d can be expressed as (418):

$$p(x_n^d) = \frac{k}{(N-d)\, c_{d,L}\, \epsilon_{n,k}^d}, \tag{12}$$

where $\varepsilon_{n,k}$ is the distance (according to the maximum norm) between x_n^d and its k-th nearest neighbour, and c_d,L is the volume of the d-dimensional unit ball under the chosen norm.

To avoid biases introduced by combining entropies of variables with different dimensions (e.g. X_n^m and $X_{n+1}{}^{m}{}_{+1}$), we adopt the formulation of Kraskov, Stögbauer, and Grassberger (418). In this setting, the entropy and the conditional entropy are respectively estimated as

$$H_{\text{knn}} = \psi(N) - \psi(k) + \frac{1}{N}\sum_{n=1}^{N}(\ln \epsilon_n), \tag{13}$$

$$CE_{\text{knn}} = -\psi(k) + \frac{1}{N-m}\sum_{n=1}^{N-m}\Big(\ln \epsilon_{n,k} + \psi(N_{X_n^m} + 1)\Big), \tag{14}$$

where $\psi(\cdot)$ denotes the digamma function, ε_n is twice the distance from (x_n) to its k-th nearest neighbor in the one-dimensional space $\varepsilon_{n,k}$ is the maximum-norm distance of the point (x_n, x_n^m) to its k-th nearest neighbour in the (m + 1)-dimensional space, and $N_{xn}{}^m$ is the number of points whose distance from x_n^m is smaller than $\varepsilon_{n,k}/2$ in the m-dimensional space.

The two key parameters of the estimator are the embedding dimension m, which controls the reconstruction of the process history, and the number of neighbours k, which determines the trade-off between variance (too small k) and bias (too large k). In this work, the number of neighbours and the embedding dimension were set to k = 10 and m = 2, respectively (404, 416, 417). The choice of m = 2 balances the ability to capture dynamic information with the limitations of short physiological time series, while the selection of k = 10 has been shown to provide stable and reliable entropy estimates in HRV analysis (404, 416, 417).

Data collected by BioTekna PPG Stress Flow equipment were automatically processed by the device, extracting a wide set of cardiovascular and autonomic indices including, among others, the Mean HR, the SDNN, the RMSSD, spectral power value in VLF, LF and HF bands, the LF/HF ratio (in both linear and logarithmic scale), percentage indices of sympathetic and parasympathetic dominance (SNS LF%, PNS HF%), bilateral flow gain (BFG) (419) for each limb, and several respiratory-related indices (real-time, auto-set, and target breath).

Only a subset of the indices provided by the device was retained, i.e. the ones conceptually corresponding to those extracted on data acquired from the MPSAS system: Mean HR, SDNN, RMSSD, VLF, LF, and HF. The selected indices were exported digitally and used as input variables in subsequent evaluations.

It is important to note that the indices extracted from the PPG STRESS FLOW device are derived from proprietary, pre-processed signals. As the raw data are not available and the specific processing pipeline is not disclosed, it is not possible to reconstruct the exact computation procedures for these indices. This may introduce discrepancies when comparing results with the MPSAS system, since differences in filtering, peak detection, or spectral estimation could significantly influence the values of HRV parameters.

Data Analysis

The analyses were aimed both at the evaluation of physiological response to the experimental protocol using data from the MPSAS research device (details in Sect. 2.3.1) and to the comparison of the computed indices with those directly provided as an output from the commercial BioTekna PPG Stress Flow device (details in Sect. 2.3.2). Of the initial cohort, data from 58 participants (42 females) were retained for analysis, after excluding two participants due to poor signal quality. The average age was 50.0 ± 3.0 years. Body mass index (BMI) was 25.6 ± 4.56 kg/m² at PRE and 25.2 ± 4.36 kg/m² at POST.

Analysis of MPSAS-Derived Data

To prove that MPSAS system-derived indices can be exploited for distinguishing physiological variations affecting autonomic and respiratory regulation due to breathing and lifestyle interventions, analyses were performed on the full set of parameters extracted from the device. The analysis encompassed two within-subject conditions, spontaneous breathing (REST) and controlled breathing (CB), as well as two experimental phases, i.e. PRE and POST. Participants were stratified into intervention and control groups to evaluate group-specific effects.

For each index, the distribution of values computed on REST and CB were compared using the Wilcoxon signed-rank test, separately for the PRE and POST phases. Longitudinal differences between PRE and POST were assessed for each breathing condition using the same test. To examine differences between the INT and CONT groups, the Mann–Whitney U test (ranksum) was used for each condition and phase. All statistical tests were two-tailed, with a significance level set at $\alpha = 0.05$; for within-subject comparisons involving multiple indices, a more stringent threshold of $\alpha = 0.005$ was adopted.

This statistical pipeline enabled a comprehensive evaluation of autonomic and respiratory dynamics modulated by controlled breathing and the intervention program, based on all the metrics extracted from the MPSAS dataset.

Cross-System Validation with the PPG Stress Flow Device

To evaluate the agreement of the indices extracted from the MPSAS device and the BioTekna PPG Stress Flow system, a targeted cross-system validation analysis was performed. The analysis aimed to assess the agreement between systems under the same experimental conditions (REST and CB), in both PRE and POST phases, and across INT and CONT groups, taking into account the six autonomic indices computed by both systems: MEAN, SDNN, RMSSD, VLF, LF, and HF.

The comparison of the distribution of indices obtained using the two systems was carried out for each parameter using the Wilcoxon signed-rank test, separately for each respiratory condition and experimental phase. Bonferroni correction for multiple comparisons was used, with a conservative adjusted threshold of $\alpha = 0.005$ applied to detect significant differences. All comparisons were conducted on matched subjects with valid data from both systems. This statistical pipeline provided a detailed insight into the agreement between the two acquisition systems and contributed to the assessment of the potential interchangeability of these devices in autonomic stress monitoring applications.

Results

This section first presents the outcomes of the statistical analysis conducted on the physiological indices extracted from the MPSAS system. Results of the cardiovascular variability indices (extracted from RRI or PPI time series) in the three domains, i.e. time (Fig. 1.24), frequency (Fig. 1.22), and information-theoretic (Fig. 1.25), are analysed across the respiratory conditions (REST, CB), experimental phases (PRE, POST), and participant groups (ALL, CONT, INT). Statistically significant differences are indicated in the figures as follows: * denotes significant differences between REST and CB conditions within the same group, # between the CONT and INT groups, and § between PRE and POST phases within the same condition.

Figure 1.21 illustrates the distributions of MEAN, SDNN and RMSSD indices computed from RRI and PPI time series. No statistically significant variations were reported for MEAN index across experimental phases or respiratory conditions. On the other hand, across all subjects (ALL), both SDNN and RMSSD were significantly higher during CB compared to REST within the same phase, reflecting enhanced parasympathetic modulation during guided respiration.

Figure 1.22 presents the distributions of VLF, HF, and LF/HF, computed from both RRI and PPI series across phases, groups, and breathing conditions. Results for the LF power were excluded for brevity, as no significant effects were observed. The VLF showed a consistent decrease from REST to CB across all groups and phases, for both RRI- and PPI-based indices. This reduction reached statistical significance only during the PRE phase, particularly in the ALL and CONT groups. A similar decreasing trend was observed for HF, likely reflecting the concentration of respiratory modulation into a narrower frequency band under paced breathing, which may reduce spectral power within the traditional high-frequency range (0.15–0.4 Hz). The LF/HF ratio increased during CB compared to REST. A statistically significant difference between the CONT and INT groups was observed during the PRE phase, specifically under the CB condition and for the RRI-derived measures. Additionally, a significant increase from PRE to POST was detected in the INT group during REST, but only for the PPI-based indices. Overall, frequency-domain indices reflected coherent respiratory- and intervention-related modulations, while interpretation of HF and LF/HF must account for the spectral shifts induced by paced respiration.

Figure 1.23 shows the results of information-theoretic indices extracted from the RRI and PPI series, i.e., H_{lin}, H_{knn}, and CE_{lin}. Results for CE_{lin} were excluded for brevity, as no significant effects were observed. For all measures, a decreasing trend from REST to CB can be observed, with this trend being most significant for CE_{lin} at PRE and H_{lin} at POST, indicating higher regularity and lower complexity during paced respiration. No significant differences between PRE and POST were observed, except for H_{lin} of the PPI series, which showed a PRE-to-POST decrease in the INT group and POST-to-PRE increase in the CONT group, respectively. A significant group difference emerged for H_{knn}, observed between REST conditions in both RRI- and PPI-derived series.

Figure 1.24 summarizes the results of analysis carried out to evaluate the agreement between the MPSAS system and the PPG STRESS FLOW device, performed on the six HRV indices extracted and compared between systems (MEAN, SDNN, RMSSD, VLF, LF, HF) across respiratory conditions (REST, CB) and experimental phases.

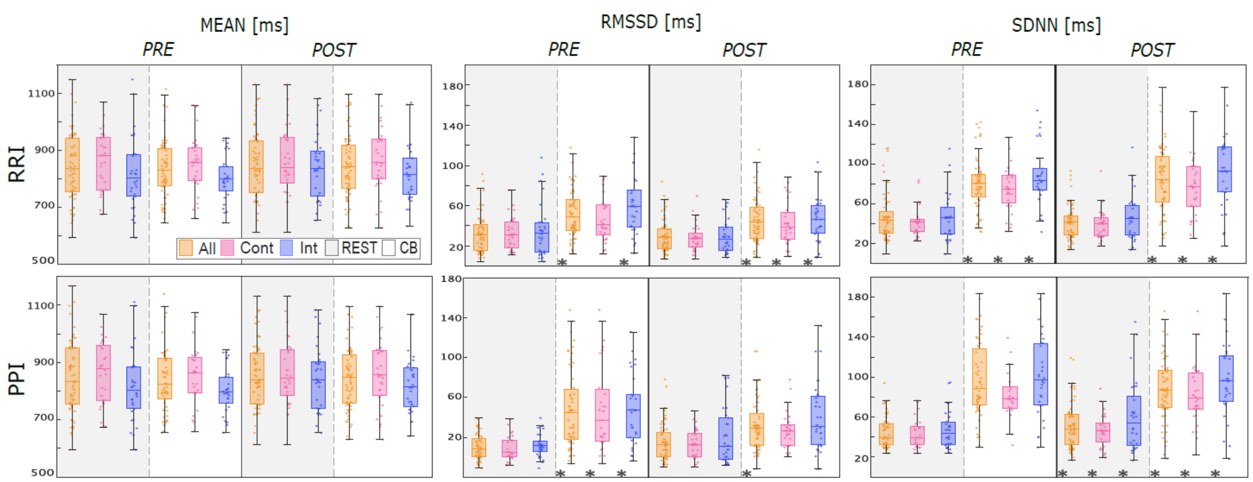


Figure 1.21 Time-domain indices (MEAN, SDNN, RMSSD computed from RRI and PPI series extracted using the MPSAS system. Results are reported for three groups: ALL (orange), CONT (magenta), and INT (blue), across both experimental phases (PRE, POST) and respiratory conditions (REST, CB). Results of statistical analysis: * denotes significant differences between REST and CB within the same phase and group according to Wilcoxon signed-rank test, # indicates differences between the CONT and INT groups

under the same phase and condition according to Mann-Witheny U test, and § highlights differences between PRE and POST within the same group and condition according to Wilcoxon signed-rank test. Bonferroni correction for multiple comparisons was used, with a conservative adjusted threshold of α = 0.05 applied to detect significant differences.

