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Exercise training in frail older adults residing in nursing homes: Exploring the muscle-related mechanisms behind its positive effect on physical function

Academic dissertation submitted with the purpose of obtaining a doctoral degree in Physical Activity and Health from the Faculty of Sport of the University of Porto, according to the Law 65/2018 from August 16th.

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KEYWORDS: FRAILTY, AGEING, EXERCISE INTERVENTION, MYOKINES, PHYSICAL FUNCTION, LONG-TERM CARE

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Table of contents

List of figures

List of Tables

Study I

Study II

Study III

Study IV

Resumo

A fragilidade caracteriza-se pela redução das reservas fisiológicas e pelo aumento da vulnerabilidade a desfechos adversos, como o declínio da função física, a dependência nas atividades básicas da vida diária e a quedas. O exercício físico é a intervenção mais eficaz na gestão da fragilidade, pois melhora a função e a resiliência de vários sistemas fisiológicos. Contudo, os mecanismos biológicos através dos quais o exercício exerce estes benefícios, ainda não são totalmente compreendidos. Também é limitada a evidência sobre a viabilidade e eficácia de programas de exercício adaptados a adultos mais velhos mais frágeis. Esta tese explora: I) a relação entre a fisiopatologia da fragilidade, as miocinas e a função física; e II) a viabilidade e eficácia de intervenções de exercício concorrente (aeróbio e força) em residentes de lares comparativamente aos cuidados habituais. Foram realizados quatro estudos: uma revisão narrativa, um estudo transversal (n= 59, idade média 82.7 anos), um ensaio de 12 semanas (n = 46, idade média 82.9 anos) e outro de 12 meses (n = 95, idade média 81.9 anos). A revisão estabeleceu a base teórica que associa o metabolismo energético comprometido à progressão da fragilidade, enfatizando o papel do músculo esquelético como órgão endócrino. Descreveu ainda como a concentração de miocinas, como a miostatina (MSTN) respondem ao exercício físico, estando envolvidas em processos fisiológicos que parecem melhorar a resiliência sistémica em adultos mais velhos. No entanto, a evidência é limitada. O estudo transversal mostrou que níveis séricos mais elevados de MSTN se associaram a menor probabilidade de fragilidade e a melhor função física. O treino concorrente de 12 semanas foi viável, melhorou o desempenho físico e a força de preensão manual, e reduziu os níveis de MSTN em residentes frágeis. Quando o mesmo treino foi aplicado durante 12 meses (ensaio pragmático) observaram-se melhorias no desempenho físico e na força de preensão manual, sem alteração da independência nas atividades básicas de vida diária, nem na taxa de quedas. Contudo, o grupo de cuidados habituais apresentou um agravamento significativo nestes desfechos, enquanto que o grupo de exercício manteve-se estável, sugerindo um efeito protetor.

No geral, esta tese apoia a viabilidade e eficácia do exercício concorrente na melhoraria da função física em residentes de lares, particularmente em indivíduos frágeis. Os resultados sugerem ainda que as miocinas, como a MSTN, podem mediar estes benefícios, justificando investigação adicional.

KEYWORDS: FRAGILIDADE, ENVELHECIMENTO, INTERVENÇÃO DE EXERCÍCIO FÍSICO, MIOCINAS, FUNÇÃO FÍSICA, CUIDADOS DE LONGA DURAÇÃO

Abstract

Frailty is characterised by reduced physiological reserves and an increased vulnerability to adverse outcomes such as physical function decline, dependency in basic activities of daily living and falls. Exercise is the most effective intervention to manage frailty, as it enhances the function and resilience of multiple physiological systems. However, the biological mechanisms through which exercise exerts such benefits are not fully understood. Evidence on the feasibility and effectiveness of exercise programs tailored to the most frail older adults is also limited. This thesis explores: I) the relationship between frailty pathophysiology, myokines, and physical function; and II) the feasibility and effectiveness of concurrent exercise (aerobic and resistance) interventions compared to usual care in nursing home residents. Four studies were conducted: a narrative review, a cross-sectional study (n= 59, mean age 82.7 years), a 12-week (n= 46, mean age 82.9 years) and a 12-month (n = 95, mean age 81.9 years) concurrent exercise trial. The review established a theoretical foundation linking impaired energy metabolism to frailty progression, emphasising the role of skeletal muscle as an endocrine organ. It also described how the concentration of myokines, such as myostatin (MSTN), responds to exercise, being involved in physiological processes that appear to improve systemic resilience in older adults. However, the evidence is limited. The cross-sectional study showed that higher serum MSTN levels were associated with lower odds of frailty and with better physical function. The 12-week concurrent training was feasible, improved physical performance and handgrip strength, and reduced MSTN levels in frail residents. When the same training was applied over 12 months (pragmatic trial), improvements in physical performance and handgrip strength were observed, with no changes in independence in basic activities of daily living or in the rate of falls. However, the usual care group showed a significant worsening in these outcomes, whereas the exercise group remained stable, suggesting a protective effect.

Overall, this thesis supports the feasibility and effectiveness of concurrent exercise in improving physical function in NH residents, particularly among frail individuals. The findings also suggest that myokines, such as MSTN, may mediate these benefits, warranting further investigation.

KEYWORDS: FRAILTY, AGEING, EXERCISE INTERVENTION, MYOKINES, PHYSICAL FUNCTION, LONG-TERM CARE

List of Abbreviations

Acetyl-CoA	Acetyl coenzyme A
ActRIIB	Activin type IIB Receptors
ADL	Activities of Daily Living
AEx	Aerobic exercise
ALM	Appendicular Lean Mass
AMPK	5’ AMP-Activated Protein Kinase
AMPKα2	AMPK α 2 Isoform
ATP	Adenosine 5’-triphosphate
AUC	Area Under Curve
BADL	Basic Activities of Daily Living
BAIBA	Beta-aminoisobutyric acid
BI	Barthel Index
BMI	Body Mass Index
BW	Body Weight
CG	Control Group
CI	Confidence Intervals
CV	Coefficients of Variation
DXA	Dual-energy X-ray Absorptiometry
DNA	Deoxyribonucleic acid
EG	Exercise Group
ELISA	Enzyme-linked Immunosorbent Assay
EV	Extracellular vesicles
FABP	Fatty Acid-Binding Proteins
FADH2	Flavin Adenine Dinucleotide
FNDC5	Fibronectin Type III Domain-containing Protein 5
FOXO	Forkhead box O
GDF	Growth/Differentiation Factor
GEE	Generalised Estimating Equations

GLMM	Generalised linear mixed model
GLP-1	Glucagon-like Peptide 1
GLUT4	Glucose Transporter Type 4
HDAC5	Histone Deacetylase 5
HGS	Handgrip Strength
HOMA-IR	Homeostatic Model Assessment for IR
ICER	Incremental Cost-effectiveness Ratio
IGF-1	Insulin-like Growth Factor 1
IL	Interleukin
IQR	Interquartile Range
IR	Insulin Resistance
IRR	Incidence Rate Ratio
IRS	Insulin Receptor Substrate
ITT	Intention-To-Treat
JAK	Janus kinases
KE	Knee Extension
KF	Knee Flexion
LPA	Light Physical Activity
Metrnl	Meteorin-like protein
MNA-SF	Mini Nutritional Assessment-Short Form
MSTN	Myostatin
mTOR	Mammalian Target of Rapamycin
MVPA	Moderate-to-Vigorous Physical Activity
NADH	Nicotinamide adenine dinucleotide hydrogen
NEFA	Non-esterified Fatty Acids
NF-κB	Nuclear Factor-kappa B
NH	Nursing homes
NLRP3	NOD-, LRR- and pyrin domain-containing protein 3
OR	Odds Ratio

OXPHOS	Oxidative Phosphorylation System
PGC-1α	Proliferator-activated Receptor-γ Co-activator 1α
PI3K	Phosphoinositide 3-kinase
PKB	Protein Kinase B
PKCθ	Protein Kinase C-theta
PP	Per-Protocol
PPAR	Peroxisome Proliferator-activated Receptor
PT	Peak Torque
RASM	Relative Appendicular Skeletal Muscle
RCT	Randomised Controlled Trial
REx	Resistance Exercise
RM	Repetitium Maximum
RNA	Ribonucleic Acid
ROC	Receiver Operating Characteristic
ROS	Reactive Oxidative Species
SD	Standard Deviation
SPPB	Short Physical Performance Battery
STAT	Signal Transducer and Activator of Transcription
TCA	Tricarboxylic acid
TGF-β	Transforming Growth Factor β
TLR4	Toll-like receptor-4
TNF-α	Tumor Necrosis Factor-alfa
UCP-1	Uncoupling Protein 1
VO2peak	Peak Value for Oxygen Uptake

Chapter I – General introduction

1. General introduction

The global population is experiencing a rapid demographic shift toward ageing. Forecasts indicate that by 2050, the number of individuals aged 65 and older will reach 1.6 billion, with those aged 80 or older being the fastest-growing segment of the population (United Nations Department of Economic and Social Affairs, 2022). While life expectancy has increased significantly over recent decades, the healthspan (e.g., years lived in good health or the period free from disease and disability) has not improved at the same rate. As longevity rises, so does the prevalence of age-related conditions such as dementia, sarcopenia, and frailty, which lead to functional decline and an increased demand for long-term care services, including institutionalisation in nursing homes (NH) during the later stages of life (Kingston et al., 2017).

Portugal is among the most aged countries globally, with individuals aged 65 and older comprising 24.1% of the total population (Instituto Nacional de Estatística, 2024). Currently, nearly 1% of the population resides in NH, with an occupancy rate exceeding 90% of the available services (Gabinete de Estratégia e Planeamento, 2024). These data underscore the pressing social and healthcare challenges associated with population ageing and the need for effective policy responses to ensure adequate support and care.

Functional impairment is a major risk factor for NH admission (Luppa et al., 2009); therefore, residents exhibit greater levels of frailty and dependency influenced by both their individual characteristics and the care environment. Indeed, NH routines often lead to sedentary behaviours and limit opportunities for physical activity (den Ouden et al., 2015). Without proper exercise interventions, residents' physical performance tends to decline rapidly over time (Masciocchi et al., 2019), leading to reduced mobility, a higher risk of falls, and loss of independence in basic activities of daily living (BADL). Additionally, there is an increased risk of frailty onset and progression (Dent et al., 2019; Wang et al., 2020). Thus, interventions to improve physical performance and functional ability are often a priority in this setting (World Health Organization, 2023).

Frailty is characterised by a state of diminished physiological reserves and increased vulnerability to adverse health outcomes, including physical decline, falls, hospitalisation, institutionalisation, disability, and death (Kim & Rockwood, 2024). Frail individuals often spend their final years with significant dependency, displaying lower intrinsic capacity and quality of life, and more frequently relying on healthcare services. This includes increased hospitalisations, extended lengths of stay, and the need for complex interventions to address complex health problems (Chi et al., 2021; Kojima, 2019). Owing to its profound impact on both individual and healthcare systems, frailty is now recognised as a major public health issue (Dent et al., 2025). However, its exact global prevalence remains uncertain, largely due to the absence of a standardised definition. A comprehensive systematic review and meta-analysis comprising data from 1,755,497 community-dwelling individuals aged 50 and older across 62 countries estimated a global frailty prevalence between 12% and 24% (O'Caoimh et al., 2021). Although these estimates are uncertain, frailty prevalence is expected to rise exponentially with age, particularly in those over 85 years, and varies by sex and setting. In this sense, frailty is particularly prevalent in NH, affecting over 50% of residents (Kojima, 2015). Despite this high prevalence, most studies are conducted on community-dwelling older adults, leaving a critical gap in interventions to improve the health and quality of life of the most frail NH residents.

Two main conceptualisations and definitions of frailty are often cited in the literature: frailty as deficit accumulation (e.g. frailty index) and the phenotype of frailty (e.g. physical frailty) (Kim & Rockwood, 2024). The first defines frailty as a state of age-related deficit accumulation, measured through the ratio of the present deficits (e.g. signs, symptoms, diseases, disabilities, laboratory values or biomarkers) to the total number of deficits assessed - frailty index (FI). A higher deficit ratio indicates a higher frailty status and increased vulnerability to adverse outcomes (Howlett et al., 2021). In contrast, physical frailty describes a phenotypic presentation of a definable biological syndrome (Fried et al., 2001). It is hypothesised that frailty arises when the dysregulation of multiple interconnected physiological and biological systems functions surpasses a

threshold (Fried et al., 2021). According to this definition, frailty is identified when an individual exhibits three or more signs and symptoms of five predefined criteria, including muscle weakness, slow gait speed, low physical activity, exhaustion, and unintentional weight loss. Individuals who met one or two criteria are classified as pre-frail, while those with none are considered robust. The higher the number of criteria, the more vulnerable an individual is (Fried et al., 2001). Both definitions recognise that individuals do not age equally, as persons of similar chronological age may exhibit differential risks of adverse health outcomes, such as mortality. However, these two conceptual frameworks tend to identify different subpopulations as frail (Howlett et al., 2021), hindering the comparison of results across studies.

In this thesis, we have defined frailty based on physical frailty (Fried et al., 2001). This definition distinguishes functional decline and disability as separate, independent entities from frailty while emphasising the critical role of skeletal muscle in its biological and physiological underpinnings (Fried et al., 2021). Furthermore, this perspective highlights the potential of exercise to modulate the onset and progression of frailty (Fried, 2016), aligning closely with the aims of this research.

Frailty presents a dynamic nature with possible natural transitions in both directions, but a progression to worsening frailty status is commonly observed (Gill et al., 2006; Xue et al., 2021). Given the multifaceted nature of frailty, identifying effective interventions is essential but challenging. With the appropriate interventions and management, it is possible to prevent, mitigate or even reverse frailty onset and progression (Kojima et al., 2019). Interventions that target multiple physiological systems, such as exercise and a comprehensive geriatric assessment, seem efficacious in preventing and reducing frailty, whereas those targeting a single physiological system do not (Fried et al., 2021). However, as frailty progresses, interventions to reverse, mitigate or manage its evolution become increasingly challenging to implement.

Among others, exercise training, including progressive resistance training either alone or as part of a multicomponent, combined or concurrent training

programme, is the recommended first-line intervention in frailty management (Dent et al., 2019). A combination of progressive resistance training and aerobic exercise is expected to produce beneficial biological effects that will potentially enhance the physiological function and resilience of multiple systems known to be dysregulated in frailty (Fried, 2016). These adaptations lead to improvements in frailty-related markers and adverse outcomes (e.g. physical decline, weakness, and exhaustion), as well as neuromuscular and cardiorespiratory function, ultimately resulting in improved overall physical function (Fried et al., 2021; Izquierdo et al., 2025; Izquierdo & Fiatarone Singh, 2023). However, the mechanisms through which exercise modulates key physiological systems to reduce frailty and enhance physical performance and functional capacity are not fully understood. One potential mechanism gaining attention is exercise-induced factors (e.g. exerkines) released from skeletal muscle and other organs into the blood in response to exercise. These molecules, which are cytokines, peptides, metabolites, nucleic acids and others, have autocrine, paracrine, or endocrine effects. They are believed to play a role in muscle regeneration, energy metabolism regulation, and anti-inflammatory actions (Chow et al., 2022). These exercise-related molecules can not only positively affect the musculoskeletal system but also sustain a network of organs and systems crosstalk, promoting geroprotector effects and enhancing the physiological function of multiple systems (Pedersen & Febbraio, 2012). Therefore, they provide a compelling theoretical framework supporting the positive effects of exercise on frailty.

The impact of physical activity and exercise in frail individuals has been studied for over three decades (Fiatarone et al., 1994). The 2018 Physical Activity Guidelines Advisory Committee (PAGAC) Scientific Report provides strong evidence that physical activity enhances physical function, improving walking, balance, strength, daily living activities, and quality of life (2018 Physical Activity Guidelines Advisory Committee Scientific Report, 2018). However, most evidence comes from trials targeting early frailty stages and community settings, likely due to challenges in implementing such interventions for the most frail individuals in long-term care environments. Logistical barriers, such as space and equipment limitations, staff constraints (particularly the lack of an exercise

specialist), and safety concerns, often hinder feasibility, alongside ethical considerations, particularly for individuals with cognitive impairment. Recruitment is also difficult due to severe cognitive and physical impairments, rapid health declines, and low motivation and confidence, which can potentially lead to high dropout rates (Provencher et al., 2014). Moreover, frail individuals often have a low tolerance for increased exertion (Lewsey et al., 2020), which may further limit participation in progressive exercise programs. A recent meta-analysis, including 105 exercise intervention studies in long-term care, highlighted key gaps in the literature, such as overall large heterogeneity in terms of exercise intervention and population characteristics, insufficient detail in reporting the exercise characteristics and a small number of studies (n = 13) targeting pre-frail and frail populations (Valenzuela et al., 2023).