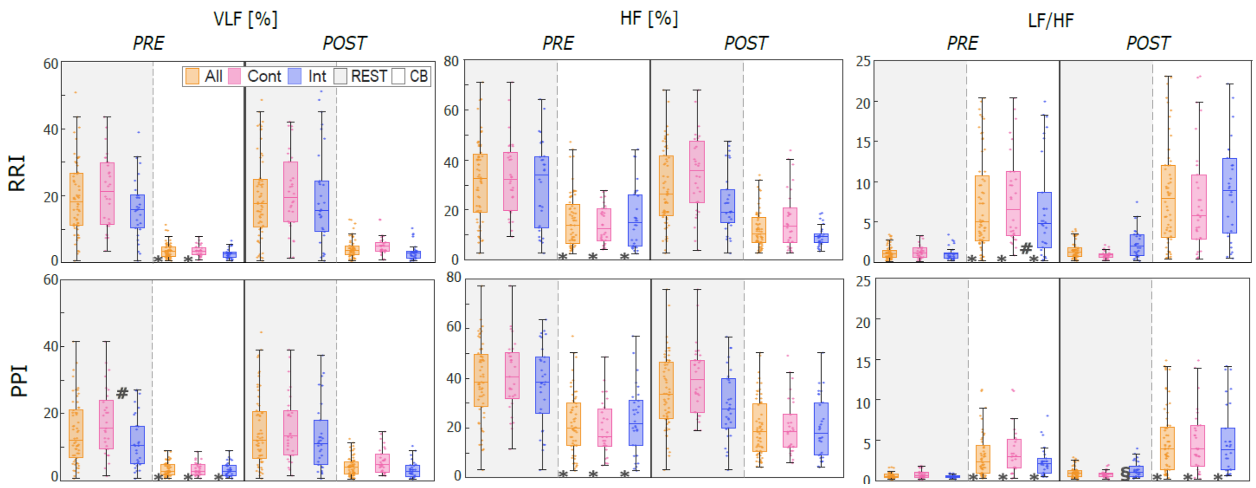


Figure 1.22 Frequency-domain indices (VLF [%], HF [%], LF/HF) computed on RRI and PPI series extracted from signals acquired with the MPSAS system. Same group and condition structure as in Figure 3.

POST). Notably, Mean HR did not show any statistically significant difference in any condition, confirming its consistency across acquisition modalities. In the PRE phase, statistically significant differences between the distributions were found for all the other parameters in the REST condition. In the CB condition, the only exception apart Mean HR was the VLF index, which did not report any statistically significant differences in PRE. In the POST phase, all five indices (apart Mean HR) exhibited statistically significant differences between the two systems under both REST and CB conditions. The above findings highlight

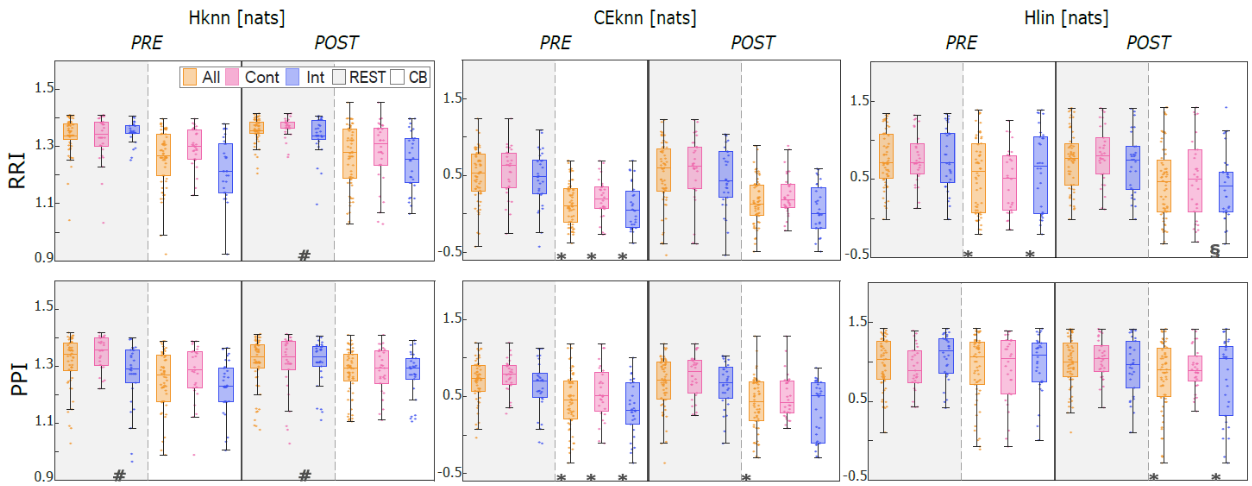


Figure 1.23 Information-theoretic indices (H_{lin}, H_{knn}, CE_{knn}) computed on RRI and PPI series extracted from signals acquired with the MPSAS system. Same group and condition structure as in Figure 3.

persistent methodological discrepancies, which may be further influenced by the effects of the intervention or changes in physiological variability.

While Mean HR appears robust across platforms, indices such as SDNN, RMSSD, LF, and HF are likely influenced by differences in time series type (RRI vs. PPI), peak detection, and processing pipelines. This suggests caution when interpreting absolute values across systems, especially for longitudinal or interventional comparisons.

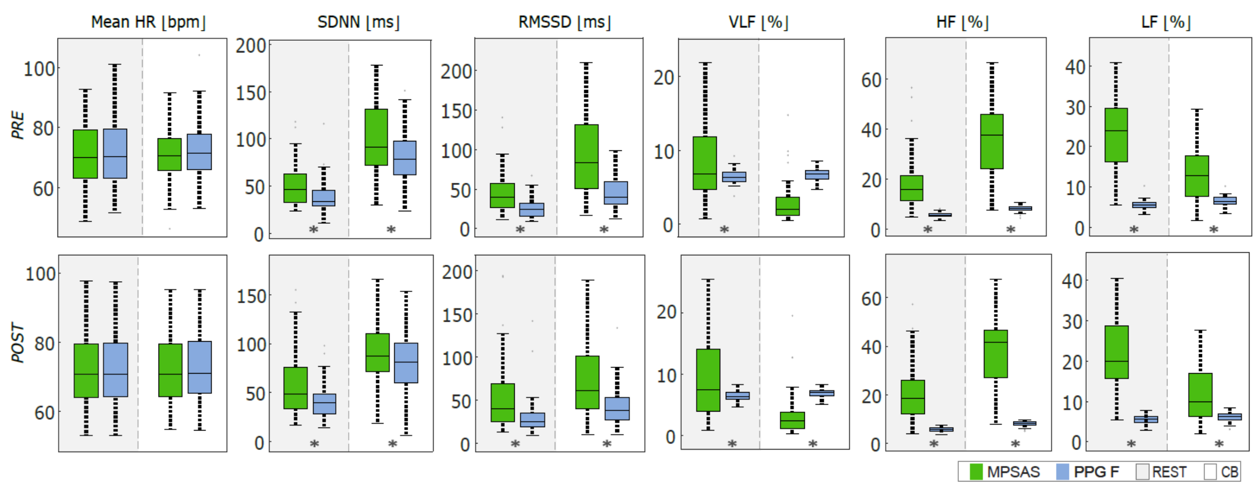


Figure 1.24 Comparison of HRV indices (Mean HR, SDNN, RMSSD, VLF, LF, HF) between the MPSAS system (in green) and the PPG STRESS FLOW device (in blue), across respiratory conditions (REST, CB)

and experimental phases (PRE, POST). Each panel shows distributions of indices computed from both systems. An asterisk indicates statistically significant differences between the two systems at the same condition (REST or CB), based on the Wilcoxon signed-rank test with Bonferroni correction ($\alpha = 0.005$).

Discussion

Heart rate variability (HRV) provides a powerful, non-invasive marker of autonomic regulation and its dynamic modulation in response to internal and external stressors. In the present study, we investigated short-term autonomic adaptations to controlled breathing and an experimental intervention targeting stress regulation, using a multimodal acquisition platform in a sample of healthcare professionals exposed to high work-related stress levels, as also demonstrated by the lower values of MCS12 scores if compared to general population. It is widely known that healthcare professionals, and in particular nursing staff, are frequently exposed to high levels of occupational stress due to organisational, emotional, and cognitive demands and a prolonged exposure to such stressors may contribute to autonomic imbalance, thus increasing the risk for cardiovascular and psychological disorders (420, 421).

Our findings confirmed that a biofeedback protocol encompassing a controlled breathing period elicits measurable increases in vagal activity, as reflected by elevated RMSSD during guided respiration. This finding aligns with previous literature highlighting the role of slow breathing in activating the parasympathetic system through baroreflex engagement and respiratory sinus arrhythmia (395, 422, 423). Such autonomic responses are functionally adaptive, promoting restoration and stress recovery. Moreover, the reduction of CE_{knn} confirms previous findings reporting that guided breathing predominantly reduces complexity (417), even if group-related effects are limited to baseline condition. Moreover, in PRE, the higher H_{knn} in REST for INT group compared to the CONT group suggests higher baseline entropy in autonomic regulation.

The reduction of VLF during CB compared with REST reflects the suppression of slow oscillatory components under paced respiration. This reduction likely indicates a withdrawal of non-respiratory autonomic influences, such as baroreflex and thermoregulatory mechanisms, in favour of respiratory driven oscillations. Particular caution is warranted when interpreting the results of other frequency-domain indices, given the multifactorial determinants of LF power and the LF/HF ratio, which are influenced by sympathetic modulation, baroreflex dynamics, and breathing patterns (424, 425). Moreover, in our protocol the paced respiration alters the location of spectral components shifting from HF to LF band, thus causing a reduction of HF normalized power and an increase of LF/HF ratio.

Therefore, the values of these indices may not reflect pure autonomic variations, but rather the combined influence of respiratory-driven spectral redistribution and intervention-related effects. Beyond respiration related modulations, the significant post-intervention increases in the LF/HF ratio observed in the INT group during spontaneous breathing suggests a reorganization of autonomic balance, potentially reflecting enhanced baroreflex sensitivity and improved coordination between sympathetic and parasympathetic components. This adaptation may indicate that the intervention facilitated a more flexible autonomic control at REST. Conversely, the absence of comparable changes in the CONT group supports the specificity of this effect. Furthermore, the group-level difference in LF/HF during the PRE phase, found only under the CB condition, suggests that distinct autonomic profiles may become evident primarily when respiratory activity is externally paced.

Beyond these respiration-related effects, the intervention group showed enhanced autonomic regulation following the experimental phase, with increases in LF/HF and a decrease of Hlin from PRE to POST during spontaneous breathing (REST). The latter finding suggests a more irregular signal structure in the intervention group at baseline. From an information-theoretic perspective, the higher entropy values (H_{knn}) consistently observed in the INT group under resting conditions, both at PRE and POST, point to a more complex and adaptable autonomic regulation pattern. Increased entropy in cardiovascular time series has been associated with greater physiological flexibility and resilience against stress-related dysregulation (426, 427). In contrast, the post-intervention decrease in linear entropy (H_{lin}) during guided breathing may reflect enhanced regularity and entrainment between cardiac and respiratory oscillations, suggesting that the intervention facilitated more efficient autonomic coupling under controlled respiration. The observed increase in entropy during controlled breathing across groups further aligns with enhanced cardiopulmonary coupling and nonlinear dynamics induced by guided respiration (428-430). Taken together, these post-intervention changes—particularly the increased LF/HF ratio and the modulation of entropy indices—suggest an improved ability to regulate autonomic output at baseline, reflecting reduced chronic stress load and greater physiological adaptability. Notably, the control group did not show such changes, supporting the specificity of the intervention effect. It is worth noting that the significant PRE–POST increase of LF/HF ratio in the INT group during REST emerged exclusively in the PPI-based indices. This finding suggests that the rebalancing of autonomic control induced by the intervention became particularly evident at rest and when assessed through peripheral rather than ECG-derived measures. Such an effect aligns with the notion that peripheral indices can be more sensitive to subtle shifts in autonomic modulation, especially in contexts where the intervention targets global physiological regulation.

Overall, our results demonstrate the usefulness of a multidomain assessment approach for characterising physiological adaptations and converge to show that paced breathing acts as a strong modulator of vagal activity across all participants, while the intervention appears to selectively enhance autonomic function and signal complexity at rest. Conversely, the comparison between acquisition systems, performed on six common HRV indices, revealed notable differences, emphasising the systems' relative sensitivity to physiological modulation rather than the similarity of absolute values. While Mean HR and VLF indices exhibited no discrepancy, the other measures showed statistically significant differences, likely due to differences in time series type and length, or peak detection algorithms and processing pipelines, including diverse filtering strategies and power spectral density estimation in the commercial device. These results suggest caution when directly comparing HRV values acquired from different devices, especially when the specifications and processing details are proprietary and not publicly documented (431). In particular, it is not specified whether the PPG Stress Flow devices estimates the power spectral density using classical or parametric methods, and not even the parameters used for the estimation. These factors contribute to the limited comparability between the two acquisition systems, particularly for frequency-domain indices.

Nevertheless, the meaningful findings at different phases and conditions obtained using our device and the consistent trends reported for most indices between the two systems when going from REST to CB favour the validity of the custom-built MPSAS system for the assessment of short-term autonomic control and of stress conditions.

Taken together, these results support the usefulness of exploiting multimodal biomedical devices for monitoring of physiological stress, offering the opportunity to track complex physiological inter-system dynamics, particularly in high-stress professions such as healthcare operators.

Conclusion

This study demonstrated the proper working of a custom-built multimodal system (MPSAS) able to synchronously acquire various physiological signals and its usefulness as a reliable, low-cost device for stress assessment in operational environments.

Our results proved the feasibility of using the device for computing short-term cardiovascular variability indices, supporting its use for monitoring autonomic activity within a network physiology framework. The results of the analysis revealed distinct patterns of autonomic modulation across respiratory conditions, experimental phases, and participant groups. Time-, frequency-, and information-domain features derived from RRI and PPI series captured the physiological effects of the paced breathing and of the intervention, with group-level comparisons shedding light on both shared and specific adaptations.

Cross-system comparison of HRV indices with a certified medical device showed contrasting results, ascribed to the differences in time series length, pre-processing algorithms and power spectral density estimation approaches in the commercial device.

Future developments could explore the use of physiological variables extracted from synchronous ECG and PPG signals, such as pulse arrival time (413), and evaluating the effect of more impactful stress-reduction interventions, including structured workplace modifications, or alternative relaxation techniques.

3.3 "Effects of Myofascial Chain Stretching Program on Work-Related Stress and Musculoskeletal Disorders in Healthcare Workers: Preliminary Results"

Antonino Scardina, Ivan Giuseppa Cammarata, Roberta Cottone, Nicoletta Salviato, Stefano Boca and Marianna Bellafiore

Introduction

Workplace health represents a crucial component of overall well-being, as adults spend a significant portion of their waking hours at work, between 8 and 11 hours per day, making the work environment one of the main determinants of sedentary behavior and its related health risks (24, 25). Prolonged sedentary behavior and low levels of physical activity are associated not only with musculoskeletal disorders but also with numerous chronic conditions, including work-related stress, depression, obesity, diabetes, arthritis, and sleep disturbances (432). From a physiological perspective, prolonged exposure to stressful situations leads to continuous activation of the autonomic nervous system and excessive production of cortisol, resulting in negative effects on cardiovascular, metabolic, and psychological health (433).

The consequences of work-related stress extend beyond individual well-being, contributing to absenteeism and presenteeism, with significant repercussions on productivity, healthcare costs, and organizational efficiency (27, 352). Although the World Health Organization (WHO) recommends between 150 and 300 minutes of moderate-intensity aerobic physical activity or between 75 and 150 minutes of vigorous-intensity activity per week (30), one in four adults worldwide fails to meet these levels. For this reason, the workplace has become a strategic setting for implementing physical activity programs aimed at reducing sedentary behavior and promoting workers' health (29-31).

Modern initiatives often integrate digital technologies, such as mobile applications and wearable devices, to encourage participation and monitor physical activity (32, 33). However, despite promising results, the effectiveness of workplace exercise-based interventions remains uncertain due to methodological heterogeneity and variations in adherence levels (34-37).

Health promotion programs in healthcare environments have gained particular relevance in recent years, especially after the COVID-19 pandemic, which intensified occupational stress among healthcare workers (46). Nurses, in particular, represent one of the most exposed professional groups, facing high physical and psychological demands, irregular schedules, and extended shifts all factors that contribute to the development of musculoskeletal disorders and burnout. Addressing work-related stress in this population requires a multidimensional approach that integrates both individual and organizational strategies, including ergonomic improvements, workload management, psychological support, and structured physical activity programs (43-47, 434, 435).

At the individual level, interventions aimed at stress reduction include cognitive-behavioral approaches, mindfulness techniques, breathing exercises, and relaxation practices, all of which have proven effective in activating the parasympathetic nervous system, lowering cortisol and ACTH levels, and promoting a state of physiological recovery (49-53). Mind-body practices such as yoga and stretching have been shown to effectively reduce stress, alleviate musculoskeletal pain, and improve sleep quality (54-56). In particular, short stretching sessions performed during the workday can help reduce accumulated muscle tension, improve postural balance, and enhance concentration and productivity (57, 58).

The present study introduces an innovative intervention based on the principle of myofascial chains, applied directly in the hospital setting for healthcare workers. This biomechanical concept describes the human body as a continuous network of muscles and connective tissues capable of transmitting tension and force between even distant regions (100, 287). According to the model developed by Myers, myofascial chains (or "Anatomy Trains") constitute integrated functional lines, such as the superficial back line, which connects the plantar fascia to the epicranial fascia, encompassing the main postural and locomotor structures. Anatomical and clinical evidence has confirmed the existence of these fascial continuities, showing that stretching one body region can influence the range of motion (ROM) in distant segments (103, 104, 234).

Based on this theoretical framework, the present interventional study aims to evaluate whether a structured program of myofascial chain stretching exercises can help reduce work-related stress and musculoskeletal disorders in a sample of nurses. The proposed protocol consists of short, simple, and easily replicable routines designed to improve flexibility, promote parasympathetic activation, and enhance physical and mental well-being within the hospital work environment.

Materials and Methods

Participants and Study Design

An original interventional study was conducted involving a group of healthcare workers who voluntarily participated in the research. Of the 59 participants initially recruited, 52 withdrew from the study; therefore, the final sample consisted of 7 individuals (5 women and 2 men). All participants were active healthcare professionals with at least five years of experience in the same hospital. The general characteristics of the sample are reported as means and standard deviations in Table 1.19. All participants signed informed consent, voluntarily participating in this study. The investigation was approved by the local ethics committee from the University of Palermo (protocol n° 129/2023) and complies with the criteria on the use of people in research, as stipulated in the Declaration of Helsinki.

Table 1.19 Descriptive characteristics of participants

	Mean	**SD**
Age (years)	50.43	5.88
Weight (kg)	70.36	14.63
Height (cm)	1.67	0.10
BMI (kg/m²)	25.02	3.15

BMI: Body Mass Index;

Measures

The assessed measures were the Stand and reach test (SRT), Tissue Hardness (TH), and the Respiratory chest test (RCE). All measures were assessed by the same investigator blinded to the administered intervention. All tests were performed three times.

Stand and reach test (SRT)

Stand and reach flexibility test: In this study, SRT test was chosen as a static flexibility measure, as experts agree that it has been used extensively as an indirect measure to simultaneously assess the hamstring and low back flexibility (436). During the test, subjects held one hand exactly on the other one and flexed their trunk slowly. Measurements were based on the maximum distance reached and held for two s. The test was performed 3 times with a 30 second interval between trials, and the best score of the three trials was included in the statistical analysis.

Tissue Hardness (TH)

The TH measurements were obtained using a portable TH meter (NEUTONE TDM-N1; TRY-ALL Corp., Chiba, Japan), capable of measuring penetration distance under a pressure of 14.71 N (1.5 kgf) (237). For the assessment of right knee flexors TH, the ischial tuberosity was used as the proximal anatomical reference, while the popliteal fossa served as the distal reference. Measurements were obtained in the lumbar region, TH measurements were performed using the third lumbar vertebra (L3) as the anatomical reference. Specifically, data were collected at two locations, positioned 2 cm to the right and left of the L3 spinous process. For the TH assessments, participants were positioned in a prone lying position on a medical bed and instructed to remain fully relaxed throughout the procedure. To enhance measurement reliability, each assessment site was precisely marked with a dermal marker before data collection. At each site, three consecutive measurements were obtained, and the mean value was calculated for subsequent analysis.

Chest Expansion Test (CET)

Patients were measured while unclothed from the waist up, standing upright with arms bent and hands resting on their heads, facing forward.