Although significant progress has been made in understanding the positive effects of exercise on frailty, several critical gaps remain. First, there is limited evidence on the feasibility and effectiveness of exercise interventions, specifically tailored for the most frail older adults in NH. Second, while some studies have explored the biological pathways through which exercise may prevent or mitigate frailty, these mechanisms are not yet fully understood. In particular, exercise-induced molecules released from skeletal muscles, known as myokines, are increasingly recognised as a key area of interest. However, their relationship with frailty and the response to both acute and chronic exercise remains unclear. Therefore, this thesis has two primary objectives: I) to refine exercise intervention strategies in NH and II) to explore the underlying biological mechanisms involved in frailty management, with a focus on the role of myokines (**Figure 1**).

To accomplish these aims, the thesis is organised into 6 chapters. Chapter 2 presents a narrative review establishing the theoretical and biological framework, including concepts of energy metabolism, frailty, and myokines. It provides essential background information to help understand the mechanisms of frailty and how exercise influences these mechanisms through myokines. Lastly, it summarises the existing evidence from trials conducted in older adults regarding myokines. Chapter 3 details the overall design and methodology employed in the studies included in this thesis. Chapter 4 presents the original studies conducted

to meet the aims of this dissertation, while Chapter 5 provides a comprehensive discussion of the findings. Chapter 6 concludes with the main insights and implications of this thesis.

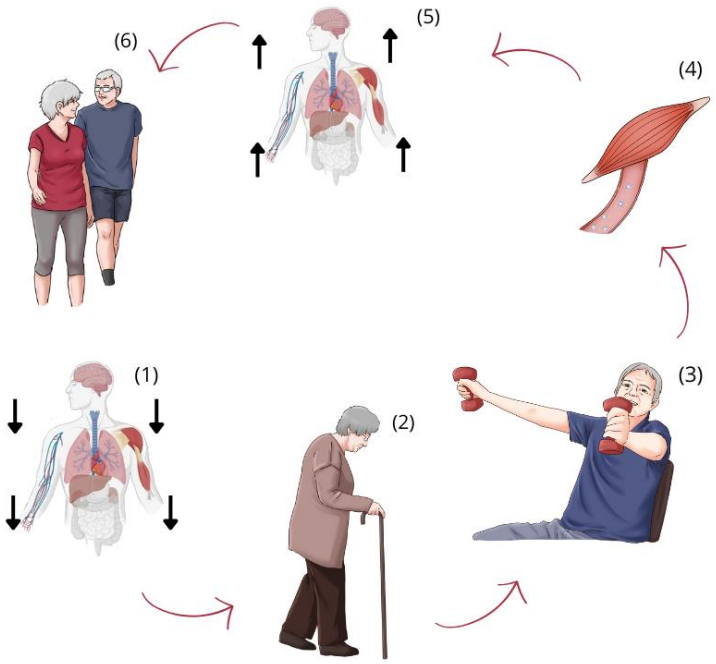

Figure 1. Conceptual framework illustrating the PhD hypothesis on the role of exercise and myokines in frailty. Age-related changes and chronic diseases reduce the physiological reserve across multiple systems (1). When dysregulation exceeds a critical threshold, homeostasis becomes severely impaired and frailty emerges (2). We hypothesise that exercise is both feasible and effective, even in individuals with advanced frailty, such as nursing home residents (3). Although the underlying mechanisms are not fully elucidated, exercise is proposed to stimulate the production and release of myokines (e.g. muscle-derived signalling molecules with both local and systemic effects) that enable organ crosstalk (4). These myokines can improve the function of biological and physiological systems that are dysregulated in frailty simultaneously (5). Ultimately, we propose that myokines mediate the beneficial effects of exercise in improving physical function and frailty status (6).

Chapter II – Theoretical framework

2. Theoretical framework

2.1 Study I

1. **Barros, D**., Marques, E. A., Magalhães, J., & Carvalho, J. (2022). Energy metabolism and frailty: The potential role of exercise-induced myokines - A narrative review. Ageing research reviews, 82, 101780. https://doi.org/10.1016/j.arr.2022.101780

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Energy metabolism and frailty: The potential role of exercise-induced myokines – A narrative review

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ABSTRACT

Frailty is a complex condition that emerges from dysregulation in multiple physiological systems. Increasing evidence suggests the potential role of age-related energy dysregulation as a key driver of frailty. Exercise is considered the most efficacious intervention to prevent and even ameliorate frailty as it up-tunes and improves the function of several related systems. However, the mechanisms and molecules responsible for these inter-system benefits are not fully understood. The skeletal muscle is considered a secretory organ with endocrine functions that can produce and secrete exercise-related molecules such as myokines. These molecules are cytokines and other peptides released by muscle fibers in response to acute and/or chronic exercise. The available evidence supports that several myokines can elicit autocrine, paracrine, or endocrine effects, partly mediating inter-organ crosstalk and also having a critical role in improving cardiovascular, metabolic, immune, and neurological health. This review describes the current evidence about the potential link between energy metabolism dysregulation and frailty and provides a theoretical framework for the potential role of myokines (via exercise) in counteracting frailty. It also summarizes the physiological role of selected myokines and their response to different acute and chronic exercise protocols in older adults.

1. Introduction

Frailty is a critical marker of aging that is defined as a state of increased vulnerability to negative health outcomes resulting from decreased reserves in multiple physiological systems (Fried, 2016). The contribution of several risk factors, such as aging, clinical and lifestyle factors (e.g. physical inactivity) in frailty onset and progression has been extensively described (Hoogendijk et al., 2019). However, frailty pathophysiology is complex, and despite the increasing interest by the scientific community, it is not consensual or well-defined. First, it is unknown if the multisystem physiological dysregulation is attributed to one specific biological driver, and second, only macro-level interventions such as physical activity seem to prevent, ameliorate and/ or reverse this condition (Fried et al., 2021). On the other hand, micro-level interventions such as monotherapies, hormonal supplementation, pharmacological interventions, or medication optimization have failed to consistently prevent and treat frailty status (Fried, 2016; Pazan et al., 2021).

Several age-related changes associated with frailty – with an emphasis on the skeletal muscle - are well recognized. These include body fat distribution resulting in increased ectopic fat accumulation, low-grade inflammation, alterations in muscle architecture, loss of muscle function and mass (sarcopenia), as well as mitochondrial dysfunction. All these changes impact muscle metabolism and insulin sensitivity (Shur et al., 2021). Interestingly, this can also be observed in younger populations that are highly physically inactive or in case of prolonged immobilization (Shur et al., 2021). Although the prevalence of frailty increases with age, not all older adults become frail. In addition, physical exercise seems to prevent, ameliorate and even reverse frailty by improving and up-tuning several interconnected physiological systems (Fried, 2016). Therefore, it can be hypothesized that these alterations are likely driven or exacerbated by physical inactivity rather than aging per se (Shur et al., 2021).

Some current knowledge gaps include the unknown mechanistic

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foundations behind physical inactivity and physical exercise that influence frailty course. The molecular and biochemical mechanisms related to exercise and inactivity are different (Booth et al., 2017), increasing their action's complexity. In brief, while physical inactivity produces a more persistent physiological stress of small magnitude inducing "negative" muscle adaptations, exercise produces (depending on its characteristics) a substantial short-term physiological stress on the muscle that causes beneficial adaptations and positive effects on metabolic health (Shur et al., 2021).

Recent evidence presents a compelling rationale for how physical inactivity could promote energetic imbalance and shrinking systems through metabolism dysregulation, which may trigger the onset and progression of frailty (Fried et al., 2021). Conversely, exercise may up-tune and regulate several systems simultaneously, partly through the secretory function of skeletal muscle, by producing and secreting hundreds of myokines with a crucial role in whole-body metabolic homeostasis, among other relevant effects (Chen et al., 2021). Nevertheless, in response to exercise, besides myokines, energy metabolism is also regulated by a complex joint work, although not yet well known, between adipokines, osteokines, and hepatokines (Antuna-Puente et al., 2008; Colaianni et al., 2020; Jensen-Cody and Potthoff, 2021). Additionally, extracellular vesicles (EVs) have also emerged as relevant players in intercellular communication due to their capacity to act as signaling vehicles and carriers of bioactive cargo such as proteins, lipids, and nucleic acids. Consequently, the recipient cells may alter their function and potentially contribute to the regulation of homeostasis and substrate metabolism in response to exercise (Murphy et al., 2020; Whitham et al., 2018).

Acute and chronic exercise elicit changes in the expression and circulating levels of myokines that are highly dependent on the exercise type and quantitative dimensions (frequency, duration, and intensity) and are linked to specific muscle fiber types (Domin et al., 2021). Nevertheless, the evidence is still scarce, and despite the novelty around these exerkines, the role of myokines in humans is still poorly understood. Some elegant reviews have been published (Lee and Jun, 2019; Pedersen, 2013; Pedersen and Febbraio, 2012; Severinsen and Pedersen, 2020); however, most data were obtained from healthy young adults (Domin et al., 2021; Gonzalez-Gil and Elizondo-Montemayor, 2020). Therefore, this review aims to describe the current evidence about the potential link between energy metabolism dysregulation and frailty and provide a theoretical framework for the potential role of myokines (via exercise) in counteracting frailty. We also explore and summarize the physiological role of selected myokines and their response to different acute and chronic exercise protocols in older adults.

2. Mechanisms of energy metabolism dysregulation linked to Frailty

Frailty is described as a physiological dysregulation that tends to appear later in life and arises when the dysregulation of multiple interconnected systems exceeds a threshold of dysfunction, critically compromising homeostasis and resulting in increased vulnerability to stress factors (Fried et al., 2021; Ghachem et al., 2021).

Recently, Fried et al. (2021) summarized the evidence about key systems responsible for homeostasis that are dysregulated in frail people, such as the energy metabolism, the stress response, and the musculoskeletal system. The specific biological drivers behind this dysregulation remain elusive, however, hallmarks of aging have been proposed as plausible drivers of frailty, particularly cellular senescence, loss of intercellular communication, and mitochondrial dysfunction (Fried et al., 2021). Interestingly, gathering evidence has interconnected cellular metabolism and mitochondrial dysfunction with other hallmarks of aging and age-related diseases (Amorim et al., 2022). There is consistent evidence that age-related traits such as chronic inflammation, increased oxidative stress and mitochondrial dysfunction are associated with frailty (El Assar et al., 2020).

Frailty is so complex that most certainly, no specific mechanism or pathway is fully responsible for its onset. In this sense, the multiple physiological dysregulation is expected to result from the joint effects of various "unfavorable" aging-related processes, and frailty phenotype emerges when a threshold is surpassed (Fried et al., 2021). Age-related changes in the immune system contribute to a low-grade inflammatory state named "Inflammageing", resulting from the imbalance between pro-inflammatory and anti-inflammatory mediators. Initially, this state was mainly attributed to the activation of macrophages. However, recent data have identified other contributing factors such as the over-activation of the adaptive immune system, senescent cells with pro-inflammatory secretory phenotype, gut dysbiosis, and mitochondrial dysfunction (Fulop et al., 2021). This state is a main pathophysiological factor underlying several age-related chronic diseases and frailty. Indeed, frail individuals tend to present higher pro-inflammatory molecules and increased counts of white blood cells, namely neutrophils and monocytes (Leng et al., 2007, 2009; Samson et al., 2019). Moreover, a sustained chronic inflammatory state seems to increase the odds of later frailty (Walker et al., 2019). In this sense, age-related frailty may present distinct pathophysiology from disease-related frailty and both often overlap (Angioni et al., 2020).

Another controversial perspective suggests that energetic imbalance may be a trigger of frailty as substrate utilization and energy exchange decrease due to aging, disuse, and stress (Fried et al., 2021). Therefore, frail and inactive individuals tend to present diminished energy flow, which increases the mismatching between structural needs and function across the organism leading to (mal)adaptations and higher vulnerability to stress factors (Fried et al., 2021). Indeed, it has been described that deleterious changes in glucose metabolism, energy-regulatory hormones, the capacity of the skeletal muscle to use energy efficiently, and mitochondrial energy production are all linked to frailty (Fried et al., 2021).

Interestingly, following this last perspective and considering that the energetic interface may have a greater role in frailty course than time (Bortz, 2009), we hypothesize that sustained physical inactivity can diminish the energy flow and consequently trigger adaptations across the organism with higher emphasis on energetic key organs (e.g., skeletal muscle and adipose tissue). In the visceral adipose tissue, physical inactivity is associated with the accumulation of fat, which in turn, when dysregulated, activates a network of inflammatory pathways (Pedersen, 2013). The increase in inter and intramuscular lipid accumulation in the skeletal muscle, may promote inflammation and mitochondrial dysfunction leading to impaired skeletal muscle responsiveness to energy-related demands (Correa-de-Araujo et al., 2020; Shoemaker et al., 2022). Moreover, these changes can result in energy metabolism dysregulation and altered substrate utilization due to impaired glucose metabolism and insulin resistance (IR), being important root causes of frailty (Booth et al., 2017; Fried et al., 2021; Kalyani et al., 2012; Shoemaker et al., 2022). On the other hand, physical inactivity might exacerbate age-related changes by amplifying the imbalance between muscle protein anabolic and catabolic pathways and consequently contribute to sarcopenia, which is considered the "biological substrate" of physical frailty (Cruz-Jentoft and Sayer, 2019; Landi et al., 2015).

As described by Booth et al. (2017), physical inactivity plays a central role in altering energy metabolism, substrate utilization and mobilization. The energy "currency" used by cells is Adenosine 5'-triphosphate (ATP) generated by mitochondrial oxidative phosphorylation. This complex process includes the utilization of acetyl coenzyme A (derived from glucose, amino acids, and fatty acids) in the tricarboxylic acid (TCA) cycle and the production of reducing equivalents in the form of nicotinamide adenine dinucleotide hydrogen (NADH) and flavin adenine dinucleotide ($FADH_2$) to supply the electron transport chain (Lanza, Sreekumaran Nair, 2010). Among others, the fuel sources for ATP-generation during physical activity are muscle glycogen stores and blood glucose, and fatty acids derived from triglycerides supply in both muscle and adipose tissue. The contribution of

these substrates to oxidative metabolism is mainly determined by activity intensity and duration. In this sense, fatty acid oxidation is more predominant than carbohydrate oxidation in low-intensity daily activities (Hargreaves and Spriet, 2020).

A recent study demonstrated that sarcopenic individuals exhibited traits of diminished metabolic "flexibility" as these individuals have an impaired ability to adapt and shift fuel utilization in response to different physiological conditions (Shoemaker et al., 2022). Sarcopenic individuals presented greater dependency on carbohydrates for energy production and showed diminished fat oxidation across several other physiological conditions compared to non-sarcopenic individuals. These results support the hypothesis that sarcopenic individuals are less able to process fat as fuel, probably due to impaired fat transport into the mitochondria or changes in the conversion of fatty acids into ATP (Shoemaker et al., 2022).

Rattray et al. (2019) discovered that metabolic dysregulation in vitamin E and carnitine shuttle energy mechanisms were associated with an increased risk of frailty, thus suggesting a potential general failure of the cell-based lipid metabolism at higher levels of frailty. These traits could result in an energetic deficit, particularly in high-energy-demanding tissues like the skeletal muscle. A recent study by van der Hoek et al. (2020) supported these results as decreased levels of total carnitines, particularly short-chain acylcarnitines, were found in pre-frail old females compared to age-matched fit peers and younger females. The authors also found that low intramuscular short-chain acylcarnitines were associated with decreased expression of genes encoding mitochondrial proteins needed for converting pyruvate to acetyl coenzyme A (acetyl-CoA), the TCA cycle, and all oxidative phosphorylation system (OXPHOS) complexes. These findings might suggest a diminished flux of acetyl-CoA into the TCA cycle and fewer electrons feeding into OXPHOS, resulting in less energy production (van der Hoek et al., 2020). The impaired state of oxidative metabolism, particularly during exercise and stress, might easily shift the metabolism toward glycolytic pathways (Ferrucci and Zampino, 2020). In fact, decreased mitochondrial function has been associated with increased utilization of anaerobic metabolism to meet energy demands, even in low-intensity activities, resulting in an accumulation of lactic acid (Liu et al., 2020). These shifts from aerobic to anaerobic-related pathways can contribute to earlier signs and symptoms of fatigue, muscle weakness, and exercise intolerance, which are consistent with the clinical presentation of frailty (Fried et al., 2001; Knottnerus et al., 2018).