This posture offered two main benefits: first, it reduced excessive contraction of the shoulder adductor muscles, particularly in young men, thereby preventing artificially high chest expansion values; second, it positioned the scapulae and breasts above the measurement line, allowing unobstructed access to the chest area.

Chest circumference was assessed using a tape measure. Each measurement was taken three times by the same examiner, and the average value was recorded. Chest expansion was calculated as the difference between the circumferences measured at deep inspiration and deep expiration, both taken at the level of the fourth intercostal space.

Interventions

The interventional protocol was implemented at the Ospedale Civico Benfratelli Di Cristina in Palermo and was conducted between January and May 2024. Over this five-month period, healthcare workers were invited to participate voluntarily in guided sessions of myofascial chain stretching exercises. Sessions were held on Tuesday and Thursday mornings, during working breaks, in a designated hospital area. Participants performed the exercises on individual mats under the supervision of the research team.

The intervention consisted of three distinct routines, labeled A, B, and C, each including three exercises and lasting approximately 15 minutes. Participants were asked to complete the exercises three times during the morning, following a randomized order of the routines to minimize sequence effects.

Routine A included:

Stretching of the Superficial Posterior Line, involving the plantar flexors, gastrocnemius, hamstrings, sacrotuberous ligament, lumbosacral fascia, spinal erectors, and epicranial fascia. Four sets of 30 seconds were performed, with 30 seconds of rest between sets.

Stretching of the Superficial Anterior Line, involving the dorsiflexors (tibialis anterior, extensor digitorum longus), patellar ligament, quadriceps (particularly rectus femoris), endothoracic fascia, and sternocleidomastoid. Four sets of 30 seconds were executed—two on the right and two on the left side—with 30 seconds of rest between sets.

Stretching of the Spiral Line, targeting the splenius capitis, rhomboids, serratus anterior, external and internal obliques, abdominal aponeurosis (linea alba), tensor fasciae latae, iliotibial tract, tibialis anterior, peroneus longus, biceps femoris, sacrotuberous ligament, thoracolumbar fascia, and spinal elevators. Four sets of 30 seconds (two per side) were completed, separated by 30 seconds of rest.

Routine B followed the same structure and duration, involving the same myofascial lines (posterior, anterior, and spiral) and adhering to identical timing: four sets of 30 seconds per exercise with 30 seconds of recovery.

Routine C also replicated the same organizational framework, including the three principal myofascial lines—superficial posterior, superficial anterior, and spiral—with identical set and recovery times (4 × 30 seconds; 30 seconds rest).

Across all routines, participants were encouraged to maintain proper alignment and controlled breathing throughout each stretch. Each session was supervised to ensure correct execution and consistency of effort across sessions.

Statistical Analysis

Means ± standard deviations were used to present the results. The normal distribution of the data was verified with the Shapiro Wilk test. A paired t-test was applied to compare pre- and post-intervention measurements on each variable.

All analyses were performed with Jamovi (The Jamovi Project, 2021). Jamovi (Version 2.3.12.0) [Computer Software]. Retrieved from https://www.jamovi.org. Significance was considered <0.05 for all analyses.

Results

No significant differences were observed in most parameters following the intervention. However, a statistically significant improvement was found in global flexibility, as indicated by higher post-intervention values in the Stand and Reach Test ($p = 0.044$).

Table 1.20 Descriptive statistics, normality test, and paired t-test results

	Fase	Mean	SD	Shapiro–Wilk *p*	Paired t-test *p*
Tissue Hardness DX	Pre	13.02	3.01	0.339	0.574
	Post	14.95	7.36	0.156	
Tissue Hardness SX	Pre	13.47	2.12	0.716	0.548
	Post	15.71	9.75	0.094	
Chest Expansion Test	Pre	5.27	1.30	0.197	0.352
	Post	4.33	1.95	0.944	
Stand and Reach Test	Pre	-4.07	6.97	0.743	0.044*
	Post	0.57	9.00	0.706	

*: Significant p<0.05

Discussion

The aim of this study was to evaluate the preliminary effects of a myofascial chain stretching program implemented in the workplace for healthcare professionals, particularly nurses. The intervention was designed as a short, simple, and easily applicable protocol that could be performed during working hours without interfering with daily activities. Specifically, the study aimed to determine whether regular participation in myofascial stretching exercises could reduce work-related stress by promoting parasympathetic activation and physiological recovery, decrease musculoskeletal discomfort associated with static postures and repetitive work tasks, and improve global flexibility. Overall, this research sought to explore the potential of myofascial stretching as an integrated workplace strategy for enhancing health and wellness among healthcare workers, providing preliminary evidence on its feasibility and possible benefits, and establishing a foundation for future studies with larger samples and longer intervention periods.

The results of the present study showed that, although no statistically significant differences were observed in most of the analysed parameters, participation in the myofascial stretching program produced a measurable improvement in global flexibility, as demonstrated by the significant increase in the Stand and Reach Test values after the intervention (p = 0.044). This finding suggests that regular engagement in short myofascial stretching sessions may positively influence the elasticity of the posterior kinetic chain and the functional mobility of the participants, even after a relatively brief intervention period.

This finding suggests that regular engagement in short myofascial stretching sessions may positively influence the elasticity of the posterior kinetic chain and the functional mobility of participants, even after a relatively brief intervention period (103, 104, 234). The absence of significant changes in tissue hardness and chest expansion parameters could be attributed to several factors, including the limited sample size, the short duration of the intervention, and the intrinsic variability of these physiological measures (237, 437). It is also possible that longer or more intensive protocols are required to produce measurable adaptations in muscle tone or thoracic mobility (57, 438). Nonetheless, the direction of the observed changes—particularly the slight improvements across most variables—suggests a potential trend toward enhanced musculoskeletal and respiratory function following the stretching routines (58, 75).

These preliminary findings align with previous literature indicating that stretching and flexibility-based interventions can reduce muscle tension, improve posture, and support psychophysiological recovery mechanisms (49, 56, 439). The improvement in flexibility may also reflect an indirect reduction in stress-related muscular rigidity, as stretching of the myofascial lines is known to influence both peripheral and central neuromuscular regulation (100, 215, 220). Moreover, considering that the participants were healthcare professionals exposed to high occupational stress, even modest improvements in flexibility and perceived well-being may have meaningful implications for the prevention of work-related musculoskeletal disorders and stress management (43, 46, 55).

Overall, while the results should be interpreted with caution due to the small sample size and the preliminary nature of the study, they provide encouraging evidence supporting the feasibility and potential benefits of implementing short myofascial stretching sessions within the workplace (24, 31). Future research with larger cohorts, longer intervention periods, and additional physiological or psychological measures is warranted to confirm these initial findings and to better understand the mechanisms underlying the observed effects (39, 440).

This study presents several limitations that should be considered when interpreting the results. The most evident limitation is the small sample size, as only seven participants completed the entire intervention. The reduced number of subjects significantly limits the statistical power of the analyses and prevents the generalization of the findings to broader populations of healthcare workers. Moreover, the high dropout rate among the initially recruited participants (52 out of 59) suggests the presence of organizational or motivational barriers that may have influenced adherence, highlighting the challenge of implementing structured physical activity programs within demanding hospital settings.

Another limitation concerns the short duration of the intervention, which lasted only five months. Although improvements in flexibility were observed in this period, longer interventions might be necessary to induce significant adaptations in muscular tone, tissue hardness, and thoracic mobility. The absence of a control group further restricts the strength of causal inferences, as it is not possible to exclude that the observed effects were partially influenced by external factors such as daily physical activity or individual variability in stress management.

Additionally, all measurements were conducted by a single examiner, which, while ensuring consistency, may introduce potential measurement bias. Some of the selected variables, such as tissue hardness and chest expansion, are also inherently influenced by individual anatomical and respiratory differences, which may have contributed to the variability of results.

Finally, the study did not include psychological or physiological biomarkers of stress (e.g., heart rate variability, cortisol levels), which could have provided a more comprehensive understanding of the relationship between myofascial stretching, stress modulation, and autonomic balance.

Despite these limitations, the study provides valuable preliminary evidence on the feasibility and potential benefits of implementing myofascial stretching protocols within hospital environments. Future research should address these issues by recruiting larger and more diverse samples, extending the duration of the intervention, incorporating objective stress indicators, and comparing results with those of a control group to strengthen the validity and reproducibility of the findings.

Conclusion

The present study provides preliminary evidence supporting the potential benefits of a short myofascial chain stretching program implemented in the workplace for healthcare professionals. Although most of the analysed parameters did not show statistically significant differences, the observed improvement in global flexibility suggests that this type of intervention may positively influence musculoskeletal function and overall physical well-being, even after a relatively brief implementation period. These findings highlight the feasibility and practicality of introducing simple, time-efficient exercise routines during working hours in hospital settings. Such interventions may help mitigate some of the physical and psychological effects associated with high workloads and occupational stress among healthcare workers, particularly nurses, who are frequently exposed to physically demanding and emotionally taxing conditions.

However, due to the small sample size, lack of a control group, and short intervention period, these results should be interpreted with caution. The study's exploratory nature nonetheless provides a valuable foundation for future research aimed at confirming and expanding these findings through larger, randomized, and longer-term trials.

In conclusion, the integration of myofascial stretching sessions within the workplace appears to be a promising, low-cost, and non-invasive strategy to promote flexibility, reduce stress, and improve the psychophysical health of healthcare professionals. With further validation, this approach could represent an effective component of occupational wellness programs designed to enhance both worker well-being and organizational performance.

3.4 Final considerations

The research work presented in this doctoral thesis aimed to explore and enhance the role of physical activity, stretching, and targeted health promotion programs in improving the physical and psychological well-being of public employees, particularly within occupational settings characterized by high levels of sedentary behavior and work-related stress. In an era where office work and digital automation have drastically reduced daily physical activity, identifying effective, sustainable, and transferable strategies to foster active lifestyles represents a public health priority and a key challenge for the sustainability of modern healthcare systems.

This thesis is situated within a research area that integrates exercise science, neuroscience, occupational psychology, and public health. It recognizes that human well-being emerges from the dynamic interaction among biological, cognitive, behavioral, and social dimensions. Health is therefore interpreted not as the mere absence of disease, but as a continuous process of adaptation and self-regulation of the organism in response to environmental, emotional, and professional demands.

From this perspective, physical activity is understood not only as a biomechanical function but also as a neuropsychological regulator—an agent that modulates stress systems, stimulates neuroplasticity, and restores psychophysiological balance.

The experimental studies conducted on different protocols of stretching, foam rolling, myofascial release, and integrated training deepened our understanding of the neurophysiological and sensory mechanisms underlying flexibility, soft-tissue adaptation, and movement perception. The evidence collected demonstrated that the beneficial effects of such interventions extend beyond joint mobility improvements, encompassing modifications in muscle tone, tissue stiffness, perceived exertion, and autonomic regulation. In particular, the increase in stretch tolerance, the nervous system's ability to accommodate higher levels of muscle tension without discomfort, emerged as a key adaptive response.

From a physiological standpoint, the results revealed that even brief and moderate-intensity interventions can elicit measurable adaptations, including increased range of motion, reduced muscle hardness, and positive modulation of heart rate variability, an established indicator of autonomic balance between sympathetic and parasympathetic branches.

On a psychological level, regular practice of stretching, mobility, and breathing exercises proved effective in reducing perceived stress, improving emotional regulation, and fostering a sense of bodily awareness and environmental control. These findings integrate cognitive and affective dimensions of well-being, confirming that mental health can be effectively supported through embodied and sensory mechanisms.

This holistic perspective aligns with the growing evidence that links movement to psychological resilience, emotional stability, and burnout prevention. The observed reduction in cortisol levels and the stimulation of endorphin, dopamine, and serotonin pathways represent the neurochemical foundations of this integrative effect, uniting physiology and behaviour in a dynamic feedback loop.

A central aspect of this research concerns the application of exercise and stretching programs among public employees, specifically teachers and healthcare professionals, two occupational groups particularly vulnerable to chronic stress. The studies conducted revealed that the implementation of brief, structured movement-based strategies during the workday not only mitigates musculoskeletal tension but also enhances mood, focus, and self-efficacy.

These findings are of major significance, as they demonstrate that occupational health and productivity can be improved through low-cost, accessible interventions without the need for radical organizational restructuring.

The concept of workplace health promotion thus emerges as a core paradigm: the workplace becomes a site of prevention and health education, integrating movement as part of the professional routine. The salutogenic approach, centered on empowerment, awareness, and active participation, proves more effective than prescriptive or punitive health models, fostering both individual and collective resilience.

Methodologically, this thesis introduced innovative tools for monitoring psychophysiological well-being, such as heart rate variability analysis and localized bioimpedance assessment to evaluate morphological and tissue changes induced by exercise. These techniques allow for objective quantification of phenomena that were traditionally assessed only subjectively, leading to more accurate and reproducible measurements of physiological and stress-related responses.

The validation of new techniques such as Positional Transversal Release (PTR) and their comparison with traditional static and proprioceptive neuromuscular facilitation (PNF) stretching protocols constitute a significant scientific contribution. The results showed that PTR can achieve comparable or even greater improvements in joint mobility with shorter application times, suggesting an alternative and more efficient approach to the management of muscular stiffness.

From an applied perspective, the outcomes of this research provide a solid foundation for designing health promotion programs targeting public sector employees and, more broadly, any occupational groups exposed to sedentary behaviour and cognitive workload.

Integrating active breaks, micro-sessions of stretching, controlled breathing, and relaxation techniques into daily routines represents a concrete strategy to enhance productivity, reduce absenteeism, and improve perceived well-being. These practices can be easily adapted to different organizational contexts and included within institutional or corporate wellness frameworks.

At the public health level, promoting physical activity among workers also carries substantial economic and social value, contributing to reduced healthcare costs, the prevention of chronic diseases, and overall quality of life improvements. When adequately supported by educational and organizational policies, regular exercise becomes an instrument of health equity and collective well-being.

Despite the methodological rigor and the richness of the data collected, some limitations must be acknowledged. Sample sizes, intervention durations, and the prevalence of cross-sectional designs suggest the need for future longitudinal studies on larger and more diverse populations.

Further research should investigate the complex interplay between physical activity, nutrition, circadian rhythms, sleep, and stress regulation, with the goal of developing personalized, evidence-based protocols tailored to individual physiological profiles.

In future perspectives, the integration of scientific research with technological innovation will be crucial. The use of wearable sensors, digital platforms, and mobile applications can enable real-time monitoring of psychophysiological parameters and foster self-regulation and behavioural adherence in everyday life.

In conclusion, this thesis demonstrates that promoting physical activity and specific mobility practices represents an effective, low-cost, and sustainable strategy for improving both physical and psychological well-being among public employees. The research reinforces the idea that health is a complex, dynamic equilibrium between body and mind, and that the culture of movement constitutes one of the fundamental pillars of health in the twenty-first century.

Encouraging active workplaces means investing in collective resilience, productivity, and human dignity. In this sense, physical exercise transcends its role as a simple fitness practice: it becomes a universal language of adaptation, balance, and growth—an essential tool for the flourishing of individuals and communities alike.

CHAPTER IV – PERSONAL SKILLS AND ACHIEVEMENTS

4.0 Professional Growth and Research Experience within the Sport and Exercise Sciences Research Unit

Throughout the PhD program, the research activity carried out within the local unit also served as a formative process. The scientific projects were conceived as practical learning experiences, through which a comprehensive series of physical, functional, and physiological evaluations were conducted.
Assessments included multiple performance tests designed to measure distinct components of physical fitness. Muscular strength was assessed using the Handgrip test and the T-Force system, while muscular endurance was evaluated through push-up and pull-up tests performed to exhaustion. Dynamic balance was examined using the standardized Y-Balance Test (Functional Movement Systems®, Chatham, USA). Lower-limb power was assessed through the Standing Long Jump (SLJ) test and Optojump analysis, and joint mobility and range of motion (ROM) were measured with the Gyko inertial sensor system (Microgate, Bolzano, Italy). Complementary physiological evaluations were also carried out. Body composition was analyzed through bioelectrical impedance analysis (BIA; AKERN 101 – AKERN SRL, RJL Systems, Detroit, USA). Heart rate variability (HRV) parameters were derived from heart rate data recorded via a Polar H10 sensor and subsequently processed using Kubios HRV software.

In addition to performance and physiological measurements, validated questionnaires were implemented to assess behavioral and psychosocial variables. Specifically, the SF-36 questionnaire was used to evaluate health-related quality of life (HRQoL), and the International Physical Activity Questionnaire (IPAQ) was applied to estimate physical activity (PA) levels.

The methodological rigor of these assessments was ensured by first learning, rehearsing, and validating all procedures through repeated practice. Only after achieving a sufficient level of technical proficiency and reliability were the protocols applied within the research projects.

A variety of stretching and intervention techniques were also explored and implemented during the doctoral studies, including Static Stretching (SS), Proprioceptive Neuromuscular Facilitation (PNF), Positional Transversal Release (PTR), and Myofascial Release (MFR) methods such as foam rolling. These interventions were used to examine their acute and chronic effects on range of motion, tissue properties, and functional performance.
Over the three years, I was also involved in the design and execution of systematic reviews and meta-

analyses, which demanded a solid understanding of research methodology, statistical modeling, and risk-of-bias assessment. This methodological expertise was progressively consolidated and applied to interpret data and strengthen the overall scientific validity of the work.

Data processing and visualization represented another essential aspect of the research. Several statistical and analytical software tools were employed, including Jamovi (v1.8.0.1), GraphPad Prism 8 (GraphPad Software, San Diego, CA), Statistica, and R. Power analyses and sample size calculations were performed using G*Power (v3.1.9.4). These programs were carefully compared and used according to their specific analytical strengths to ensure the precision, consistency, and reliability of all statistical outcomes.

Furthermore, but not less importantly, I took part in the design and development of an innovative seismocardiography (SCG) system conceived to enhance the accuracy and robustness of cardiac vibration detection through synchronized multi-sensor acquisition and advanced signal fusion algorithms. The motivation underlying this research arises from the inherent limitations of conventional single-sensor SCG systems, which are often affected by noise, motion artifacts, and operator dependency. To address these constraints, the proposed system was designed as a distributed sensing architecture composed of a central processing unit and multiple satellite modules, each equipped with high-bandwidth MEMS accelerometers (LSM6DSV16X) operating in externally triggered mode. The hardware was meticulously engineered to achieve precise temporal synchronization among all sensors by employing a master–slave timer configuration, thus ensuring that every acquisition event shares a common time reference. This synchronization is a critical prerequisite for effective sensor fusion, which in turn significantly improves the signal-to-noise ratio and the fidelity of the acquired cardiac vibrations, allowing for a more accurate representation of both primary and secondary heart sounds.
The central processing board integrates a high-performance STM32H743ZI microcontroller operating at 400 MHz with 1 MB of RAM, providing ample computational resources for real-time acquisition, signal processing, and wireless communication via an ESP-12E (ESP8266) module. Each satellite board mirrors the sensing setup of the main unit and is connected through independent SPI interfaces buffered by TXU0304-Q1 components, allowing efficient Direct Memory Access (DMA) data transfers that minimize CPU load and latency. Data originating from the accelerometers and gyroscopes are periodically transferred from the sensors' internal FIFOs to the main memory, producing six data arrays per device—three from acceleration and three from angular velocity—which can be locally stored, processed, or transmitted via USB or Bluetooth for further analysis. Preliminary experimental results demonstrated that the correlation-based sensor fusion algorithm, coupled with a heartbeat detection method inspired by the Pan–Tompkins

ECG model, achieved a noise reduction of approximately 6 dB, thereby enhancing both the readability and diagnostic interpretability of the SCG signal.

The proposed system thus represents a significant advancement toward the development of a cost-effective, high-performance platform for non-invasive cardiovascular monitoring. Its modular design ensures scalability, allowing future iterations to incorporate a greater number of synchronized sensors distributed over the thoracic surface. This configuration would enable the simultaneous capture of complex mechanical phenomena related to cardiac activity, including multi-point detection of heart sounds and chest wall motion with unprecedented temporal resolution. Beyond its evident clinical and diagnostic implications, this technology holds great promise for applications in sports science and exercise physiology. In these fields, the implementation of SCG-based monitoring could allow continuous, real-time evaluation of cardiac function during training and competition, facilitating the analysis of cardiovascular performance, fatigue, and recovery dynamics. Ultimately, this research paves the way for the integration of biomechanical cardiac sensing into wearable technologies designed to optimize athletic performance, prevent overtraining, and support personalized health and fitness monitoring.

Baiamonte, G., Vella, A., Valvo, F. L., Chiarello, G., Sapienza, S., Alfano, T., ... & Giaconia, G. C. (2024, September). Synchronized Vibration Data Acquisition for Seismocardiography. In International Conference on Applications in Electronics Pervading Industry, Environment and Society (pp. 380-386). Cham: Springer Nature Switzerland.

4.1 International Mobility and Scientific Collaborations

During the three-year PhD program in *Health Promotion and Cognitive Sciences*, I had the opportunity to spend six months abroad, from March 5th to September 14th, 2024, at the *Faculty of Rehabilitation Sciences* of *Nishi Kyushu University* in Kanzaki, Saga Prefecture, Japan. This experience represented a crucial stage in my academic and professional development, allowing me to work closely with Professor Masatoshi Nakamura and his research group on several projects focused on neuromuscular physiology, eccentric exercise, and recovery strategies.