Accordingly, recent untargeted proteomic analyses showed an association between higher expression of lipid metabolism-related proteins (i.e., fatty acid-binding proteins – FABP3/4) and frailty (Sathyan et al., 2020). These proteins are involved in intracellular long-chain fatty acid transport and lipolysis, which may enhance the rate of fatty acid uptake leading to the accumulation of lipids intermediates. A lower rate of fatty acid oxidation is also a favorable trait for lipid ectopic accumulation, particularly in skeletal muscle. Recently, Ahiawodzi et al. (2020) verified that non-esterified fatty acids (NEFAs) were positively associated in a dose-dependent manner with a higher risk of incident frailty and associated with self-reported exhaustion (a core frailty criterion). NEFAs can promote mitochondrial dysfunction and contribute to decreased production of ATP, resulting in insufficient intracellular energy production (Jang et al., 2020).

Altered mitochondrial dysfunction has been potentially linked to the onset and development of frailty, as pre-frail individuals presented a slower phosphocreatine recovery (a commonly used index of mitochondrial oxidative phosphorylation and energy repletion after exercise) and lower levels of respiratory complex protein content and activity in muscle when compared to non-frail / active individuals (Andreux et al., 2018; Varadhan et al., 2019). In a series of fatigability tests, frail, non-frail, and control groups showed similar levels of high-energy phosphate stored in skeletal muscle at fatigue (Lewsey et al., 2020). However, the rate of high-energy phosphate decline during exercise was 4–10-fold faster in frail individuals compared to the other groups, and is associated with exercise intolerance. High-energy phosphate depletion in skeletal muscle was inversely correlated to maximal mitochondrial oxidative capacity, and this latter was reduced by nearly 50% among older participants compared to middle-aged individuals. Frail individuals also presented a markedly increased muscle fat accumulation compared with the other groups. Taken together, these findings suggest that fast skeletal muscle energetic decline is associated with significantly reduced age-related mitochondrial oxidative capacity and augmented muscle fat accumulation in frail individuals (Lewsey et al., 2020).

Age-related changes in metabolism and body composition can result in sarcopenic obesity, which is the co-existence of excessive adiposity with low muscle function/mass (Batsis and Villareal, 2018). The mechanisms underlying this condition are complex to decipher but reinforce the cross-talk between adipose tissue and skeletal muscle. Excessive adipose tissue is associated with adipocyte hypertrophy and hyperplasia that induces dysregulated production of several adipokines and infiltration of immune cells (e.g., macrophages), consequently resulting in the production of pro-inflammatory cytokines and chemokines, thus leading to low-grade systemic inflammation (Franceschi et al., 2018; Surmi and Hasty, 2008). This pro-inflammatory state contributes to accelerated aging, being a risk factor for multiple diseases and a key feature in the pathogenesis of IR (de Luca and Olefsky, 2008; Ferrucci and Fabbri, 2018). The exacerbated production of lipids tends to accumulate ectopically in skeletal muscle. Moreover, excessive inter and intramuscular lipid accumulation may induce mitochondrial dysfunction by impairing β-oxidation and increasing reactive oxygen species (ROS) formation, thus creating a lipotoxic environment and promoting the secretion of pro-inflammatory myokines with deleterious effects (e.g., Myostatin) (Kalinkovich and Livshits, 2017). The self-sustained vicious cycle between adipose tissue and skeletal muscle inflammation is the primary driver of sarcopenic obesity. This condition increases the risk for adverse health outcomes such as disability, mortality, comorbidities, and frailty (Batsis and Villareal, 2018).

Evidence shows that age-related decreases in mitochondrial function also contribute to ectopic lipid accumulation and IR in the muscle (Petersen et al., 2003). Ectopic accumulation of intracellular lipids in skeletal muscle has also been associated with IR, even without peripheral and visceral adiposity. The mechanisms behind it are attributed to diacylglycerols responsible for triggering IR through the activation of protein kinase C-theta (PKCθ) in muscle (Shulman, 2014).

Insulin binds to an insulin receptor (a tyrosine kinase receptor) at the surface of muscle cells or fibers, which phosphorylates its corresponding receptors (IRS-1 and IRS-2), leading to sequential phosphoinositide 3-kinase (PI3K) and protein kinase B (PKB)/Akt activation. The activation of this pathway results in the translocation of insulin-sensitive glucose transporter type 4 (GLUT4) to the plasma membrane and a concomitant increase of glucose uptake into muscle (Leto and Saltiel, 2012). In this sense, IR is associated with impaired insulin-mediated glucose uptake (Honka et al., 2018).

In older adults, higher glucose levels and IR seem to significantly increase the risk for frailty (Peng et al., 2019; Rodríguez-Mañas et al., 2021; Zaslavsky et al., 2016). Indeed, Kalyani et al. (2012) found that altered glucose-insulin dynamics were associated with frailty as glucose and insulin responses were more exaggerated and prolonged in the frail compared to the other groups. Moreover, 6.5 % or more of hemoglobin A1c levels (a marker of diabetes) were associated with a greater prevalence of prefrail and frail status, independent of body mass index, inflammation, and chronic diseases (Blaum et al., 2009).

For the time being, the described evidence on the potential link between energy dysregulation and frailty onset is still controversial and reflects the current state of art. Nevertheless, such evidence is consistent with the theory of symmorphosis, suggesting that different structures are fine-tuned to varying levels of energy flow. This implies that reduced production and supply of energy at the cellular level could theoretically require the whole organism to operate below an "energetic optimum", as

described by Bortz (2008), leading to a decline in multisystem function and resilience (Bortz, 2002, 2008). Gathering evidence has shown that skeletal muscle can produce a cluster of endocrine factors (i.e., myokines) in response to muscle contractions that are responsible for physiological and metabolic adaptations in numerous tissues. Moreover, these exerkines allow crosstalk between organs and systems. This could also partly explain how physical exercise can refine several physiological systems at the same time by improving their function and resilience, increasing the energy flow, improving several age-related cellular features and the whole-body metabolism, and even reverting frailty (Fried, 2016; Garatachea et al., 2015).

3. Myokines linked to frailty

Skeletal muscle is the primary site for metabolic regulation of both energy and protein metabolism and is also a major site of glucose disposal in the postprandial period (Meng et al., 2017). Nowadays, it is considered a crucial secretory organ with endocrine functions (Steensberg et al., 2000) that can produce and secrete exercise-related molecules, the so-called myokines. These molecules are cytokines and other peptides released by muscle fibers that elicit autocrine, paracrine, or endocrine effects (Pedersen et al., 2003). Currently, it is proposed that myokines mediate, at least in part, some of the positive effects promoted by physical exercise in skeletal muscle as they might provide a feedback loop for muscle modulation, being involved in several muscular processes, such as muscle proliferation, differentiation, and regeneration (Henriksen et al., 2012). Importantly, during muscular contraction, myokines act not only within the muscle but also mediate muscle crosstalk with several other organs (e.g., key organs involved in the regulation of energy homeostasis), namely the adipose tissue, liver, pancreas, bone, and brain (Severinsen and Pedersen, 2020) by being secreted in a classical secretion pathway or by a delivery mechanism such as EVs into the bloodstream (Safdar and Tarnopolsky, 2018). It is assumed that EVs are messengers between cells communication mediating cross-organ signaling. Some studies support that EVs abundance and cargo are altered in response to exercise, being plausible carriers of exercise factors (e.g., myokines) (Darragh et al., 2021; Safdar and Tarnopolsky, 2018). For example, Whitham et al. (2018) observed an increase of 322 proteins after acute exercise; of those, a large proportion is thought to constitute EVs. The authors also demonstrated through enrichment analyses that EVs can deliver glycolytic enzymes to recipient cells and potentially alter their glycolytic rate, in response to exercise. Interestingly, Estébanez et al. (2021) verified that resistance training might modulate and attenuate inflammation in older adults. These authors found an increase of ~6.8% EV marker CD63 in the training group compared to ~42.5% increase in the control group, in the absence of changes in inflammatory mediators such as microRNA-146a and circulating cell-free DNA. In this sense, increasing evidence supports the role of EVs in delivering the positive effects of exercise across organs. Moreover, as these molecules can be found in circulation and other body fluids, there is an increased recognition that EVs can have relevant value as biomarkers of underlying pathophysiological conditions, including frailty (Carini et al., 2022; Ipson et al., 2018). However, despite the growing interest, the evidence regarding their biological activity, physiological relevance, and methodological approaches is still circumstantial and incomplete (Brahmer et al., 2020). A detailed discussion of these molecules is beyond the scope of this review, and we recommend the reader to see the following articles that provide an updated state of the art (Brahmer et al., 2020; Darragh et al., 2021; Denham and Spencer, 2020; Nederveen et al., 2020).

From a frailty viewpoint, myokines released from muscular contraction may partly synchronize nutrient sensing, delivery, uptake, and utilization, which is vital to maintaining whole-body homeostasis, from cells to physiological systems (Murphy et al., 2020). We speculate that by increasing physical activity (through exercise training), the energy flow and metabolic demands also increase; therefore, the organism has to adapt by up-tuning its function and structure and impacting systems resilience. In fact, decreased expression of these molecules could compromise the crosstalk between skeletal muscle and other organs (Pedersen and Febbraio, 2012), leading to the metabolic dysregulation observed in frail individuals.

Until now, only a few of these myokines have been biologically characterized for their activity and function in humans (Lee and Jun, 2019). Moreover, there is a lack of evidence about the effect of acute and chronic exercise on myokines expression and release in specific muscle atrophy models (Lee and Jun, 2019), such as aging, sarcopenia, and frailty. For this review, we selected myokines based on (1) being produced and secreted in the skeletal muscle and (2) having a role in energy metabolism or skeletal muscle remodelling, as this organ is highly affected in frail individuals, after searching high-quality recent reviews about myokines (Chow et al., 2022; Lee and Jun, 2019; Pedersen, 2013; Pedersen and Febbraio, 2012; Severinsen and Pedersen, 2020). The role of Myostatin, Decorin, IL-6, Irisin, IL-15, Meteorin-like protein (Metrnl), and Beta-aminoisobutyric acid will be further discussed as fair evidence of their action on energy metabolism and potential relationship with frailty is available. In each molecule, basic research data was firstly presented, followed by clinical evidence.

3.1. *Myostatin*

Myostatin (MSTN), also known as growth/differentiation factor-8 (GDF-8), is a member of the transforming growth factor β (TGF-β) superfamily (McPherron et al., 1997) that is a negative regulator of muscle growth and strength (Muramatsu et al., 2021) in an autocrine and paracrine manner. It is also closely involved in the maintenance of metabolic homeostasis and modulation of adipose tissue supporting muscle-fat crosstalk (Pedersen and Febbraio, 2012). Interestingly, myostatin diminishes muscle glucose uptake via inhibiting GLUT4 and 5' AMP-activated protein kinase (AMPK) (Paris et al., 2020).

Myostatin activity seems to be regulated by TGF-β family members antagonists, such as Follistatin and its related factors (FSTL1, FSTL3, and Decorin), mainly by blocking the binding of myostatin to the receptors (Gonzalez-Gil and Elizondo-Montemayor, 2020). Deletion of the myostatin gene leads to muscle hypertrophy seen in mice, cattle, and humans (Schuelke et al., 2004). It acts by mainly binding to activin type IIB receptors (ActRIIB), leading to the activation of Smad 2/3, which in turn reduces muscle protein synthesis by inhibiting the Akt/mammalian target of rapamycin (mTOR) pathway and stimulating forkhead box O (FOXO)-mediated muscle atrophy (Bowen et al., 2015; Hitachi et al., 2014). Myostatin may also induce oxidative stress by producing ROS in myotubes through tumor necrosis factor-alfa (TNF-α) signaling via nuclear factor-kappa B (NF-κB) and NADPH oxidase (Sriram et al., 2011). Early reports suggest that this myokine may also play a role in muscle-bone communication as it directly influences osteocyte function and inhibits osteoblastic differentiation (Qin et al., 2017), which supports muscle-bone crosstalk.

Studies with disease models of muscle wasting show an upregulation of myostatin (Anker et al., 2020); however, evidence about the relationship between myostatin and parameters of both sarcopenia and frailty is ambiguous. This controversy may reflect the confounding influence of age and total muscle mass, as most studies only report absolute myostatin levels (Baczek et al., 2020) or due to difficulties in its measuring since it has a close homology with other TGF-β members, namely growth/differentiation factor-11. For example, Arrieta et al. (2019) presented controversial findings as they observed higher concentrations of absolute serum myostatin in fitter, more active and non-frail individuals than in frail participants among institutionalized older adults. Conversely, Chew et al. (2019) observed that age-adjusted myostatin levels normalized for total body lean mass were higher in frail individuals with low relative appendicular skeletal muscle (RASM) when compared to their counterparts with normal RASM. Also, no differences in myostatin were observed among robust individuals,

independent of having normal or low RASM.

Previous studies reported that muscle myostatin mRNA and protein expression were higher in older adults than in younger males and females (Bertrand Léger et al., 2008; Raue et al., 2006). However, recent findings did not support a correlation between myostatin and age (Baczek et al., 2020; Ryan et al., 2017), meaning that myostatin is not likely to be a direct feature of aging but rather an indirect consequence of it. Nevertheless, some studies support that myostatin is associated with muscle mass and physical performance in older adults, which are core markers of frailty (Choi et al., 2021; Fife et al., 2018; Furihata et al., 2016; Peng et al., 2018).

From a frailty perspective, myostatin is a primary target as increased levels of myostatin may contribute to muscle atrophy while inhibiting insulin signaling, muscle mitochondrial biogenesis, and lipid oxidation. These traits may favor the onset of sarcopenic obesity (Consitt and Clark, 2018). The multiple adverse physiologic effects of myostatin have sparked an enormous interest in developing myostatin inhibitors as therapeutic agents for treating several muscle wasting conditions. However, many trials were unsuccessful (Anker et al., 2020). In this sense, exercise plays a key role in reducing myostatin expression, probably through the release of antagonist exerkines such as Follistatin (Hepatokine) and Decorin (Pedersen and Febbraio, 2012).

3.2. Decorin

Decorin is a small leucine-rich proteoglycan that is part of the extracellular matrix associated with collagen fibrils in the connective tissue and is released from contracting human myotubes in response to exercise (Kanzleiter et al., 2014). This myokine has been proposed as a counter-regulator of MSTN by directly binding and inhibiting the activation of the Smad 2/3 complex in a zinc-dependent manner (Miura et al., 2006). *In vivo,* overexpression of Decorin promoted an up-regulation of pro-myogenic factors Mighty, Myod1, Follistatin, and downregulation of muscle atrophy markers, such as Atrogin-1 and MuRF-1 (Kanzleiter et al., 2014; Zhu et al., 2007). Recent data suggest that this myokine is also an important factor in maintaining glucose tolerance (Svärd et al., 2019). Decorin mRNA expression significantly decreases in an age-dependent manner (Kanzleiter et al., 2014). However, despite the relevance of this novel myokine, more studies are needed to address its relationship with frailty (Coelho-Junior et al., 2019).

3.3. IL-6

IL-6 is a pleiotropic cytokine that is better known for its role as a pro-inflammatory cytokine, as it stimulates the secretion of acute-phase proteins, supporting the immune system (Lehrskov and Christensen, 2019). However, several studies support that IL-6 is also produced by skeletal muscle cells during exercise in the absence of inflammatory markers (Keller et al., 2006; Pedersen et al., 2003).