During my stay, I contributed to the writing of two scientific manuscripts: one investigating the effects of microwave hyperthermia therapy applied the day before eccentric exercise on elbow flexor muscle damage, and another examining sex differences in the *repeated bout effect*, that is, the protective adaptation developed after repeated sessions of eccentric exercise. At the same time, I participated in two additional experimental data collection projects: one concerning an eccentric training program for Japanese older adults, carried out in collaboration with Associate Professor Yoshihiro Katsura from Kogakuin University of Tokyo, and another focused on localized bioelectrical impedance analysis (*L-BIA*) to evaluate the effects of massage roller application on the plantar flexor muscles in young Japanese adults.

The experimental work conducted in Japan allowed me to deepen my understanding and practical use of various instruments for physiological and functional assessment. Measurements of muscle strength and performance were carried out using an isokinetic dynamometer (*Biodex System 3.0*), which enabled the analysis of maximal voluntary torque during isometric, concentric, and eccentric contractions of the elbow flexors. Changes in joint range of motion were measured with a goniometer, while muscle soreness perception was assessed through a 100 mm *Visual Analog Scale* (VAS). The pressure pain threshold was evaluated using an algometer (*NEUTONE TAM-22 BT10*, TRYALL, Chiba, Japan), applied at specific points along the biceps brachii, whereas muscle thickness was measured using an ultrasound device (*LOGIQ eV2*, GE Healthcare Japan, Tokyo), allowing for non-invasive monitoring of structural changes in muscle tissue before and after eccentric exercise protocols.

One of the most innovative aspects of this experience was the use of microwave hyperthermia therapy, delivered through a *Microwave Therapy ME-7250* device (OG Wellness Technologies, Okayama, Japan). This system, applied for 30 minutes at 80 W and at a distance of 10 cm from the target muscle, enabled the administration of controlled local hyperthermia with the purpose of evaluating whether pre-exercise tissue heating could modulate muscle damage induced by eccentric activity. Alongside this, localized bioelectrical impedance analysis (*L-BIA*) was employed to observe acute changes in muscle composition and hydration following mechanical stimulation such as massage roller application.

Data collection and analysis were supported by integrated acquisition systems, including the *PowerLab 8/30* (AD Instruments, Colorado Springs, USA), which was connected to the dynamometer for digital recording and processing of force signals. Statistical analyses were performed using *Jamovi* (version 2.3.12.0), which was used for repeated-measures ANOVA and Bonferroni post-hoc corrections, while data visualization and graphical representation were carried out with *GraphPad Prism 8* (GraphPad Software, San Diego, CA), ensuring accurate and intuitive illustration of results.

This period in Japan was not only an opportunity to acquire advanced technical skills but also a profound lesson in scientific rigor, methodological precision, and the importance of systematic experimentation. I learned how to design and implement controlled exercise protocols, analyse complex physiological responses, and interpret data with both statistical accuracy and clinical awareness. Working in a multicultural research environment also taught me the value of interdisciplinary collaboration, clear scientific communication, and meticulous attention to detail at every stage of the experimental process.

Overall, this experience greatly enriched my PhD journey, providing me with practical and theoretical tools to approach research in exercise and rehabilitation sciences with deeper awareness, stronger competence, and a genuinely international perspective.

The methodological insights gained during the Japanese research experience also had a direct influence on several choices made in the studies later carried out in Italy. Working within a laboratory environment where experimental procedures were highly standardized taught me the importance of strict control over measurement timing, participant preparation, and environmental conditions. This approach shaped the way I structured the Italian protocols, encouraging the adoption of clearly defined warm-up routines, consistent pre- and post-test procedures, and precise monitoring of external variables that could influence outcomes.

In addition, the opportunity to use advanced instruments for assessing neuromuscular and tissue properties played a key role in selecting the most appropriate tools for my subsequent projects. Although the equipment available in Italy differed from that used in Japan, the underlying methodological principles remained the same: combining mechanical, perceptual, and functional parameters; integrating both acute and chronic assessments; and relying on repeated measures designs to capture temporal adaptations. These principles guided the construction of my experimental protocols and contributed to ensuring that the research maintained internal consistency and alignment with international methodological standards.

Overall, the overseas period did not merely expand my technical skill set; it provided a framework for thinking about experimental design that directly shaped the methodological structure of the studies undertaken after my return to Italy.

4.2 Attended conferences

1. "XIII National Congress **SISMES** Milan, Italy, 4–6 November 2022 – **Poster presentation**"

2. "XIV National Congress **SISMES** Napoli, Italy, 2–4 November 2023 – **Oral presentation**"

3. "XV National Congress **SISMES** Chieti, Italy, 19-21 September 2024 – **Poster presentation**"

4. "20th Annual Conference **EAS**, AWF Katowice (Poland), 1-3 October 2024 – **Oral presentation**"

5. "**SIGOT** Congress, Palermo, 6-7 March, 2025 – **Oral presentation**"

6. "30th Annual Congress **ECSS**, Rimini - Italy, 1-4 July 2025 – **Poster presentation**"

7. "16th Conference of **HEPA**, Kaunas, Lithuanian, 17-19 September 2025 – **Oral presentation**"

8. "XVI National Congress **SISMES** Parma, Italy, 6-8 November 2025 – **Oral presentation**"

4.3 Co-authored papers

1. **Scardina, A.,** Tabacchi, G., Thomas, E., Navarra, G. A., Petrigna, L., Caramazza, G., Palma, A., & Bellafiore, M. (2024). Relationship between Lifestyle Determinants and Perceived Mental and Physical Health in Italian Nursery and Primary School Teachers after the COVID-19 Lockdown. Journal of functional morphology and kinesiology, 9(1), 33. https://doi.org/10.3390/jfmk9010033

2. Pillitteri, G., Thomas, E., Battaglia, G., Navarra, G. A., **Scardina, A.,** Gammino, V., Ricchiari, D., & Bellafiore, M. (2021). Validity and Reliability of an Inertial Sensor Device for Specific Running Patterns in Soccer. Sensors (Basel, Switzerland), 21(21), 7255. https://doi.org/10.3390/s21217255

3. Petrigna, L., Thomas, E., **Scardina, A.,** Rizzo, F., Brusa, J., Camarazza, G., Galassi, C., Palma, A., & Bellafiore, M. (2022). Methodological Considerations for Movement Education Interventions in

Natural Environments for Primary School Children: A Scoping Review. International journal of environmental research and public health, 19(3), 1505. https://doi.org/10.3390/ijerph19031505

4. Navarra, G. A., **Scardina, A.**, Thomas, E., Battaglia, G., Agnese, M., Proia, P., Palma, A., & Bellafiore, M. (2022). How Does the Amount of a Physical Education Intervention Affect Gross Motor Coordination in Early Childhood? Journal of functional morphology and kinesiology, 7(4), 96. https://doi.org/10.3390/jfmk7040096

5. Navarra, G. A., Tabacchi, G., **Scardina, A.**, Agnese, M., Thomas, E., Bianco, A., Palma, A., & Bellafiore, M. (2023). Functional fitness, lifestyle and demographic factors as predictors of perceived physical and mental health in older adults: A structural equation model. PloS one, 18(9), e0290258. https://doi.org/10.1371/journal.pone.0290258

6. Rossi, C., Roklicer, R., Drid, P., Milovancev, A., Trivic, T., **Scardina, A**., Carraro, A., & Bianco, A. (2024). Left Ventricular Hypertrophy in Male and Female Judo Athletes. International journal of sports medicine, 45(5), 377–381. https://doi.org/10.1055/a-2252-1239

7. Tabacchi, G., Navarra, G. A., **Scardina, A.**, Thomas, E., D'Amico, A., Gene-Morales, J., ... & Bellafiore, M. (2025). A multiple correspondence analysis of the fear of falling, sociodemographic, physical and mental health factors in older adults. Scientific Reports, 15(1), 6341.

8. **Scardina, A.**, Nakamura, M., Kasahara, K., Murakami, Y., Yoshida, R., Kawashima, M., and Ikegami R. Sex differences in repeated bout effect of eccentric exercise on elbow flexors. Submitted to the Journal Musculoskeletal Care

9. **Scardina, A** Nakamura, M., Kasahara, K., Murakami, Y., Yoshida, R., Kawashima, M., and Ikegami R. Effects of microwave hyperthermia therapy on the day before eccentric exercise on elbow flexor muscle damage. Submitted to the Journal of Bodywork Movement Therapies

10. Smirni, D., Tabacchi, G., Cottone, R., Navarra, G. A., Muscarnera, G., Di Noto, V., **Scardina, A.**, & Bellafiore, M. (2025). Cognitive performance, sleep quality and physical activity as predictors of functional mobility in older adults. Frontiers in aging neuroscience, 17, 1649682. https://doi.org/10.3389/fnagi.2025.1649682

11. Agnese, M., Cottone, R., Forte, C., **Scardina, A.,** Navarra, G. A., Buttitta, D., ... & Bellafiore, M. (2025). Effects of a Feldenkrais method-based protocol on body fluid balance and stress-related measures in healthy adult women: A pilot study. Journal of Bodywork and Movement Therapies, 45, 812-818.

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APPENDIX

1. **Abstracts of published studies**

"Relationship between Lifestyle Determinants and Perceived Mental and Physical Health in Italian Nursery and Primary School Teachers after the COVID-19 Lockdown"

Antonino Scardina, Garden Tabacchi, Ewan Thomas, Giovanni Angelo Navarra, Luca Petrigna, Giovanni Caramazza, Antonio Palma, Marianna Bellafiore

The COVID-19 lockdown and the consequent distance school learning made epochal changes in children's lifestyles; however, little is known about the lockdown effects on schoolteacher habits. The aim of this observational study is to examine differences in the lifestyle of nursery (NS) and primary (PS) schoolteachers after one of the COVID-19 lockdowns and investigate the relationship between perceived physical/mental health and demographics, weight status and lifestyle determinants, such as adherence to the Mediterranean Diet (MD) and physical activity level (PAL). A total sample of 265 participants (49.22 ± 6.95 years) filled out an online information questionnaire and standardized questionnaires to collect data on the Physical Component Summary (PCS), Mental Component Summary (MCS), PAL and MD-adherence. A t-test or ANOVA analysis was used to assess differences between quantitative variables: Mann–Whitney U or Kruskal–Wallis tests for qualitative variables. Spearman's correlations and multinomial logistic regression analyses were performed to identify categorical factors associated with classes of PCS and MCS. Teachers showed sufficient/high PAL, with a significantly higher level in the PS group ($p < 0.05$). This last showed a higher PCS score ($p < 0.05$). No differences between groups were found for MD-adherence, which was moderate/fair in both groups, and MCS, which was sufficient/good. Logistic regression showed that the only positive predictor of a high PCS was being physically active (OR 2.10, 95%CI 1.05–4.2, $p < 0.05$), while MCS was positively associated with normal weight status (OR 0.48, 95%CI 0.33–0.78, $p = 0.020$). This study highlights that PS teachers are more active than the NS group and perceive a higher physical health level. Mere predictors of physical and mental well-being are PA practice and being normal weight, respectively. This suggests that interventions to improve perceived health in this work category should be focused on the promotion of physical activity practice and on the maintenance of an optimal weight status.