This myokine works as an energy sensor as intramuscular IL-6 mRNA expression, and protein release are intensified when intramuscular glycogen content levels are low (Pedersen, 2012a). IL-6 has a critical role in glucose metabolism homeostasis during exercise as it is responsible for the increase of endogenous hepatic glucose production during sustained exercise (Febbraio et al., 2004), while it increases glucose uptake, followed by translocation of GLUT4 to the plasma membrane in skeletal myotubes (Carey et al., 2006). Concomitantly, it increases lipolysis and fatty acid oxidation in the whole body, possibly via AMPK activation (Carey et al., 2006; van Hall et al., 2003). This myokine is also implicated in pancreatic β-cell metabolism and insulin secretion. Studies found that the secretion of exercise-induced glucagon-like peptide 1 (GLP-1) is regulated by IL-6 signaling (Ellingsgaard et al., 2011, 2020). Moreover, IL-6 stimulates nutrient uptake and catabolism (e.g., glucose and free fatty acids) into myofibers during exercise in an osteocalcin-dependent manner (osteokine), and this positive feedback loop is required to increase muscle function (Chowdhury et al., 2020). Taken this finds together, this myokine can be considered a key energy coordinator during exercise (Kistner et al., 2022).

In addition to its critical role in "fuel" metabolism, this myokine also mediates the anti-inflammatory effect of exercise as its increases are followed by an augmented expression of other anti-inflammatory cytokines, such as IL-1 receptor antagonist and IL-10, and inhibition in the production of the pro-inflammatory cytokine TNF-α (Petersen and Pedersen, 2005).

Although IL-6 secreted from skeletal muscle promotes hypertrophy via satellite cell proliferation and differentiation (Serrano et al., 2008), this cytokine is generally investigated regarding its relationship with low-grade inflammation and its detrimental impact on muscle mass and functionality in older adults. Indeed, findings suggest that IL-6 levels are systematically increased in an age-dependent manner (Puzianowska--Kuźnicka et al., 2016), and they seem to be increased in individuals with sarcopenia (Rong et al., 2018), in pre-frail and frail older adults (Soysal et al., 2016). Moreover, numerous studies confirmed a negative correlation between chronic inflammation, namely basal IL-6, and skeletal muscle strength, quality, and function (Brinkley et al., 2009; Cohen et al., 1997; Custodero et al., 2020; Grosicki et al., 2020; Tay et al., 2019).

Taken together with the described evidence, IL-6 has high clinical relevance in frailty as this pleiotropic cytokine acts as a multi-organ energy coordinator in response to exercise, and is responsible for orchestrating both pro- and anti-inflammatory processes.

3.4. Irisin

Irisin is a multifunctional adipomyokine that is released into the bloodstream in response to exercise (Boström et al., 2012). Early findings reported that exercise induces the expression of the transcriptional regulator peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α) in skeletal muscle, a master regulator that interacts with a broad range of transcription factors that are involved in a wide variety of biological responses, such as the expression of fibronectin type III domain-containing protein 5 (FNDC5) (Boström et al., 2012). This latter is proteolytic cleaved and secreted into the bloodstream as irisin. This myokine is thought to trigger the conversion of white fat cells into brown-in-white or beige cells (browning effect) and increases thermogenesis through the expression of uncoupling protein 1 (UCP-1) (Pedersen, 2012b). In addition to the critical role in energy homeostasis, irisin has also been described as an important myokine in the bone, brain, and skeletal remodulation (Estell et al., 2020; Islam et al., 2021; Lourenco et al., 2019).

In vitro studies showed that irisin might have anti-inflammatory properties, as irisin treatment polarized M0 and M1 macrophages towards an M2 phenotype leading to an increased expression of anti-inflammatory factors and decreased expression of pro-inflammatory cytokines (e.g., TNF-α), particularly in visceral and subcutaneous fat tissue (Li et al., 2019; Ye et al., 2020). Another recent study found that irisin helps to mitigate β-cell IR and inflammatory response through the activation of PI3K/Akt/FOXO1 insulin signaling pathway and the inhibition of Toll-like receptor-4 (TLR4)/NF-κB inflammatory signaling pathway in vivo and in vitro models (Zheng et al., 2021). In addition, irisin might increase mitochondrial respiration and glycolysis in visceral adipocytes by a UCP-1 independent pathway (Li et al., 2019).

In mice, irisin functions as a pro-myogenic factor that increases myogenesis via IL-6 signaling. Also, in response to a skeletal muscle injury, irisin seems to improve regeneration and induce hypertrophy through the activation of satellite cells and enhanced protein synthesis (Reza et al., 2017). Irisin is also involved in muscle growth through the induction of Insulin-like growth factor 1 (IGF-1) and suppression of myostatin (Huh et al., 2014a).

In humans, irisin is associated with muscle mass, quality, strength, and glucose and lipid metabolism through AMPK activation (Chang

et al., 2017; Kurdiova et al., 2014; Park et al., 2019; Planella-Farrugia et al., 2019). Older adults tend to present lower circulating levels of irisin than younger adults (Huh et al., 2014b). In this sense, this myokine has been linked to sarcopenia (Chang et al., 2017; Park et al., 2019; Zhao et al., 2020). In fact, Chang et al. (2017) found that serum irisin was associated with the incidence of sarcopenia, even after adjustment for important confounders, such as sex, age, and fat indices. Nevertheless, it is important to acknowledge that a recent study found conflicting results (Baek et al., 2022).

In sum, irisin might play a key role in frailty status since it acts in multiple tissues and organs. This myokine might improve general energy metabolic balance while enhancing cellular homeostasis by optimizing autophagy. It promotes mitochondrial quality control, reduces ROS production, and mitigates inflammatory responses due to its anti-inflammatory role.

3.5. IL-15

IL-15 is a cytokine that belongs to the IL-2 superfamily and communicates downstream effects via the Janus kinases (Jak) 1 and 3, signal transducer and activator of transcription (STAT) 3 and 5 pathways (Nelke et al., 2019). It was originally described as a cytokine involved in T-cell growth factor (Berard et al., 2003). The evidence about the effect of aging in this myokine is still conflicting. While some animal models have shown an increase in IL-15 mRNA, others demonstrated a decrease in serum and protein expression (Quinn and Anderson, 2011). In humans, serum IL-15 concentrations seem to follow a descending non-significant trend with age, however in very old individuals (95 or more) it appears to be significantly elevated (Gangemi et al., 2005).

Preclinical studies demonstrated that IL-15 is associated with the regulation of lipid and glucose metabolism in skeletal muscle (Lutz and Quinn, 2012; Nielsen et al., 2008) by activating the Jak3/STAT3 signaling pathway to mediate glucose uptake (Krolopp et al., 2016). Also, its overexpression improved glucose tolerance and promoted GLUT4 translocation via the AMPK pathway (Fujimoto et al., 2019). Concomitantly, it seems that IL-15 also ameliorates mitochondrial oxidative function (Nadeau et al., 2019) and mitochondrial activity through the peroxisome proliferator-activated receptor (PPAR) delta PPARδ-dependent mechanism in skeletal muscle cells (Thornton et al., 2016), while reducing intracellular ROS production and attenuating oxidative stress (Li et al., 2014). Findings from mice studies demonstrated that circulating IL-15 has a role in regulating body composition by modulating adipose tissue deposition (Quinn et al., 2009). In fact, it was later confirmed that IL-15 correlates negatively with adiposity indexes, particularly visceral fat, in humans (Hingorjo et al., 2018).

IL-15 is believed to promote myogenesis and even act as a compensatory factor, expressed to mitigate inflammatory processes related to skeletal muscle atrophy (Duan et al., 2017; O'Leary et al., 2017). Controversial evidence points out that IL-15 might not be an anabolic factor, at least in healthy organisms (Nadeau and Aguer, 2019), but this topic remains under discussion. A study suggests an association between low levels of plasma IL-15 and sarcopenia in older adults (Yalcin et al., 2018); however, evidence is still scarce.

From a frailty perspective, this myokine could act as an energy regulator by improving mitochondrial function, downregulating lipids' accumulation and improving lipid and glucose metabolism through muscle-fat crosstalk.

3.6. Meteorin

Meteorin-like protein (Metrnl) is a novel myokine that increases energy expenditure by stimulating the expression of genes associated with beige fat thermogenesis, stimulates anti-inflammatory cytokines, and improves glucose tolerance in response to exercise (Jung et al., 2018a; Rao et al., 2014). Indeed, in an in vitro study, Metrnl increased glucose uptake via the calcium-dependent AMPK α 2 Isoform (AMPKα2) pathway and increased the phosphorylation of Histone Deacetylase 5 (HDAC5), which later resulted in the activation of GLUT4 transcription (Lee et al., 2020). In the same study, the authors also found that the injection of recombinant Metrnl improved glucose tolerance in mice with obesity or type 2 diabetes. Therefore, Metrnl may have a beneficial role in glucose metabolism through AMPKα2 (Lee et al., 2020). In skeletal muscle cells, Metrnl may also have a role in muscle regeneration and repair through Stat3/IGF-1 mechanism (Baht et al., 2020).

Studies in mice showed that adipocyte-specific overexpression of Metrnl promotes adipogenesis and controls insulin sensitivity locally through the PPAR gamma (PPARγ) pathway (Li et al., 2015). Metrnl seems to mediate muscle-fat crosstalk during exercise to inhibit NLRP3 inflammasome activation and inflammation in adipose tissues by suppressing macrophage-induced IL-1β secretion (Javaid et al., 2021).

The potential role of Metrnl on metabolic and inflammatory diseases has been studied, but data from clinical studies are contradictory (Miao et al., 2020). While an increased expression of Metrnl was found in type 2 diabetes and obese individuals (AlKhairi et al., 2019), decreased serum levels of Metrnl were found in individuals with coronary artery disease or type 2 diabetes and negatively correlated to inflammatory markers (i.e., IL-6 and TNF-α) when compared to controls (Dadmanesh et al., 2018). Nevertheless, a recent systematic review and meta-analysis showed that circulating Metrnl levels are not significantly different in type 2 diabetes patients compared with normal glucose tolerance controls. The authors argued that these inconsistencies could be due to the influence of confounders, such as age, body mass index (BMI), homeostatic model assessment for IR (HOMA-IR), and sample size (Wu et al., 2020).

Evidence suggests that Metrnl seems to be inversely correlated with visceral fat obesity in individuals with type 2 diabetes (Du et al., 2020), and lower serum Metrnl level is correlated with weight loss and the severity of cardiac dysfunction among older adults with chronic heart failure (Cai et al., 2021). A novel study also demonstrated that Metrnl can protect against cardiac hypertrophic development in mice (Rupérez et al., 2021).

Taken together, these findings suggest that Metrnl may mediate the possible positive effects of exercise against cardiometabolic disorders by playing a role in muscle-fat crosstalk (Das et al., 2020), exerting anti-inflammatory effects and improving insulin resistance. However, the evidence in humans is limited.

3.7. Beta-aminoisobutyric acid

Beta-aminoisobutyric acid (BAIBA) is a non-proteinogenic amino acid consisting of two enantiomers, L-BAIBA and D-BAIBA. BAIBA was recently discovered as a myokine secreted from myocytes after muscle contraction under the control of PGC-1α (Yang and Kwon, 2020). This myokine induces browning of white adipose tissue and stimulates hepatic fatty acid oxidation through a PPARα-dependent pathway, thereby increasing energy expenditure. L-BAIBA also acts as a bone-protective factor that prevents osteocyte cell death induced by ROS; however, this capacity seems to be lost with aging (Kitase et al., 2018). In addition, BAIBA attenuates IR, suppresses inhibitory κBα phosphorylation, NF-κB nuclear translocation and downstream inflammatory cytokines, and induces fatty acid oxidation via the AMPK-PPAR delta pathway in skeletal muscle of mice (Jung et al., 2015, 2018b). In line with these findings, Roberts et al. (2014) found that BAIBA levels were inversely correlated with fasting glucose, insulin, HOMA-IR, triglycerides, and total cholesterol in adults. Moreover, it was recently reported that physical inactivity was associated with lower plasma levels of BAIBA (Molfino et al., 2019).

Interestingly, a study demonstrated that frail older adults presented lower levels of BAIBA when compared with non-frail peers (Fazelzadeh et al., 2016). However, it was also observed that BAIBA levels were higher in the healthy older subjects compared with younger subjects (Fazelzadeh et al., 2016). Thus, it is reasonable to assume that other

unknown confounding factors could affect BAIBA expression and studies exploring the full action of this myokine (in addition to its promising role in energy metabolism) are needed.

Taken together, BAIBA seems to enhance mitochondrial β-oxidation, reduce lipid accumulation and attenuate IR while protecting against inflammation, which may improve energy regulation in frail status.

3.8. Implication of myokines actions on frailty

Exercise is known to improve frailty, frailty core signs and symptoms, and underlying biological markers (Angulo et al., 2020). Indeed, lifelong exercise seems to delay age-related skeletal muscle decline by maintaining skeletal muscle structure, function, bioenergetic characteristics, and phenotype (Zampieri et al., 2015). Exercise seems to improve the function of multiple interconnected systems related to frailty (Fried, 2016). This effect may be partly mediated by myokines that act as mediators between organs crosstalk and could exert their biological action in an autocrine, paracrine, or endocrine manner.

Regarding inflammation, exercise seems to induce the release of many factors that synergistically orchestrate an anti-inflammatory milieu. Exercise suppresses inflammation in adipose tissue through the suppression of macrophage infiltration and the phenotypic switching of macrophages (Kawanishi et al., 2010), and myokines may be mediating these effects. In response to exercise, increases in IL-6 seem to raise the expression of IL-1 receptor antagonist and IL-10, and inhibit the production of the pro-inflammatory cytokine TNF-α (Pedersen and Febbraio, 2012). Concomitantly, increases in irisin seem to shift macrophages phenotype action on adipose tissue from M1 (pro-inflammatory) toward M2 (anti-inflammatory), while decreasing the expression of TLR4, TNF-α, IL-1β and increasing IL-10 (Ye et al., 2020). In addition, BAIBA might suppress the secretion of pro-inflammatory cytokines such as TNFα (Jung et al., 2015, 2018b) and Metrnl can inhibit NLRP3 inflammasome activation and inflammation in adipose tissues, by suppressing macrophage-induced IL-1β secretion (Javaid et al., 2021). Moreover, Metrnl can indirectly stimulate M2 polarization by inducing eosinophils to secrete IL-4 and IL-13 (Rao et al., 2014). IL-15 might mitigate the action of TNF-α while stimulating adiponectin release, which attenuates the inflammatory response (Quinn et al., 2005). Decorin might bind and inactivate myostatin, thus suppressing its pro-inflammatory actions (Kanzleiter et al., 2014). TNF-α and other proinflammatory factors induce IR by directly disrupting the canonical insulin signaling pathway or by promoting the activation of other additional inflammatory pathways (Tilg and Moschen, 2008). In this sense, myokines-induced suppression of inflammation, namely TNF-α, can reduce IR. In addition, most of these myokines are insulin sensitizers, improving glucose uptake and tolerance mainly via GLUT4 translocation.

In response to exercise, stimulated lipolysis can lead to increased circulation of free fatty acids, which, if not metabolized in the liver or skeletal muscle, could ectopically accumulate elsewhere resulting in higher levels of inflammation, oxidative stress, and IR (Gonzalez-Gil and Elizondo-Montemayor, 2020). Myokines such as irisin (Boström et al., 2012), BAIBA (Roberts et al., 2014), and Metrnl (Rao et al., 2014) can trigger the conversion of white fat cells into brown-in-white or beige cells "browning effect" which could increase energy expenditure and the whole-body capacity to oxidize fat, thus, counteracting ectopic lipid accumulation (Gonzalez-Gil and Elizondo-Montemayor, 2020). In addition, these myokines, under the control of PGC-1α, seem to play an important role in increasing fatty acids oxidation, which could directly improve mitochondrial function (Pang et al., 2021).

In summary, myokines appear to be implicated in reducing inflammation by promoting an anti-inflammatory milieu, which could disrupt the vicious cycle of chronic inflammation, energy dysregulation, IR, mitochondrial dysfunction, and ectopic lipid accumulation, which are traits of frail individuals (Fried et al., 2021). Lastly, these exercise messengers also exert positive benefits in distant organs, which could also explain their usefulness in improving the function of several interconnected systems (Severinsen and Pedersen, 2020).

4. Myokines response to exercise

Myokines are key players that make possible the fine orchestra of inter-organs communication triggered by exercise (Priest and Tontonoz, 2019). Exercise elicits muscle adaptations by increasing the expression of numerous enzymes involved in aerobic and anaerobic metabolism. It also increases the sensitivity of adipose tissue to epinephrine-stimulated lipolysis and the utilization of intramuscular triglycerides (Priest and Tontonoz, 2019). The metabolic demands to exercise greatly vary depending on the exercise intensity and the ATP needed; therefore, many myokines can be highly dependent on exercise structure and muscle fiber type (Domin et al., 2021; Nielsen et al., 2007).