Scardina, A., Tabacchi, G., Thomas, E., Navarra, G. A., Petrigna, L., Caramazza, G., Palma, A., & Bellafiore, M. (2024). Relationship between Lifestyle Determinants and Perceived Mental and Physical Health in Italian Nursery and Primary School Teachers after the COVID-19 Lockdown. Journal of functional morphology and kinesiology, 9(1), 33. https://doi.org/10.3390/jfmk9010033

"Effective Strategies for Promoting Physical Activity through the Use of Digital Media among School-Age Children: A Systematic Review"

Giovanni Angelo Navarra, Ewan Thomas, Antonino Scardina, Mohammad Izadi, Daniele Zangla, Stefano De Dominicis, Pietro Cataldo, Patrizia Proia and Marianna Bellafiore

Digital media are widespread among school-age children, and their incorrect use may lead to an increase in sedentary levels and the consequences associated with it. There are still few studies that have investigated whether physical activity levels could be increased through their use. The aim of this study was to systematically review the scientific literature in order to identify whether digital strategies and technologies are capable of increasing the level of physical activity. A literature search was performed using the following databases: Pubmed, Scopus, and Web of Science. The main outcomes evaluated the increase in physical activity levels, the number of steps, and the reduction of sedentary behaviors. Two trained researchers independently assessed eligible studies against eligibility criteria, extracted data, and assessed the risk of bias. The Downs and Black checklist was used to assess the quality of the included studies. A total of 15 studies (1122 children) were included in this systematic review, with a mean age of 8.45 ± 0.70 years. Quality assessment of the studies observed a "moderate quality" of the included records. The results of this systematic review highlight that digital media can be applied as a way to improve the levels of physical activity in children to contrast a sedentary lifestyle. The main limitations of the study are the heterogeneity within the exercise protocols and the paucity of studies involving school-age children. More research is needed to confirm our findings also due to continuing technological progress.

"Validity and Reliability of an Inertial Sensor Device for Specific Running Patterns in Soccer"

Guglielmo Pillitteri, Ewan Thomas, Giuseppe Battaglia, Giovanni Angelo Navarra, Antonino Scardina, Viviana Gammino, Dario Ricchiari, Marianna Bellafiore

Electronic performance tracking devices are largely employed in team sports to monitor performance and improve training. To date, global positioning system (GPS) based devices are those mainly used in soccer training. The aim of this study was to analyse the validity and reliability of the inertial sensor device (ISD) in monitoring distance and speed in a soccer-specific circuit and how their performance compare to a GPS system. 44 young male soccer players (age: 14.9 ± 1.1, range 9–16, years, height: 1.65 ± 0.10 m, body mass: 56.3 ± 8.9 kg) playing in a non-professional soccer team in Italy, participated in the study. We assessed the players trough a soccer running sport-specific circuit. An ISD and a GPS were used to assess distance and speed. Data was compared to a video reference system, and the difference were quantified by means of the root mean square error (RMSE). Significant differences were found for both GPS and ISD devices for

distance and speed. However, lower error for distance (dRMSE 2.23 ± 1.01 m and 5.75 ± 1.50 m, respectively) and speed (sRMSE 0.588 ± 0.152 $m.s^{-1}$ and 1.30 ± 0.422 $m.s^{-1}$, respectively) were attained by the ISD compared to the GPS. Overall, our results revealed a statistically significant difference between systems in data monitoring for either distance and speed. However, results of this study showed that a smaller error was obtained with the ISD than the GPS device. Despite caution is warranted within the interpretation of these results, we observed a better practical applicability of the ISD due to its small size, lower cost and the possibility to use the device indoor.

Pillitteri, G., Thomas, E., Battaglia, G., Navarra, G. A., Scardina, A., Gammino, V., Ricchiari, D., & Bellafiore, M. (2021). Validity and Reliability of an Inertial Sensor Device for Specific Running Patterns in Soccer. Sensors (Basel, Switzerland), 21(21), 7255. https://doi.org/10.3390/s21217255

"Methodological Considerations for Movement Education Interventions in Natural Environments for Primary School Children: A Scoping Review"

Luca Petrigna, Ewan Thomas, Antonino Scardina, Federica Rizzo, Jessica Brusa, Giovanni Camarazza, Claudia Galassi, Antonio Palma, Marianna Bellafiore

Background: Education is the ideal setting for carrying out projects to improve primary students' capacities. In recent years, interventions in natural environments have been more frequently proposed, but there is still a lack of standardization, making deeper study of the topic necessary. This review aims to report on what previous scientific research has been carried out, and eventually, to propose standard operating procedures for future interventions.

Methods: This is a scoping review that adopted the PRISMA guidelines. Primary school children have been included, and the interventions had to be proposed adopting nature as the primary element of the learning process.

Results: A total of 19 studies have been included, and a wide range of methodological differences has been detected regarding the included intervention protocols.

Conclusions: Learning in nature is a feasible intervention that, despite the high heterogeneity of interventions, demonstrates positive outcomes in the learning sphere of children.

Petrigna, L., Thomas, E., Scardina, A., Rizzo, F., Brusa, J., Camarazza, G., Galassi, C., Palma, A., & Bellafiore, M. (2022). Methodological Considerations for Movement Education Interventions in Natural Environments for Primary School Children: A Scoping Review. International journal of environmental research and public health, 19(3), 1505. https://doi.org/10.3390/ijerph19031505

“Does Learning Through Movement Improve Academic Performance in Primary Schoolchildren? A Systematic Review”

Luca Petrigna, Ewan Thomas, Jessica Brusa, Federica Rizzo, Antonino Scardina, Claudia Galassi, Daniela Lo Verde, Giovanni Caramazza, Marianna Bellafiore

Physically active children have greater motor competence and a faster maturation compared with their sedentary peers. Recent research also suggests that physical activity during childhood may also promote cognitive development and therefore improve academic performance. The aim of this study was to understand if physically active academic lessons may improve academic achievement in primary schoolchildren. A systematic review following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines was conducted. The search was performed on the following database: PubMed, Web of Science, Scopus, Education Resources Information Center (ERIC), and PsycINFO (APA). Studies evaluating schoolchildren aged between 3- and 11-years taking part in educational contexts that include physical activity and natural environments evaluating physical fitness and/or educational outcomes were included. A total of 54 studies (for a total sample of 29,460 schoolchildren) were considered eligible and included in the qualitative synthesis. The Effective Public Health Practice Project risk-of-bias assessment revealed a moderate quality of the included studies with only two considered weeks. Despite differences in the retrieved protocols, physically active academic lessons improve the total time engaged in physical activity, motor skills, and/or academic performance. The results of this review suggest that learning through movement is an effective, low-cost, and enjoyable strategy for elementary schoolchildren.

Petrigna, L., Thomas, E., Brusa, J., Rizzo, F., Scardina, A., Galassi, C., Lo Verde, D., Caramazza, G., & Bellafiore, M. (2022). Does Learning Through Movement Improve Academic Performance in Primary Schoolchildren? A Systematic Review. Frontiers in pediatrics, 10, 841582. https://doi.org/10.3389/fped.2022.841582

“How Does the Amount of a Physical Education Intervention Affect Gross Motor Coordination in Early Childhood?”

Giovanni Angelo Navarra, Antonino Scardina, Ewan Thomas, Giuseppe Battaglia, Massimiliano Agnese, Patrizia Proia, Antonio Palma, Marianna Bellafiore

Little is known about the dose–response of physical education interventions on motor coordination in preschoolers. Our aim was to investigate whether the development of motor skills changed depending on different amounts of a physical education program (PEP) in children aged 3–5 years. One hundred forty-five children were recruited from kindergartens and randomly divided into a control group (CG, n = 28), which did not perform any PEP, and two intervention groups, which performed 4 h/week (I1, n = 78) and 10 h/week (I2, n = 39) of a PEP for 16 weeks. Each lesson was set in the form of a programmed game in order to produce fun, thus increasing enthusiasm for participation. Before and after the intervention, locomotor and object control skills and the gross motor development quotient were assessed with the Italian version of the gross motor development test. Both intervention groups showed a significant increase in the motor skills compared with the control group. Moreover, the level of performance was significantly higher after 10 h/week compared to 4 h/week. These findings can be useful for standardizing PEPs in preschool settings so that they can be applied by teachers for planning effective programs for developing motor skills in early childhood.

Navarra, G. A., Scardina, A., Thomas, E., Battaglia, G., Agnese, M., Proia, P., Palma, A., & Bellafiore, M. (2022). How Does the Amount of a Physical Education Intervention Affect Gross Motor Coordination in Early Childhood?. Journal of functional morphology and kinesiology, 7(4), 96. https://doi.org/10.3390/jfmk7040096

"Functional fitness, lifestyle and demographic factors as predictors of perceived physical and mental health in older adults: A structural equation model"

Giovanni Angelo Navarra, Garden Tabacchi, Antonino Scardina, Massimiliano Agnese, Ewan Thomas, Antonino Bianco, Antonio Palma, Marianna Bellafiore

Over the past 50 years, socioeconomic development has brought a reduction in birth rates, an increase in life expectancy and consequently in the elderly population. For this reason, there has been an increasing focus on physical and mental health of the elderly, promoting the concept of healthy aging. The aim of this study was to explore the associations between perceived physical and mental health of older adults and a variety of determinants, such as demographic factors, physical functional fitness, physical activity level, adherence to the Mediterranean diet and anthropometric indices, through a structural equation modelling (SEM). A cross-sectional observational study involved 208 elderly (24 men and 184 women) over the age of 60, fully independent and autonomous. Perceived physical and mental health were assessed with the Short Form 12 questionnaire. Basic sociodemographic information was collected; anthropometric features were directly measured, functional fitness was assessed with the Senior Fitness Test, and physical activity level was determined through the International Physical Activity Questionnaire; adherence to Mediterranean Diet (MD) was also collected through the MEDAS questionnaire. The SEM analysis revealed that functional fitness, which was a latent variable of the model described by the six administered fitness tests, was a strong predictor both of perceived physical and perceived mental health in the sample of elderly. Physical activity level was as predictor of the perceived physical component, but not of the mental health, while score of metabolic equivalent task did not result a predictor, as well as the sociodemographic factors and adherence to MD. The present findings suggest that it would be strongly recommended for elderly subjects to engage in physical activity specifically targeted to aged populations, in order to enhance their fitness abilities and enable them to improve the perception of their own health status.