For this narrative review, PubMed and Scopus were searched using the following keywords: "Myostatin", "IL-6", "IL-15", "Decorin", "Beta-aminoisobutyric acid", "Meteorin-like protein", "Meteorin", "Irisin" in a set of queries. For each specific query, the following Boolean search strategy was used: myokine AND ("older adults" OR "Elders" OR "Seniors") AND ("exercise" OR "training" OR "Bout"). Additionally, the search was complemented by a manual review of reference lists from relevant articles.

Inclusion criteria comprised (1) experimental design studies that (2) were written in English from inception to January 31, 2022. (3) Studies that involved both younger and older populations were included, but only data on older adults were analysed. Articles conducted exclusively in younger populations, animal studies, observational studies, editorials, and commentaries were excluded. Studies were also excluded if exercise characteristics were not provided or if the article was written in another language than English.

The selected articles were screened for eligibility by looking at the title and abstract. The full text was analysed if the abstract did not provide appropriate information. Afterwards, relevant articles were read more carefully. From our search, we were able to include 12 and 32 studies about the acute and chronic effects of exercise, respectively.

4.1. The acute effect of exercise on myokines

The summary of the available 12 studies of acute exercise-induced myokines in older adults (with a total of 243 subjects) is presented in Table 1.

Most studies were on older men with sample sizes ranging from 6 to 37 individuals (41.7 % of the studies had 10 or fewer individuals in one group). Resistance exercise – using a variable-resistance machine or elastic resistance band – was the most common type of acute intervention (8/12, 66.7 %) (Hulmi et al., 2007; Kim et al., 2005; Raue et al., 2006; Rioux et al., 2021; Snijders et al., 2014; Urzi et al., 2019; Wessner et al., 2019; Ziegler et al., 2019). Overall, resistance exercise intensity ranged between 65 % and 80 % of 1 repetition maximum (1-RM), and aerobic exercise intensity was set from 40% to 70% of peak power output or at 50 % of maximum attained peak value for oxygen uptake (VO_2peak) or at 50–75% of heart rate reserve.

Overall, studies exploring the acute effect of exercise on myokines concentration /expression have shown a reduction of myostatin (Hulmi et al., 2007; Kim et al., 2005; Raue et al., 2006; Snijders et al., 2014; Wessner et al., 2019), an increase of IL-6 levels (Bizjak et al., 2021; MacNeil et al., 2020; Ziegler et al., 2019), whereas inconsistent results were found on irisin (Bizjak et al., 2021; Rioux et al., 2021; Tsai et al., 2021) and IL-15 concentrations (MacNeil et al., 2020; Urzi et al., 2019). Our review supports that myokines response to an acute bout of exercise aims to maintain metabolic homeostasis and adapt fuel utilization, along with an equilibrium between acute inflammation and anti-inflammatory mediators in older adults, similar to younger populations. By contrast, when exercise is repeated and sustained, these myokines can provide a feedback loop for the muscle to adapt to chronic exercise (Huh, 2018) in

Table 1
Summary of myokine studies: effects of an acute bout of exercise.

Study	Study Groups	Total Sample size[a] (% male), Group, sample size (mean age ± SD)	Exercise session	Blood Sampling time	Main findings[c]
	Myostatin				
Wessner et al. (2019)	EG1: Former men weightlifters EG2: Untrained Men	n = 16 (100%) EG1 = 8 (61.0 ± 8.9 yrs) EG2 = 8 (61.4 ± 8.1 yrs)	REx: 3 sets of unilateral knee extension exercise using a variable-resistance machine until voluntary fatigue at 70–75% of 1-RM, on the dominant leg	Resting and 3 h after exertion[b]	EG1: ↓ EG2: ↓ EG1 vs EG2: =
Snijders et al. (2014)	EG1: Older Men	n = 10 (100%) EG1 = 10 (73 ± 1 yrs)	REx; 6 sets of 10 reps of leg extension and leg press using a variable-resistance machine at 70% of 1-RM	Resting, 12 h after exertion, 24 h after exertion,48 h after exertion and 72 after exertion[b]	EG1: ↓ 24–72 h
Hulmi et al. (2007)	EG1: Untrained Healthy EG2: Untrained Healthy	n = 18 (100%) EG1 = 11 (60.9 ± 5.0 yrs) EG2 = 7 (63.9 ± 7.4 yrs)	REx: 5 sets of bilateral knee extension exercises using a variable-resistance machine at 10RM	30 min before exertion, 1 h after exertion and 48 after exertion[b]	EG1: ↔1 h/48 h before training; ↓48 h after 21 wks of REx EG2: ↔ 1 h/48 h before training; ↔ 1 h/48 h after 21wks of normal routine EG1 vs EG2: NR
Raue et al. (2006)	EG1: Older women	n = 6 (0%) EG1 = 6 (85 ± 1 yrs)	REx: 3 sets of 10 repetitions of bilateral knee extension using a variable-resistance machine at 70% of 1-RM	Before exertion and 4 h after exertion[b]	EG1: ↓
Kim et al. (2005)	EG1: Older Women EG2: Older Men	n = 18 (50%) EG1 = 9 (64.3 ± 1.0 yrs) EG2 = 9 (65.1 ± 1.7 yrs)	REx: 3 sets of 8–12 reps of bilateral squat, leg press, knee extension using a variable-resistance machine at 80% of 1 RM	Before exertion and 24 h after exertion[b]	EG1: ↔ EG2: ↓ EG1vs EG2: NR
	IL-6				
Ziegler et al. (2019)	EG1: Older adults EG2: Older adults	n = 25 (72%) EG1 = 13 (67, 62–69 yrs)[d] EG2 = 12 (66, 62–70 yrs)[d]	REx: 3 sets of 12 reps of unilateral knee extension exercise using a variable-resistance machine at 70% of 1-RM, on the dominant leg	Resting, 3 h after exertion	EG1: ↑ before training; ↑ after 48 wks of REx training EG2: ↑ before training; ↑ after 48wks of normal routine EG1 vs EG2: =
MacNeil et al. (2020)	EG1: Sedentary EG2: Physically active	n = 37 (64.9%) EG1 = 23 (71.8 ± 7.1 yrs) EG2 = 14 (71.7 ± 5.6 yrs)	AEx: a 30-min cycling session at 50% of maximum attained VO2peak	Resting and immediately after exertion	EG1: ↑IL-6; ↔IL-15 EG2: ↑IL-6; ↑IL-15 EG1 vs EG2: NR
Windsor et al. (2018)	EG1: Lower physical fitness EG2: Higher physical fitness	n = 30 (88.7%) EG1 = 16 (72 ± 6 yrs) EG2 = 14 (69 ± 6 yrs)	AEx1: 24-min moderate-intensity continuous cycling at 40% of peak power output; AEx2 higher-intensity interval cycling (12 at 70% of peak power output x 1 min at 10% of peak power output; C: 24-min rest in an upright seated position.	Immediately before exertion, immediately after exertion, 20 min and 90 min after exertion	EG1: AEx1: ↑; AEx2: ↑; C: ↑ EG2: AEx1: ↑; AEx2: ↑; C: ↑ EG1 vs EG2: =
Bizjak et al. (2021)	EG1: Higher Physical Fitness EG2: Lower Physical Fitness	n = 28 (42.9%) EG1 = 14 (74.4 ± 5.7 yrs) EG2 = 14 (76.1 ± 5.2 yrs)	AEx: cardiopulmonary exercise test – starting at 25 W with a continual increase of 15 W/min on a stationary bicycle	Immediately before exertion, immediately after exertion	EG1: ↑ IL-6; ↔ Irisin EG2: ↑IL-6; ↔ Irisin EG1 vs EG2: NR
	Irisin				
Rioux et al. (2021)	EG1: Inactive Overweight	n = 14 (38.46%) EG1 = 14 (67.7 ± 4.1 yrs)	REx: 3 sets of 2–15 reps of 6 full-body exercises using free weights, variable-resistance exercise machines, and the participant's body weight at 65–70% of 1-RM	Immediately before exertion, at 15 min, 30 min, and 45 min of exertion, 45 min after exertion	EG1: ↔ at 15 min, ↔ at 30 min, ↔ at 45 min, ↔ 45 min after
Tsai et al. (2021)	EG1: Healthy Older adults	n = 21 (47.6%) EG1 = 21 (60.62 ± 4.96 yrs)	AEx1: 30 min of high-intensity intervals (1 min at 70–75% heart rate reserve) on a stationary adjustable bicycle; AEx2: 30 min of moderate-intensity exercise at 50–55% heart rate reserve on a stationary adjustable bicycle; C: 35 min of rest (i.e., sitting and reading magazines)	Immediately before exertion, immediately after exertion	EG1: AEx1: ↑; AEx2: ↔; C: ↔ AEx1 vs AEx2 vs C: + AEx1
	IL-15				
Urzi et al. (2019)	EG1: Female nursing home residents EG2: Female nursing home residents	n = 20 (0%) EG1 = 11 (84.4 ± 7.7 yrs) EG2 = 9 (88.9 ± 5.3 yrs)	REx: 45–50 min, 2–3 sets of 5 reps of 10 elastic bands exercises at 12–14 BRPE scale	Immediately before exertion, immediately after exertion, 2 h after exertion	EG1: ↔ EG2: ↔ EG1 vs EG2: =

Abbreviation: AEx: Aerobic exercise, BRPE: Borg Rate of Perceived Exertion scale, C: Control, EG: Exercise group, min: minutes, NR= not reported, reps: repetitions, REx: Resistance exercise, VO2peak: peak oxygen uptake, wk: week, years= yrs, ↑ significant increase, ↔ no change, ↓significant decrease, + Significant superior intervention effect compared to the other group, = Non significant intervention effect

[a]number of participants included in the analysis (final sample size).

[b]Biopsy sampling time.

[c]Baseline vs post-exercise, between-group differences.
[d](mean, range).

terms of long-term metabolic adaptations and decreased inflammation (Chow et al., 2022).

Myostatin plays a role in the adaptive remodelling of skeletal muscle in response to exercise; thus, it is expected that a reduction in myostatin expression or function might be needed for muscle hypertrophy, probably mediated by c-Jun N-terminal kinase (Lessard et al., 2018). As observed in animal models and suggest by Kim et al. (2005), myostatin expression seems closely related to mechanical load.

In response to acute resistance exercise, myostatin RNA expression seems to decrease in older adults (Hulmi et al., 2007; Kim et al., 2005; Raue et al., 2006; Snijders et al., 2014; Wessner et al., 2019), in a similar way to younger individuals, regardless of gender (Kim et al., 2005; Raue et al., 2006).

Current knowledge suggests that myostatin mRNA expression decreases between 2 and 24 h after exertion (Louis et al., 2007), which could explain why Hulmi et al. (2007) have not detected significant changes 1 h after exertion. However, the author detected a significant decrease at 48 h post-exercise after a 21-week training. It is reasonable to assume that myostatin plays a role during recovery and that older individuals may be less efficient in downregulating myostatin compared to younger individuals (Snijders et al., 2014). Also, although these results may suggest that training status could potentially affect myostatin expression in response to acute exercise, Wessner et al. (2019) demonstrated that untrained and trained older adults responded in a very similar way. Nevertheless, differences in session protocol and biopsy sampling time could potentially explain differences in results (Allen et al., 2011).

Currently, it remains unknown whether myostatin is responsive to acute aerobic exercise in older as in younger adults (Allen et al., 2011). Moreover, it is not clear whether changes in muscular myostatin mRNA levels are followed by parallel changes in muscle myostatin protein content or plasma myostatin, as this myokine is also expressed in other non-muscle cells and tissues. Nevertheless, some evidence in the younger population suggests that serum myostatin levels are upregulated till 24 h after an acute bout of exercise (Domin et al., 2021).

Skeletal muscle can adapt to many physiological demands by shifting energy substrate utilization and mobilization during exercise. This is possible via myokines, such as IL-6, Irisin, and IL-15 (Huh, 2018).

Upon muscle contraction, IL-6 seems to increase in circulation, acting as a short-term energy allocator, particularly when intramuscular glycogen content levels are low, by having a critical role in glucose and lipid metabolism (Kistner et al., 2022). It is well known that plasma concentration of IL-6 increases in intensity and duration-dependent-manner during exercise, whereas the type of exercise has little effect (Pedersen, 2012a). These findings are supported by a systematic review that indicates that a single bout of moderate to high-intensity exercise seems to increase IL-6 regardless of the type of exercise in untrained adults (Brown et al., 2015), and these results seem consistent among older adults (Bizjak et al., 2021; MacNeil et al., 2020; Ziegler et al., 2019). Interestingly, MacNeil et al. (2020) demonstrated that active older adults displayed an increased response compared to their sedentary peers (mean of 3.3-fold vs 1.7-fold, respectively). The authors further discussed that this could be due to insufficient muscle power and/or mass to produce physiological signaling during exercise. However, no differences in IL-6 secretion were found between untrained vs trained, or higher fitness vs low fitness subjects (Bizjak et al., 2021; Ziegler et al., 2019), therefore, other factors could play a role in IL-6 response to exercise.

While evidence about the effect of an acute exercise session on irisin levels in animals (e.g., mice) seems consistent (Brenmoehl et al., 2014), it remains erratic in humans due to major methodical problems observed when using enzyme-linked immunosorbent assay (ELISA) kits as they lack on required specificity, and cross-reactivity with other proteins can potentially happen (Albrecht et al., 2015; Maak et al., 2021).

A meta-analysis concluded that acute exercise, lasting at least 10 min, could increase the irisin levels by nearly 15% in adults, independently of exercise type (Fox et al., 2018). However, findings in older adults are inconsistent. Two studies have not found a significant effect (Bizjak et al., 2021; Rioux et al., 2021) possible due to exercise features (e.g., intensity and duration) or the presence of metabolic disorders (e.g., obesity) that seem to influence changes in irisin concentrations. Bizjak et al. (2021) exercise session comprised a cardiopulmonary exercise test, which could have been of short duration and, therefore, unable to produce a potent stimulus for irisin release. On the other hand, Rioux et al. (2021) performed a prolonged high-intensity acute exercise session; however, their sample comprised older obese individuals, and irisin response in these individuals seems to be blunted (Fox et al., 2018; Zügel et al., 2016). Moreover, there might exist sex differences in irisin response (Löffler et al., 2015; Zügel et al., 2016), which could influence results in small samples with both sexes. Fitness levels may also play a role in irisin response as fit individuals tend to present higher post-exercise irisin concentrations (Fox et al., 2018), and therefore, it is expected that older adults exhibit a lower response. Nevertheless, Tsai et al. (2021) verified that high-intensity exercise seems to significantly increase irisin concentrations, while a positive but not significant effect was observed in moderate-intensity aerobic exercise. Therefore, high-intensity exercise can potentially upregulate PGC-1α, which in turn synthesizes FNDC5 (Boström et al., 2012). Moreover, these findings could suggest an important role of exercise intensity, at least in aerobic exercises, which is supported by previous evidence in mice (Brenmoehl et al., 2014), adolescents, and young adults (Löffler et al., 2015).

IL-15 might have potential actions in stimulating mitochondrial biogenesis, fat oxidation, glucose uptake, and myogenesis in skeletal muscle, and thus, it may mediate the exercise-induced muscle-fat crosstalk (Huh, 2018). Exercise seems to up-regulate IL-15 expression in rodents (Quinn et al., 2013); however, evidence is less clear in humans. In humans, IL-15 mRNA is predominantly expressed by type II muscle fibers (Nielsen et al., 2007). Therefore, few studies in young adults verified that an acute resistance exercise seems to stimulate IL-15 secretion within the first hour of recovery (Domin et al., 2021).

To our knowledge, only two studies analysed the acute effect of resistance exercise on IL-15 concentrations in older adults and found inconsistent effects (MacNeil et al., 2020; Urzi et al., 2019). In one study (Urzi et al., 2019), the acute session was performed using elastic bands, which could have led to a lower intensity threshold than required. Moreover, this study comprised a very old small sample ($n = 20$, mean age $= 84 \pm 8$ years) that included some sarcopenic individuals, and as described by Domin et al. (2021), fitness levels may influence IL-15 response to exercise. Another study reinforces this hypothesis as IL-15 increased after exertion in physically active older adults, whereas no significant changes were observed among sedentary individuals (MacNeil et al., 2020). Another more plausible perspective suggests that acute exercise increases muscular IL-15 mRNA levels may not be followed by parallel changes in muscle IL-15 protein content or plasma IL-15 (Nielsen et al., 2007; Rinnov et al., 2014), as muscle IL-15 may exist in a translationally inactive pool (Nielsen et al., 2007). Therefore, the non-significant changes in plasma IL-15 following a bout of exercise reinforce the idea that IL-15 may be acting in an autocrine-manner.