Navarra, G. A., Tabacchi, G., Scardina, A., Agnese, M., Thomas, E., Bianco, A., Palma, A., & Bellafiore, M. (2023). Functional fitness, lifestyle and demographic factors as predictors of perceived physical and mental health in older adults: A structural equation model. PloS one, 18(9), e0290258. https://doi.org/10.1371/journal.pone.0290258

"Left Ventricular Hypertrophy in Male and Female Judo Athletes"

Carlo Rossi, Roberto Roklicer, Patrik Drid, Aleksandra Milovancev, Tatjana Trivic, Antonino Scardina, Attilio Carraro, Antonino Bianco

Changes in cardiac geometry develop after intense and prolonged training. Left ventricular enlargement, increased relative wall thickness, and growing mass of the left ventricle occur after strenuous exercise. Combat sports such as judo can lead to left ventricular hypertrophy. Previous studies have found that there are differences in left ventricular chamber size and thickness between the sexes, with female athletes having smaller wall diameters and less hypertrophy than male athletes. The research aims to examine heart muscle adaptations and remodeling of cardiac geometry among elite judo athletes and to evaluate differences between males and females. A cross-sectional study included a group of 19 (males n = 10, females n = 9) professional judokas between 20 and 30 years. Demographic and anthropometric data were collected. Cardiac geometry was determined by two-dimensional transthoracic echocardiography. In terms of left ventricular mass and the left ventricular mass index significant differences were found between male and female judokas (233.44 ± 68.75 g vs. 164.11 ± 16.59 g, p = 0.009), (105.16 ± 24.89 vs. 84.66 ± 15.06, p = 0.044), respectively. A greater enlargement of the heart muscle is observed in male athletes compared to the female group. Left ventricle enlargement is likely to occur among elite-level judokas.

Rossi, C., Roklicer, R., Drid, P., Milovancev, A., Trivic, T., Scardina, A., Carraro, A., & Bianco, A. (2024). Left Ventricular Hypertrophy in Male and Female Judo Athletes. International journal of sports medicine, 45(5), 377–381. https://doi.org/10.1055/a-2252-1239

“A multiple correspondence analysis of the fear of falling, sociodemographic, physical and mental health factors in older adults”

Garden Tabacchi, Giovanni Angelo Navarra, Antonino Scardina, Ewan Thomas, Antonella D’Amico, Javier Gene-Morales, Juan C. Colado, Antonio Palma and Marianna Bellafiore

Fear of falling (FoF) is a disabling condition due to different factors. The present study assessed potential FoF predictors, among sociodemographic, physical, and mental health domains, and explored their structural patterns. This cross-sectional study is part of the Physical Activity Promotion & Domestic Accidents Prevention (PAP & DAP) project and was targeted to a sample of 229 independent older people (M 14.0%, F 86.0%) aged over 60 (mean 70.5 ± 5.96), both normal and overweight (median BMI 25.8 kg/m2, Interquartile Range 5.24). Standardized tools were used to assess the variables: the Short Falls Efficacy Scale International for the FoF; an information questionnaire for the socio-demographic variables, the presence of diseases, and previous falls; the International Physical Activity Questionnaire for the PA level; the Senior Fitness Test for physical fitness data; the Short Form 12 questionnaire for variables in the mental domain; and the Psychological Well-Being Scale 24 for the psychological well-being. Correlation/regression analyses were used to explore relationships between FoF and the considered variables. A Multiple Correspondence Analysis (MCA) was conducted to show graphical patterns projected into space dimensions. A percentage of 59.0 of the sample showed moderate/high concern of falling. The multiple regression model showed the following variables being significant predictors ($p < 0.05$) of the FoF: BMI (coeff 0.44, SE 0.104), musculoskeletal disease (1.55, 0.681), upper body strength (0.33, 0.117), mobility and balance (0.76, 0.320), perceived physical health (− 0.21, 0.047), and self-acceptance (− 0.52, 0.190). MCA evidenced two dimensions: the first one explained 41.8% of the variance and was described mostly by FoF (square residuals 0.721), gender (0.670), leg strength (0.617), perceived mental health (0.591), musculoskeletal diseases (0.572), and PA level (0.556); the second dimension (12.3% of the variance), was characterized mainly by perceived physical health (0.350), life objectives (0.346), education (0.301), upper body strength (0.278), and living in family/alone (0.260). The pattern elicited by MCA was characterized by older subjects with moderate/high FoF having low education or no occupation, being overweight and inactive, suffering from different diseases, having low physical fitness, and declaring low perceived physical and mental health. These results suggest that interventions aimed at reducing FoF should be addressed to this specific profile of older people.

Tabacchi, G., Navarra, G. A., Scardina, A., Thomas, E., D’Amico, A., Gene-Morales, J., ... & Bellafiore, M. (2025). A multiple correspondence analysis of the fear of falling, sociodemographic, physical and mental health factors in older adults. Scientific Reports, 15(1), 6341.

“Sex differences in repeated bout effect of eccentric exercise on elbow flexors”

Antonino Scardina, Masatoshi Nakamura, Kazuki Kasahara, Yuta Murakami, Riku Yoshida, Masato Kawashima, and Ryo Ikegami

This study aimed to compare the repeated bout effect (RBE) between sexes by implementing a muscle damage protocol in two consecutive eccentric-exercise workouts.

21 participants were analyzed during 5 days for two bouts after implementing the muscle-damaging protocol. Outcome variables included extension-ROM, muscle soreness (MS), pain pressure threshold (PPT), muscle thickness (MT), and maximum voluntary contraction (MVC-ISO, CON, ECC) of the elbow joint. Measures were conducted before(pre), immediately after(post) intervention, and on days 1, 2, 3, and 4 during follow-up visits. Significant($p<0.01$) main effects by time were observed for ROM, MS, PPT, and MT during the two bouts. A significant interaction($p<0.001$) showed decreased MVC-ISO, MVC-CON, and MVC-ECC values in both bouts, with increased values for all contraction types only in males during the 2nd bout.

These results suggest that adaptations induced by eccentric-exercise do not differ between sexes. Females show a lower tendency to fatigue with a lower reduction in MVC-ISO, CON, and ECC; however, the RBE magnitude is similar between sexes.

The manuscript has been submitted to the journal Musculoskeletal Care

“Effects of microwave hyperthermia therapy on the day before eccentric exercise on elbow flexors muscle damage”

Antonino Scardina, Masatoshi Nakamura, Kazuki Kasahara, Yuta Murakami, Riku Yoshida, Masato Kawashima, Ryo Ikegami

Background: This study aims to investigate the effects of microwave heat therapy (MHT) on the elbow flexor muscle group using hyperthermia before administering an eccentric exercise-induced muscle damage protocol.

Methods: This study categorized 35 young sedentary students (20.9 ± 0.99 years; 58.6 ± 9.02 kg; 166.5 ± 8.34 cm) into control and preheat groups and analyzed them within 5 days after MHT (80 W for 30 min) 24 h before the muscle damage protocol (50 eccentric exercises with elbow flexor of the dominant side). Range of motion (ROM), muscle soreness (MS), pain pressure threshold, muscle thickness (MT), and maximum voluntary isometric, concentric, and eccentric contraction of the elbow joint were measured before (pre), immediately after (post) intervention, and 24, 48, 72, and 96 h after the muscle damage protocols.

Results: A significant difference was observed only for ROM (after 24 h) and MT (after 48 h) with greater increases in the preheat group. No statistically significant effects of MHT on eccentric exercise-induced strength reductions were observed.
Conclusion: The effects of MHT 24 h before muscle damage protocol administration caused no observable neuromuscular changes.

The manuscript has been submitted to the Journal of Bodywork Movement Therapies

"Cognitive performance, sleep quality and physical activity as predictors of functional mobility in older adults"

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Objective: This study investigated the relationships between functional mobility and cognitive performance, sleep quality and physical activity in older adults according to age, sex, weight status and education, and whether these variables could be predictors of functional mobility and fall risk. Method: Eighty-five physically independent older adults (72.3 ± 5.67 years; 83.5% female), without significant cognitive impairments, were involved in this cross-sectional study. Functional mobility, cognitive functions as phonemic fluency, attention, memory, processing speed, and manual dexterity were, respectively, assessed with Timed Up and Go (TUG) test, phonemic fluency test, Grooved Pegboard Test, (GPT) and Symbol Digit Modalities Test, Stroop Color-Word Test. Sleep quality and physical activity were evaluated with self-reported questionnaires. Correlational and multiple regression analyses were performed. Results: Poorer TUG performance was significantly associated with older age ($\rho = 0.46$, $p < 0.001$), lower educational level, reduced GPT performance, and lower scores on working memory, and processing speed. Age and GPT performance showed the strongest associations with TUG results. Regression analysis confirmed age and manual dexterity as significant predictors of functional mobility. Conclusion: Older age and reduced manual dexterity were linked to greater fall risk. These findings suggest that early interventions targeting fine motor skills may help preserve mobility and prevent falls in aging populations.

Smirni, D., Tabacchi, G., Cottone, R., Navarra, G. A., Muscarnera, G., Di Noto, V., Scardina, A., & Bellafiore, M. (2025). Cognitive performance, sleep quality and physical activity as predictors of functional mobility in older adults. Frontiers in aging neuroscience, 17, 1649682. https://doi.org/10.3389/fnagi.2025.1649682

“Effects of a Feldenkrais method-based protocol on body fluid balance and stress-related measures in healthy adult women: A pilot study”

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The Feldenkrais Method® (FM) is a somatic education approach aimed at enhancing awareness and movement. Evidence regarding its effects on autonomic regulation and stress adaptation is limited. This pilot study examined whether a brief FM protocol could influence physiological and psychological outcomes in sedentary women. Twenty-one women (26–59 years) completed the study (IG, n = 12; CG, n = 9). The IG attended eight consecutive Awareness Through Movement® lessons (90 min/day). Assessments included heart rate variability (HRV), total body water (TBW), extracellular water (ECW), hypothalamic-pituitary-adrenal (HPA) axis index, and questionnaires: the Psychological Well-Being Scale (PWBS) and the Self-Compassion Scale (SCS). In the IG, TBW and ECW decreased significantly ($p = 0.002$, $p < 0.001$). HRV parameters showed no significant changes, although the HPA index was higher post-intervention versus CG ($p = 0.019$). Psychological measures indicated increases in total self-compassion ($p = 0.033$), self-judgment ($p = 0.012$), and over-identification ($p = 0.017$). Between-group analysis showed improved self-acceptance in the IG ($p = 0.047$). Findings suggest that FM may influence body fluid balance, though effects on physiological and psychological measures require further study. However, interpretation is limited by small sample size, baseline imbalances, and short intervention. An 8-day FM protocol appears feasible and may support physiological and psychological well-being. Larger randomized studies are necessary.

Agnese, M., Cottone, R., Forte, C., Scardina, A., Navarra, G. A., Buttitta, D., ... & Bellafiore, M. (2025). Effects of a Feldenkrais method-based protocol on body fluid balance and stress-related measures in healthy adult women: A pilot study. Journal of Bodywork and Movement Therapies, 45, 812-818.