4.2. The chronic effect of exercise on myokines

Evidence about the chronic effect of exercise on myokines concentrations among older adults is more consistent, and it is described in detail in Table 2. Most of the summarized evidence (32 studies with a total of 1858 individuals) comes from randomized controlled trials (RCT, $n = 22$), with a higher number of studies being exclusively

Table 2
Summary of myokine studies: effects of chronic exercise.

Study	Study design	Comparator group	Intervention Group	Total sample size[a] (% male), Group sample size (mean age ± SD)	Exercise intervention	Main findings
	Myostatin					
Mafi et al. (2019)	RCT	CG: Non-exercise placebo control	EG1: REx EG2: EP EG3: REx+EP	n = 62 (100%) EG1 = 14 (69 ± 2.44 yrs) EG2 = 17 (68.60 ± 3.13 yrs) EG3 = 15 (69 ± 3.08 yrs) CG = 16 (68 ± 3.04 yrs)	Supervised REx: 3x/wk for 55 min/session, 3 sets of 8–12 reps, 8 full-body exercises using variable-resistance machines at 60%− 80% of 1-RM, 8 wks Supervised REx+EP: Same REx + daily intake of EP (1 mg/kg), 8 wks	EG1: ↓ EG2: ↔ EG3: ↓ CG: ↔ CG vs EG1: + EG1 CG vs EG3: + EG3 EG2 vs EG3: + EG3
Binns et al. (2017)	RCT	CG: Same exercises as EG, except no external weight was used.	EG: REx	n = 33 (24.2%) EG = NR (age = NR) CG = NR (age = NR) [Total sample age = 77 ± 6.4 yrs][b]	Supervised REx: 2x/wk for ~60 min/session, 3 sets of 8 reps, 8 full-body exercises using variable-resistance machines at 50–70% of 1-RM, 20 wks	EG: ↔ CG: ↔ EG vs CG: =
Hofmann et al. (2016)	RCT	CG: Cognitive training	EG1: REx EG2: REx+S	n = 91 (0%) EG1 = 33 (82.9, 71.7–92.2 yrs)[c] EG2 = 28 (83.9, 65.0–92.2 yrs)[c] CG = 30 (84.5, 69.4–91.8 yrs)[c]	Supervised REx: 2x/wk for 60 min/session, 1–2 sets of 15 reps, 6–12 full-body elastic band[d] exercises, 24 wks Supervised REx+S: same REx + supplementation in every morning breakfast and after training sessions, 24 wks	EG1: ↔ EG2: ↔ CG: ↔ EG1 vs CG: NR EG2 vs CG: NR
Mero et al. (2013)	RCT	CG: Non-exercise control	EG: REx	n = 38 (100%) EG = 18 (61.2 ± 4.1 yrs); CG = 10 (42.5 ± 20.0 yrs)	Supervised REx: 2x/wk for ~40 min/session, 2–5 sets of 5–15 reps, 10 full-body exercises using variable-resistance machines at 40%− 80% of 1RM, 21 wks	EG: ↑ CG: ↔ EG vs CG: NR
Hulmi et al. (2007)	RCT	CG: Non-exercise control	EG: REx	n = 18 (100%) EG = 11 (60.9 ± 5.0 yrs) CG = 7 (63.9 ± 7.4 yrs)	REx: 2x/wk for ~60 min/session, 2–5 sets of 5–20 reps, 12 full-body exercises using variable-resistance machines at 40–85% of 1-RM, 21 wks	EG1: ↑ CG: ↔ EG1 vs CG: NR
Negaresh et al. (2019)	UT	NA	EG1: REx EG2: REx	n = 31 (100%) EG1 = 16 (age = NR) EG2 = 15 (age = NR) [Total sample age = 55–70 yrs][b]	REx: 3x/wk for ~60 min/session, 4 sets of 10 reps, 9 full-body exercises using variable-resistance machines at 50–85% of 1-RM, 8 wks	EG1: ↓ EG2: ↓ EG1 vs EG2: =
Bagheri et al. (2020)	RCT	CG: Non-exercise control	EG1: REx+ AEx EG2: AEx + REx	n = 30 (100%) EG1 = 10 (63.8 ± 3.6 yrs) EG2 = 10 (61.1 ± 3.3 yrs) CG = 10 (65 ± 3.9 yrs)	Supervised REx+ AEx: 3x/wk for 40–60 min/session, 2–3 sets of 8–16 reps, 6 full-body exercises using variable-resistance machines at 40–75% of 1RM + 15–30 min cycling at 55–70% maximal heart rate, 8 wks Supervised AEx + REx: 3x/wk for 40–60 min/session, 15–30 min cycling at 55–70% maximal heart rate + 2–3 sets of 8–16 reps, 6 full-body exercises using variable-resistance machines at 40–75% of 1RM, 8 wks	EG1: ↓ EG2: ↓ CG: ↔ EG1 vs CG: + EG1 EG2 vs CG: + EG2
(Micielska et al., 2021)	UT	NA	EG1: AEx1 EG2: AEx2	n = 32 (28.1) EG1 = 18 (age = NR) EG2 = 14 (age = NR) [Total sample age = 61 ± 12 yrs][b]	Supervised AEx1: Nordic Walking 3x/wk for 65–75 min/session at 60–70% HRmax intensity, 12 wks. Supervised AEx2: high intensity interval training 3x/wk for 25 min/session, 10x 1 min cycling + 10x 1 min rest at 80–100 rpm.min^{-1} with an intensity of 90% HRmax, 2 wks	EG1: ↓Myostatin, ↔ Decorin EG2: ↔ Myostatin, ↔ Decorin EG1 vs EG2: + EG1
Ryan et al. (2013)	UT	NA	EG: AEx	n = 33 (48.5%) EG = 33 (61 ± 1 yrs)	Supervised AEx + weekly weight loss classes and daily caloric restriction: 3x/wk for 40–70 min/session at ~50–60% heart rate reserve, then, 60–80% VO2max on a treadmill, 24 wks	EG ↓
Konopka et al. (2010)	UT	NA	EG: AEx	n = 9 (0%) EG = 9 (70 ± 2 yrs)	AEx: 3–4x/wk for 20–45 min/session, 60–80% heart rate reserve on a cycle ergometer, 12 wks	EG: ↓
Arrieta et al. (2019)	RCT	CG1: Low-intensity routine activities CG2: Low-intensity routine activities	EG1: MT EG2: MT	n = 112 (29.5%) EG1: Women = 28 (age = NR) CG1: Women = 31	Supervised MT + unsupervised walking: 2x/wk for 45 min, 2 sets of 8–12 reps, 3–5 full-body exercises using free weights at 40–70% 1-RM,	EG1: ↔ CG1: ↔ EG1 vs CG1: = EG2: ↔

(*continued on next page*)

Table 2 (*continued*)

Study	Study design	Comparator group	Intervention Group	Total sample size[a] (% male), Group sample size (mean age ± SD)	Exercise intervention	Main findings
				(age = NR) [Total Women age = 86.2 ± 6.8 yrs][b] EG2: Men = 14 (age = NR) CG2: Men = 15 (age = NR) [Total Men age = 82.0 ± 6.3 yrs][b]	2–3 balance exercises + walking 5–20 min/ day, 24 wks	CG2: ↔ EG2 vs CG2: + EG2
IL-6						
Kirk et al. (2021)	RCT	CG1: Non-exercise control	EG1: REx EG2: REx + Protein Supplementation EG3: Protein supplementation	n = 100 (48%) EG1 = 24 (66.63 ± 3.92 yrs)[b] EG2 = 22 (68.59 ± 5.70 yrs)[b] EG3 = 23 (71.83 ± 6.51 yrs) CG1 = 31 (68.16 ± 5.85 yrs)[b]	Supervised REx + functional circuit: 3x/wk for 50 min/session, 2 sets of reps to fatigue, 8 full-body exercises using free weights and variable-resistance machines. Functional circuit consisted of 3 sets of 12 stations with 1 min of exercise performed at each at 7–10 BRPE, 16 wks	EG1: ↔ EG2: ↔ EG3: ↔ CG1: ↔ EG1 vs EG2 vs EG3 vs CG1: =
Furtado et al. (2020)	RCT	CG: Non-exercise control	EG1: REx EG2: MT	n = 60 (0%) EG1 = 21 (81 ± 4.79 yrs) EG2 = 20 (80 ± 8.19 yrs) CG = 19 (80 ± 10.01 yrs)	Supervised REx: 2–3x/wk for 45 min, 2–3 sets of 10–15 reps, 8–10 chair-based exercises using elastic bands at 56–75% of maximum heart-rate, 28 wks Supervised MT: 2–3x/wk, 2–4 sets of 8–16 reps, 7–9 bodyweight exercises in sitting and standing position at 56–85% of maximum heart-rate, 28 wks	EG1: ↔ EG2: ↔ CG: ↔ EG1 vs CG: NR EG2 vs CG: NR
Ziegler et al. (2019)	RCT	CG: Non-exercise control	EG: REx	n = 25 (72%) EG = 13 (67 (62–69 yrs))[d] CG = 12 (66 (62–70 yrs))[d]	Supervised REx: 3x/wk, 1–3 sets of 15–6RM, 7 full-body exercises using variable-resistance machines at 15RM-6RM, 48 wks	EG: ↔ CG: ↔ EG vs CG: NR
Tomeleri et al. (2018a)	RCT	CG: Non-exercise control	EG: REx	n = 46 (0%) EG = 24 (71.0 ± 5.4 yrs) CG = 22 (68.8 ± 4.6 yrs)	Supervised REx: 3x/wk for 45–50 min/session, 3 sets of 8 full-body exercises using a combination of free weights and variable-resistance machines at 10–15RM, 12 wks	EG: ↓ CG: ↔ EG vs CG: + EG
Tomeleri et al. (2018b)	RCT	CG: Non-exercise control	EG: REx	n = 45 (0%) EG = 22 (72.1 ± 6.3 yrs) CG = 23 (68.8 ± 4.9 yrs)	Supervised REx: 3x/wk for 45–50 min/session, 3 sets of 8 full-body exercises using a combination of free weights and variable-resistance machines at 10–15RM, 12 wks	EG: ↔ CG: ↔ EG vs CG: =
Tomeleri et al. (2016)	RCT	CG: Non-exercise control	EG: REx	n = 38 (0%) EG = 19 (66.8 ± 3.2 yrs) CG = 19 (69.5 ± 4.7 yrs)	Supervised REx: 3x/wk for 45–50 min/session, 3 sets of 8 full-body exercises using a combination of free weights and variable-resistance machines at 10–15RM, 8 wks	EG: ↓ CG: ↔ EG vs CG: + EG
Bruunsgaard et al. (2004)	RCT	CG: Social activities 2x/ wk	EG: REx	n = 21 (9.5%) EG = 10 (88.6 (86–95 yrs))[b,d] CG = 11 (90.6 (86–95 yrs))[b,d]	Supervised REx: 3x/wk for 45 min/session, 3 sets of 8 reps, knee extension and flexion exercise using a variable-resistance machine at 50–80% of 1-RM, 12 wks	EG: ↔ CG: ↔ EG vs CG: NR
Hangelbroek et al. (2018)	UT	NA	EG: REx	n = 61 (34.4%) EG = 61 (78.4 ± 7.7 yrs)	Supervised REx: 2x/wk for ~45 min/session, 3–4 sets of 8–15 reps, 6 full-body exercises using variable-resistance machines at 50%− 75% of 1-RM, 24 wks	EG: ↔
Forti et al. (2016)	UT	NA	EG1: High REx EG2: Low REx EG3: Mixed low REx	n = 51 (47.1%) EG1 = 17 (67.86 ± 4.36 yrs) EG2 = 16 (68.85 ± 5.34 yrs) EG3 = 18 (67.61 ± 6.02 yrs)	Supervised High REx: 3x/wk for ~40 min/ session, 2 sets of 10–15 reps, 2 lower-limb exercises using variable-resistance machines at 80% of 1RM, 12 wks; Supervised Low REx: 3x/wk for ~40 min/ session, 1 set of 80–100 reps, 2 lower-limb exercises using variable-resistance machines at 20% of 1RM, 12 wks; Supervised Mixed Low REx: 3x/wk for ~40 min/ session, 1 set of 60 reps at 20% of 1 RM then 1 set of 10–20 reps at 40% of 1-RM of 2 lower-limb exercises using variable-resistance machines, 12 wks.	EG1: ↔ EG2: ↔ EG3: ↔ EG1 vs EG2 vs EG3: =
Chupel et al. (2018)	Non-RCT	CG: Usual care	EG1: REx+AEx EG2: Taurine Supplementation EG3: REx+AEx	n = 48 (0%) EG1 = 13(83.5 ± 7.3 yrs) EG2 = 12 (85 ±	Supervised REx+AEx: 2x/wk for 60 min/session, 2–3 sets of 15–20 reps, 4–8 exercises using elastic bands at 5–7 OMNI perceived exertion scale + 4–8 chair-based exercises and walking.	EG1: ↓ EG2: ↔ EG3: ↔ CG: ↔

(*continued on next page*)

Table 2 (*continued*)

Study	Study design	Comparator group	Intervention Group	Total sample size[a] (% male), Group sample size (mean age ± SD)	Exercise intervention	Main findings
			+ Taurine Supplementation	4.5 yrs) EG3 = 11 (83.8 ± 8.6 yrs) CG = 12 (82 ± 7.5 yrs)		EG1 vs EG2 vs EG3 vs CG: =
Lima et al. (2015)	RCT	CG: Non-exercise control	EG1: AEx + REx EG2: AEx	n = 44 (11.4%) EG1 = 15 (67.8 ± 5.2 yrs) EG2 = 15 (67.8 ± 4.3 yrs) CG = 14 (69.9 ± 5.5 yrs)	Supervised AEx+REx: 3x/wk for ~60 min/ session 2 sets of 15–20 reps, 9 full-body exercises using variable-resistance machines at 50–60% of 1-RM and 50–80% training heart rate on a treadmill, 10 wks Supervised AEx: 3x/wk for 40 min/ session at 50–80% training heart rate on a treadmill, 10 wks	EG1: ↔ EG2: ↓ CG: ↔ EG2 vs CG: + EG2
Kohut et al. (2006)	UT	NA	EG1: MT EG2: AEx	n = 97 (30.1%) EG1 = 49 (70.3 ± 4.6 yrs)[b] EG2 = 48 (69.8 ± 5.5 yrs)[b]	Supervised MT: 3x/wk for 50 min/session, 10–15 reps, session included elements of yoga and Tai chi, Flex bands, free hand weights, stability balls and variable weight machines. Supervised AEx: 3x/wk, 50 min of aerobic exercise in an aerobic exercise equipment at 45–80% of maximal cardiac effort, 40 wks	EG1: ↔ EG2: ↓ EG1 vs EG2: + EG2
Sadjapong et al. (2020)	RCT	CG: Non-exercise control	EG: MT	n = 64 (39.1%) EG = 32 (76.68 ± 1.14 yrs) CG = 32 (78.87 ± 1.32 yrs)	Supervised MT: 3x/wk for 60 min/ session, 15 min of chair-based aerobic training at 40–65% maximum heart rate, 2–3 sets of 8–12 reps, 10 full-body elastic band exercises at 60%− 90% of 1RM and 8 balance exercises, 12 wks	EG: ↓ CG: ↔ EG vs CG: + EG
Nicklas et al. (2008)	RCT	CG: Health education sessions plus 5–10 min of upper extremity stretching	EG: MT	n = 369 (31.9%) EG = 183 (76.4 ± 4.1 yrs)[b] CG = 186 (77.0 ± 4.4 yrs)[b]	Supervised MT: 2–3/wk for 60 min/session plus≥ 3/wk of home-based exercises. Sessions comprised lower extremity strengthening exercises, followed by lower extremity stretching exercises at 15–16 BRPE and walking at 12–13 BRPE, 48 wks	EG = ↓ CG = ↑ EG vs CG: + EG
Grosicki et al. (2020)	UT	NA	EG1: MT	n = 99 (54%) EG = 99 (78 ± 5 yrs)	Supervised REx+AEx: 3x/wk for 60 min/session, 30 min of walking + 20 min of lower extremity strengthening exercises (2 set of 10 reps at 15/16 BRPE) + balance exercises, 24 wks	EG: ↔
	Irisin					
Planella-Farrugia et al. (2019)	RCT	CG: NR	EG1: REx EG2: REx + nutritional support	n = 44 (9.1%) EG1 = 14 (64.9 ± 5.5 yrs)EG2 = 9 (71.2 ± 3.3 yrs) CG = 20 (66.4 ± 4.6 yrs)	Supervised REx: 2x/wk for 45 min /session, 7 low-intensity full-body exercises, 16 wks Supervised REx+ nutritional support: Same as REx + protein intake, 16 wks	EG1: Myostatin ↔, irisin ↑ EG2: Myostatin ↔, irisin ↑ CG: myostatin ↓, irisin ↔ EG1 vs CG: NR EG2 vs CG: NR
Zhao et al. (2017)	RCT	CG: Non-exercise control	EG: REx	n = 17 (100%) EG = 10 (62.3 ± 3.5 yrs) CG = 7 (61.9 ± 3.1 yrs)	REx: 2x/wk for 55 min/session consisting of leg muscle strength and core strength training, 12 wks	EG: ↑ CG: ↔ EG vs CG: + EG
Kim et al. (2015)	RCT	CG: 60 min- stretching 1x/wk plus health education	EG: REx	n = 30 (0%) EG = 22 (74.45 ± 0.62 yrs) CG = 8 (76.05 ± 2.01 yrs)	Supervised elastic bands REx: 2x/wk for 60 min/ session, 2–3 sets of 12–15 reps at BRPE 12–13 + 3x/wk of home-based exercises consisting in flexibility and REx, 12 wks	EG: ↑ CG: ↔ EG vs CG: NR
Miyamoto-Mikami et al. (2015)	Non-RCT	CG: Non-exercise control	EG: AEx	n = 28 (42.9%) EG = 14 (65 ± 8 yrs) CG = 14 (69 ± 6 yrs)	Supervised AEx: 3x/wk for 55 min/session at 60–70% peak oxygen uptake on a leg ergometer, 8 wks	EG: ↑ CG: ↔ EG vs CG: + EG
	IL-15					
Urzi et al. (2019)	RCT	CG: Non-exercise control	EG: REx	n = 20 (0%) EG = 11 (84.4 ± 7.7 yrs) CG = 9 (88.9 ± 5.3 yrs)	Elastic bands REx: 3x/wk for 45–50 min/session, 3 sets of 5–12 reps, 10 full-body exercises at BRPE 12–14, 12 wks	EG: ↔ CG: ↔ EG vs CG: =
Nishida et al. (2015)	RCT	CG: Non-exercise control	EG: AEx	n = 62 (0%) EG = 31 (70.4 ± 5.8 yrs) CG = 31 (69.7 ± 6.6 yrs)	Unsupervised home-based AEx: 3x/day for 10–20 min/session bench step exercise program for a goal of ≥ 140 min/wk, 12 wks	EG: IL-6 ↔, IL-15 ↔ CG: IL-6 ↔, IL-15 ↔ EG vs CG: NR

Abbreviation: AEx: Aerobic exercise, BRPE: Borg Rate of Perceived Exertion scale, CG: Control group, EG: Exercise group, Epicatechin: EP, min: minutes, MT: multicomponent, NA: not applicable, NR: not reported, RCT: randomized controlled trial, reps: repetitions, REx: Resistance exercise, RM: repetition maximum, UT: Uncontrolled trial; VO2max: maximal oxygen uptake, wk: week, yrs: years, ↑ a significant increase, ↔ no change, ↓ a significant decrease, + Significant superior

intervention effect compared to the other group, = Non significant intervention effect
[a]number of participants included in the analysis (final sample size)
[b]Baseline characteristics
[c]median, range
[d]mean, range

performed on women (10 studies) than men (6 studies). The sample size ranged between 9 and 369 subjects. The most common type of training was resistance exercise (62.5%), performed at least two times per week, three studies combined resistance exercise and aerobic exercise (Bagheri et al., 2020; Chupel et al., 2018; Lima et al., 2015), and six studies combined in a single session three components (resistance, aerobic, and balance training) (Arrieta et al., 2019; Furtado et al., 2020; Grosicki et al., 2020; Kohut et al., 2006; Nicklas et al., 2008; Sadjapong et al., 2020). Resistance exercise interventions were mostly performed using variable-resistance machines with an intensity ranging between 40% and 85% of 1RM. Five studies explored the effect of aerobic training alone (Konopka et al., 2010; Micielska et al., 2021; Miyamoto-Mikami et al., 2015; Nishida et al., 2015; Ryan et al., 2013) with an intensity ranging between 50% and 80% heart rate reserve or 60–90% maximum heart rate or 60–80% of maximal oxygen consumption (VO_2max). All outcomes were measured at baseline and the end of each intervention period, ranging from 2 weeks to 48 weeks.

Overall, evidence suggests that chronic exercise decreases myostatin (Bagheri et al., 2020; Konopka et al., 2010; Mafi et al., 2019; Micielska et al., 2021; Negaresh et al., 2019; Ryan et al., 2013) and increases irisin (Kim et al., 2015; Miyamoto-Mikami et al., 2015; Planella-Farrugia et al., 2019; Zhao et al., 2017), whereas mix results were found in IL-6 (Bruunsgaard et al., 2004; Chupel et al., 2018; Forti et al., 2016; Furtado et al., 2020; Grosicki et al., 2020; Hangelbroek et al., 2018; Kirk et al., 2021; Kohut et al., 2006; Lima et al., 2015; Nicklas et al., 2008; Sadjapong et al., 2020; Tomeleri et al., 2018a, 2016, 2018b; Ziegler et al., 2019) and no significant changes on decorin (Micielska et al., 2021) and IL-15 concentrations in older adults (Nishida et al., 2015; Urzi et al., 2019).

The results on the chronic effect of exercise on myostatin expression or concentrations in older adults are consistent with previous reports in adults (Baczek et al., 2020; Domin et al., 2021), as overall, myostatin tends to decrease after long-term exercise, regardless of exercise type. Mafi et al. (2019) and Negaresh et al. (2019) verified that 8 weeks of three-times a week resistance exercise decreased myostatin levels, and this reduction was concomitant to increases of its antagonist - follistatin - in both healthy and sarcopenic older adults. Comparable results were obtained by Bagheri et al. (2020) in a concurrent exercise program, independently of components order (e.g., aerobic followed by resistance exercise or resistance exercise followed by aerobic), with similar frequency and intervention period. Contradictory findings were presented by Hofmann et al. (2016) that verified an increase in follistatin concentration in the resistance exercise group after the intervention; however, this finding was not parallelly followed by significant changes in myostatin levels.

Aerobic exercise interventions performed three times per week seem consistent in reducing myostatin levels among older adults (Konopka et al., 2010; Micielska et al., 2021; Ryan et al., 2013), similar to younger adults (Hittel et al., 2010). However, these results should be interpreted with caution as these were uncontrolled studies and need further confirmation in RCTs.

Some differences in sample characteristics and exercise intervention structure can potentially explain why some studies have failed to find significant results. Generally, exercise intervention performed twice a week failed to decrease myostatin levels (Arrieta et al., 2019; Binns et al., 2017; Hofmann et al., 2016; Hulmi et al., 2007; Mero et al., 2013). Therefore, frequent overloading may be needed to chronically affect myostatin levels in older adults.

Decorin is an antagonist of myostatin (Miura et al., 2006) and may have a role in exercise-induced skeletal muscle hypertrophy (Kanzleiter et al., 2014). To our knowledge, only one study verified the effect of chronic exercise in the regulation of this myokine among older adults (Micielska et al., 2021). Micielska et al. (2021) found that 12 weeks of Nordic walking decreased myostatin, while a trending inversely correlated increase of decorin levels was observed ($p = 0.06$). Previous evidence in adults suggests that resistance exercise or combined exercise (aerobic plus resistance exercise) can increase decorin levels (Bugera et al., 2018; Kanzleiter et al., 2014). Moreover, it was proposed that training volume or total muscle mass involvement may be important predictors of the magnitude of decorin secretion (Bugera et al., 2018). However, further studies examining the effect of exercise on this myokine are required.

The chronic effects of exercise in IL-6 in older adults have been extensively reported in recent systematic reviews and meta-analyses (Bautmans et al., 2021; Zheng et al., 2019). Briefly, consistent evidence of RCTs seems to support the anti-inflammatory effects of exercise in lowering circulating levels of IL-6 among older adults, which are less noticeable in frail compared to healthy subjects (Bautmans et al., 2021). However, the included studies in this review showed mixed results.

In seven RCTs of resistance exercise, only two found a significant decrease in IL-6 (Tomeleri et al., 2018a, 2016). Two uncontrolled studies of resistance exercise also failed to find a significant effect (Forti et al., 2016; Hangelbroek et al., 2018). Conversely, combined training (Chupel et al., 2018), aerobic exercise (Kohut et al., 2006; Lima et al., 2015), or multicomponent (Nicklas et al., 2008; Sadjapong et al., 2020) seem to have a positive effect on IL-6 concentrations, independent of study design, which could reinforce the role of aerobic training in improving the inflammatory status.

As described, the accumulation of abdominal adiposity stimulates macrophage infiltration of adipose tissue resulting in a pro-inflammatory immune response. Then, M1 macrophages increase the release of TNF-α, IL-1β, and IL-6 (Wueest and Konrad, 2020). Data from a systematic review and meta-analysis suggest a significant positive effect of aerobic training in reducing the visceral adipose tissue, whereas resistance training failed in overweight and obese adults (Ismail et al., 2012). Moreover, recently Wedell-Neergaard et al. (2019) demonstrated that IL-6 is required for exercise (aerobic exercise) to reduce visceral adipose tissue mass. Other factors such as training protocol (intensity and the muscle mass involved during exercise), blood sampling time (as IL-6 may be upregulated till 24 h after a bout of exercise), and baseline IL-6 scores, particularly in participants with inflammatory underlying conditions, may potentially explain the inconsistencies found in the literature.

It is important to highlight that although basal levels of IL-6 are reduced after chronic exercise, the muscular expression of the IL-6 receptor seems to be upregulated and markedly increases in response to exercise, which suggests that trained skeletal muscle becomes more sensitive to IL-6 action (Pedersen, 2012a). A recent hypothesis also highlighted this link as it has been hypothesized that muscle adaptation to physical inactivity and disuse may lead to IL-6 resistance, probably through Jak/STAT3 pathway, which is responsible for skeletal muscle atrophy (Pérez-Baos et al., 2018). Therefore, the elevated basal circulating levels of IL-6 observed in these models can also hypothetically represent a compensatory mechanism or a possible resistance to IL-6 signaling (Pedersen, 2013).

Evidence on the chronic effect of exercise on irisin concentration is consistent. Irrespective of the exercise protocol (type of exercise, duration, or intensity of exercise training), all the included studies suggest an increase in the circulating levels of irisin after a chronic exercise among older adults (Kim et al., 2015; Miyamoto-Mikami et al., 2015;

Planella-Farrugia et al., 2019; Zhao et al., 2017), particularly when the training is demanding and progressive in terms of intensity (Cosio et al., 2021). Initially, irisin was thought to be more responsive to aerobic training as it is a PGC1-α-dependent myokine (Boström et al., 2012). However, resistance exercise seems to elicit superior irisin responses compared with endurance exercise or combined (resistance and endurance exercises) (Tsuchiya et al., 2015), probably because muscle mass is an important predictor of circulating irisin (Huh et al., 2012).

Interestingly, Planella-Farrugia et al. (2019) and Kim et al. (2015) verified that chronic resistance exercise resulted in increased circulating irisin concomitant with strength and walking speed improvements. Moreover, exercise-induced increases in irisin levels also seem related to improvements in body fat percentage (Zhao et al., 2017) and abdominal visceral fat (Miyamoto-Mikami et al., 2015). These findings suggest that low levels of circulating irisin might be a marker for muscle weakness, atrophy, and sarcopenia (Chang et al., 2017; Park et al., 2019).

Data on the effect of regular exercise on IL-15 among humans is conflicting and scarce. In adults, Rinnov et al. (2014) demonstrated that 12 weeks of regular aerobic exercise increased basal skeletal muscle IL-15 protein content without changes in either muscle IL-15 mRNA or plasma IL-15 levels. In addition, BrunelIi et al. (2015) found increased serum concentrations of IL-15 after 24 weeks of combined training in obese men. However, other studies found that prolonged exercise does not affect IL-15 levels (Riechman et al., 2004) or may even decrease them (Pérez-López et al., 2021). These inconsistent findings are also noticed in a recent review of healthy adults (Domin et al., 2021).

Similarly, chronic exercise does not seem to affect IL-15 concentration in older adults (Nishida et al., 2015; Urzi et al., 2019). The lack of consistent evidence limits us from drawing specific conclusions; however, some studies suggest that physical fitness and body composition should be taken into account as these factors seem to affect IL-15 concentrations (Domin et al., 2021). These questionable results may be due to differences in the exercise protocol, sample characteristics, blood sampling time, or the mechanisms of IL-15 secretion, given the short life of IL-15 in plasma.

To our knowledge, previous work about the effects of acute and chronic exercise in upregulating and increasing serum/plasma levels of Metrnl (Eaton et al., 2018) and BAIBA (Roberts et al., 2014; Stautemas et al., 2019) has only been limited to young adults.

Taken as a whole, research on myokines among older adults presents several limitations, such as very few studies, with most of them having a small sample size and specific sample characteristics (i.e., women), and lack of necessary statistical adjustments to relevant covariates. Moreover, the heterogeneity across exercise regimens and myokines measurement (e.g., blood plasma or serum or muscle biopsies) hamper further analyses and generalizations. These limitations should be addressed in well-designed future studies.

One of the main limitations of the present study is that studies including physical exercise together with other types of interventions (e.g., nutritional supplementation) were not excluded. Moreover, uncontrolled studies were also included, which could have blurred the results.

Although data is scarce, it holds promising results for future research. As we entered in the "omics" era, several novel molecules and mechanisms involved in organs crosstalk during exercise will be discovered with high potential as biomarkers or therapeutics agents. This technology will increase our understanding on how exercise improves health, resilience, and regulates multiple physiological systems (Chow et al., 2022), which is crucial in the era of complex syndromes such as frailty.

5. Conclusions and future directions

As presented above, the metabolic adaptation to exercise in organisms is very complex and depends on integrative crosstalk between several organs to achieve whole-body homeostasis. Failure in these mechanisms can highly impact structure and function in cells, tissues, and systems, consequently diminishing multisystem function and resilience and increasing vulnerability to stressor factors.

As summarised by Gonzalez-Gil and Elizondo-Montemayor (2020), the benefits of myokines include skeletal muscle adaptation to exercise through hypertrophy and increased efficiency in substrates (glucose and free fatty acids) utilization and browning of white adipose tissue. It also induces an anti-inflammatory milieu, improving insulin sensitivity and creating a favourable environment for brown adipose tissue to function.

Although the evidence presented in this review only showed early suggestions about the effect of exercise on myokines among older adults, studies in this population seem to be increasing at a fast pace. Future studies should address the current controversies around inconsistency between the acute and chronic myokine response, between studies in animals and humans and the variability in outcomes and sampling protocols (Chow et al., 2022).

This speculative theory linking energy dysregulation to frailty development and progression needs further confirmation. Moreover, several questions remain to be answered and deserve further attention. For example, are the expression and secretion of myokines decreased in frail older adults when compared to non-frail after adjusting for important covariates (i.e., appendicular skeletal muscle mass or age)? Does chronic exercise elicit the same effects among frail and non-frail older adults? Is the skeletal muscle of frail older adults resistant to myokines actions? Can acute changes in myokine levels be used as a biomarker of sarcopenia or frailty?

Understanding the pathways through which exercise improves frailty might disentangle novel mechanisms and pathways related to frailty onset and progression. Future research is needed to verify how energy metabolism can shape frailty trajectories. Also, it is necessary to assume an integrative view about muscle-organs crosstalk, exercise, and frailty as more myokines and other exercise-secreted factors are discovered.

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CRediT authorship contribution statement

Duarte Barros: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Elisa Marques**: Conceptualization, Methodology, Writing – review & editing. **José Magalhães:** Writing – review & editing. **Joana Carvalho**: Writing – review & editing, Supervision, Funding acquisition. All authors approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Data Availability

No data was used for the research described in the article.

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Chapter III – General methodology

3. General methodology

At the end of this chapter, **Figure 3** provides an overview of the study designs, duration, sample characteristics, primary and secondary outcome variables, and main statistical analyses of each included study.

To achieve the aims of this thesis, a prospective cohort study titled “The Observatory of the Older Person” was conducted in NH across the Porto Metropolitan Area, Portugal. The study sought to address four key objectives:

(I) To collect comprehensive data on the overall function, health status, and frailty of the resident population, filling a significant gap in the existing literature regarding the health profiles of older adults in Portuguese NH;
(II) To screen participants for inclusion in the exercise intervention trials, ensuring that eligible individuals were identified for subsequent studies;
(III) To identify NH with an adequate number of eligible participants, ensuring both statistical power and logistical feasibility for the interventions;
(IV) To track yearly changes in key outcomes, such as frailty status, independence in BADL, rate of falls, and physical performance.

The cohort study was conducted from September 2021 to December 2024 and was retrospectively registered with ClinicalTrials.gov (NCT06825026) in January 2025.

3.1 Design and Settings

Nested within this cohort, three studies were conducted: a cross-sectional study (**Study II**), a cluster-randomised controlled crossover trial (**Study III**), and a pragmatic controlled trial (**Study IV**).

Trial designs were chosen to optimise internal and external validity, bridging the gap between experimental conditions and real-world practice, and enabling the translation of evidence into care strategies.

3.1.1 Studies timeline

This research was conducted between September 2021 and December 2024 and divided into distinct phases associated with each study (**Figure 1**).

Recruitment of NH to participate in the cohort study started at the beginning of September 2021, followed by participant evaluations in each participating institution from September 2021 to December 2021. NH with a minimum number of eligible participants for the trial intervention were subsequently recruited for Study III, with baseline assessments carried out in the early part of January 2022, and the first intervention cycle running until August 2022. The recruitment for a second cycle of intervention took place from September 2022 to December 2022 to reach the necessary sample size, with the second intervention cycle occurring from early January 2023 to August 2023.

Study II used data collected from participants in Studies III and IV. Data collection for Study II took place from January 2022 to July 2024. For Study IV, the recruitment period coincided with the follow-up assessments, which took place between July 2023 and September 2023, with a 12-month intervention running until September 2024. The final assessments for Study IV were completed in September 2024.

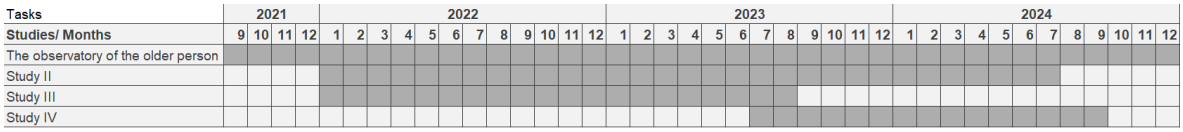

Figure 1. Experimental timeline

3.2 Recruitment and Eligibility Criteria

3.2.1 Recruitment

A structured recruitment strategy was implemented to engage NH and facilitate their participation. Initial contact was made via email to NH directors,

accompanied by an informational flyer detailing the study's objectives, methodology, and participation criteria. To further elucidate study procedures, follow-up online meetings were conducted, some in collaboration with municipal authorities to optimise outreach and simultaneously engage multiple NH.

Despite these efforts, some NH either did not respond to the outreach or chose not to participate, mainly due to concerns related to the COVID-19 pandemic, which led to significant logistical feasibility challenges to the proposed research. Between 2021 and 2024, 18 NH participated in the cohort study, each evaluated at least once. Their geographical distribution within the Porto Metropolitan Area is presented in **Figure 2**, with a notable concentration in the municipality of Vila Nova de Gaia.

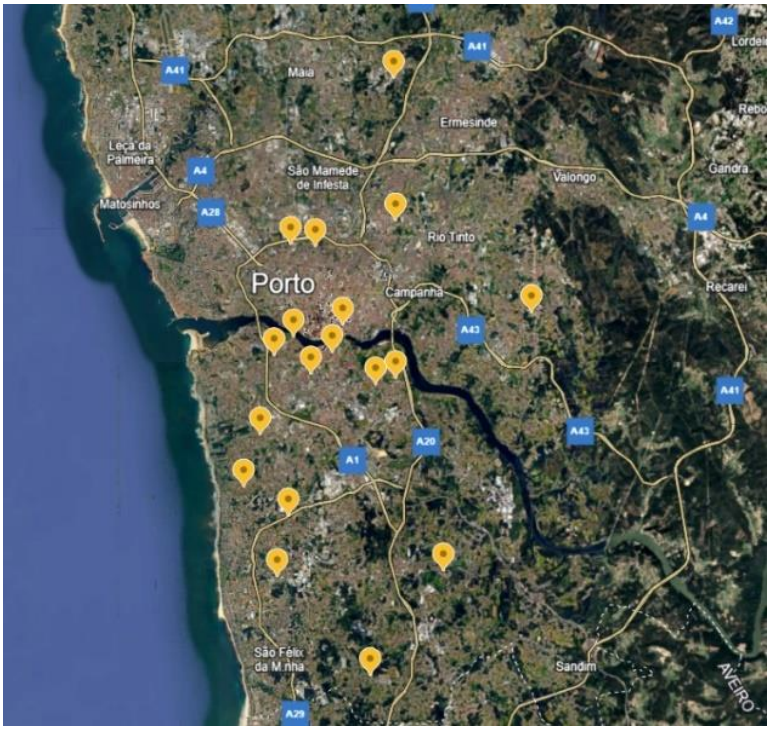

Figure 2. Geographical location of the participating NH (image from Google Earth)

3.2.2 General eligibility criteria

Participants were required to be aged 65 or older, able to walk independently, with or without an assistive device and in stable health, free of conditions that

could interfere with study testing or interventions (e.g., uncontrolled medical conditions or terminal illness). Those with fractures within the last three months were excluded to avoid complications during physical testing.

Specific eligibility criteria for each study are detailed in the respective sections of each article.

3.3 Data collection

Data collection in the cohort study was conducted annually during a single in-person assessment session, which lasted approximately 45 minutes per participant at each NH. To maintain consistency across data points, all measurements were conducted by a single trained researcher, who conducted the assessments at multiple time points throughout the study period. Standardised protocols were followed for each outcome measure to ensure the accuracy and reliability of the data. All collected data were encoded and securely stored in an encrypted database to protect participant confidentiality and privacy.

In Studies III and IV, data were collected at baseline and after each intervention by the same assessors, who were not responsible for conducting the exercise sessions. All assessors were blinded to participants' group allocation, except for the researcher responsible for assessing physical performance (e.g. SPPB), who also managed the project.

3.3.1 General Outcomes Measures

A structured questionnaire was completed by the nursing staff of each nursing home using the residents' medical records. The questionnaires covered data on sociodemographic characteristics, social activities and visits, fall history, utilisation of healthcare services, general clinical information, medication use, and independence in BADL. To ensure a comprehensive health evaluation, participants were assessed on their physical frailty, physical performance,

cognitive function, body composition and anthropometrics, nutritional status and physical activity levels. Specific laboratory tests, including blood analyses, body composition assessment using dual-energy X-ray absorptiometry (DXA), and isokinetic knee strength measurements, were performed at the faculty's research facilities. Detailed methodologies and assessment protocols are outlined in the methods section of each respective study.

3.4 Exercise interventions

In addition to receiving usual care, participants in the intervention group engaged in supervised concurrent exercise sessions lasting 50–60 minutes. Detailed exercise characteristics such as frequency, intensity, volume, and duration are provided in the Methods sections of Studies III and IV.

The programs were tailored to the functional limitations of frail nursing home residents, with an emphasis on improving lower-extremity strength and mobility. Furthermore, sessions were structured with the resistance training component preceding the aerobic training, to minimise the potential interference effect and enhance neuromuscular adaptations (Izquierdo et al., 2025). To maximise safety, several risk mitigation strategies were employed. Sessions were conducted in small groups (with a maximum of six frail participants) to reduce fall or injury risk, starting with seated exercises and gradually transitioning to standing movements. Additionally, the nursing home staff was informed about the importance of ensuring participants wore appropriate clothing, and hydration was provided during each session to prevent both hyperthermic and hypothermic states (Izquierdo et al., 2025).

Generally, exercise sessions consisted of a warm-up, resistance and aerobic training, and a 5-minute cool-down. The warm-up involved 1 set of 10 repetitions of later resistance exercises performed without load. The resistance training comprised 1 to 3 sets of 10 to 15 repetitions with an external load equivalent to 30% to 70% of the 1-repetition maximum (1RM) for upper and lower body exercises. Target loads were determined using a 10-repetition maximum (10RM)

test, with the testing protocol described in detail in both Study III and Study IV. The sit-to-stand exercise was performed as fast as possible according to each participant's capacity without external load. The recovery between sets lasted 1 to 2 minutes. The same exercises were performed in all sessions, including biceps curl, knee extension, knee flexion, calf raise and sit and stand. The aerobic training included walking exercises with changes in pace and direction. Intensity was monitored through observer-rated perceived exertion of signs and symptoms (e.g. sweating, difficulty talking while walking, and respiratory rate) due to participants often presenting cognitive impairment. The sessions ended with stretching exercises.

The same exercise professional led all sessions and was not involved in any assessment.

3.5 Ethics

This thesis project was approved by the Faculty of Sports, University of Porto Ethics Committee (CEFADE 012021). In full compliance with the Helsinki Declaration, all participants and nursing home staff involved received a complete explanation of the study's purposes, risks, and procedures. After receiving medical approval from each nursing home medical team, all participants and their legal representatives signed an informed consent before enrolment.

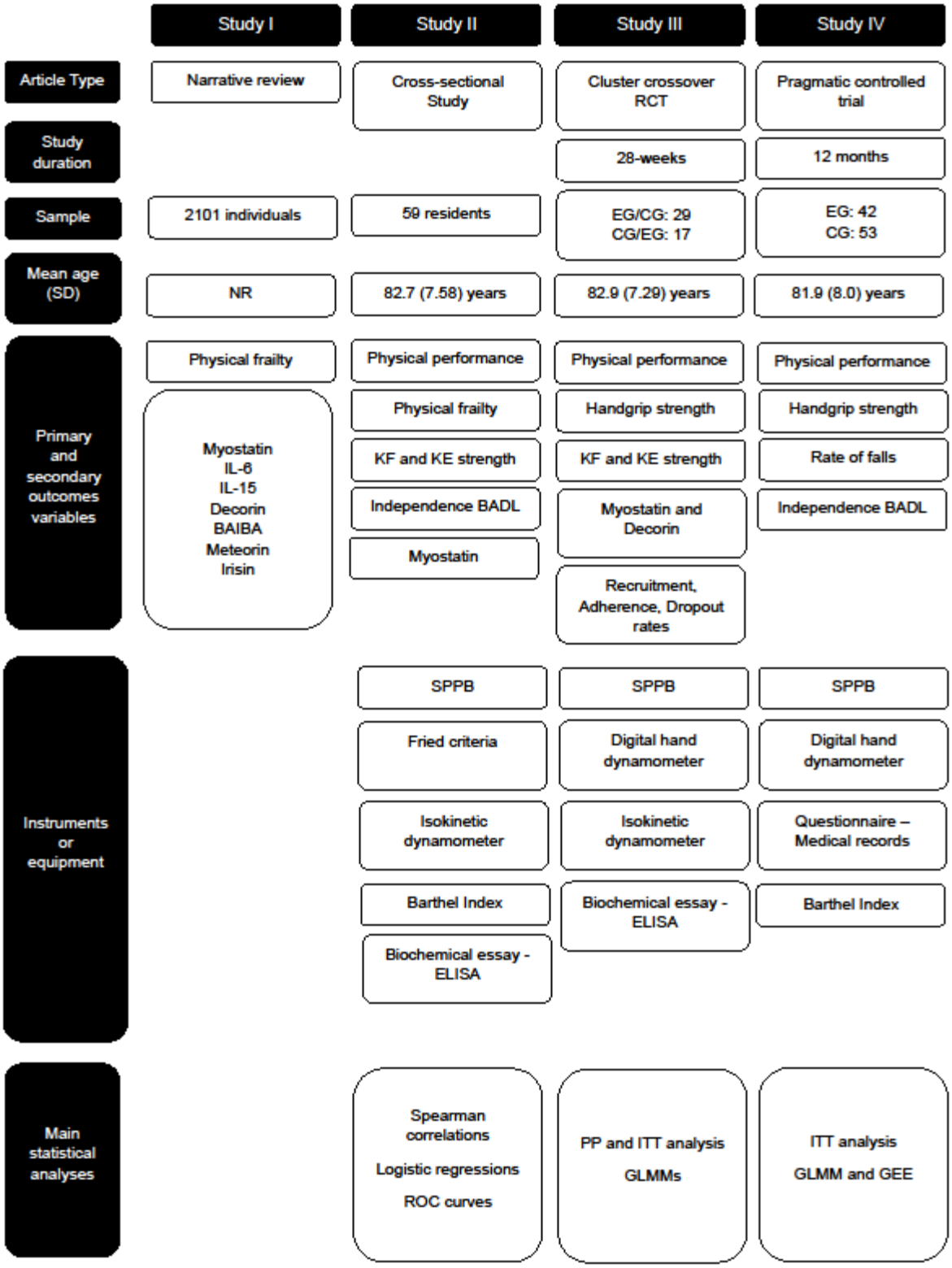

Figure 3. Methodological overview of the studies included in this thesis.

Note: BADL, basic activities of daily living; BAIBA, Beta-aminoisobutyric acid; CG, control group; EG, exercise group; ELISA, Enzyme-Linked Immunosorbent Assay; GEE, generalised estimating equations; GLMM, generalised linear mixed models; IL, interleukin; ITT, intention to treat; KE, knee extension; KF, knee flexion; NR, non-reported; PP, per-protocol; RCT, randomised controlled trial; ROC, Receiver Operating Characteristic; SPPB, short physical performance battery.

Chapter IV – Original Studies

4. Original Studies

4.1 List of publications

1. **Barros, D**., Marques, E. A., Magalhães, J., & Carvalho, J. (2022). Energy metabolism and frailty: The potential role of exercise-induced myokines - A narrative review. *Ageing research reviews*, 82, 101780. https://doi.org/10.1016/j.arr.2022.101780

2. **Barros, D**., Silva-Fernandes, A., Martins, S., Guerreiro, S., Magalhães, J., Marques, E. A., & Carvalho, J. (2025). Relationship between myostatin, frailty status and physical function: An exploratory analysis. *To be submitted.*

3. **Barros, D**., Silva-Fernandes, A., Martins, S., Guerreiro, S., Magalhães, J., Carvalho, J., & Marques, E. A. (2024). Feasibility and Effectiveness of a 12-Week Concurrent Exercise Training on Physical Performance, Muscular Strength, and Myokines in Frail Individuals Living in Nursing Homes: A Cluster Randomized Crossover Trial. *Journal of the American Medical Directors Association*, 25(11), 105271. https://doi.org/10.1016/j.jamda.2024.105271

4. **Barros, D.**, Johansson, J., Wilsgaard, T., Magalhães, J., Carvalho, J., & Marques, E. A. (2025). One-year concurrent training improves physical performance and handgrip strength in nursing home residents. *GeroScience*. Advance online publication. https://doi.org/10.1007/s11357-025-01770-y

4.2 Study II

Barros, D., Silva-Fernandes, A., Martins, S., Guerreiro, S., Magalhães, J., Marques, E. A., & Carvalho, J. (2025). Relationship between myostatin, frailty status and physical function: An exploratory analysis. *To be submitted.*

Title: Relationship between myostatin, frailty status and physical function: An exploratory analysis

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Abstract

Myostatin (MSTN) is a well-established negative regulator of muscle mass, however, its relationship with frailty and physical function in older adults remains controversial. This study examined the associations between absolute and relative serum MSTN levels, frailty status, and physical function in pre-frail and frail nursing home residents. This cross-sectional study included 59 older adults (median age, 83 years; 66.1% female). Frailty was determined using the Frailty Phenotype. Physical function was evaluated through the Short Physical Performance Battery (SPPB), Barthel Index (BI) for activities of daily living, and knee extension/flexion strength through the Isokinetic strength test. Serum MSTN levels were quantified using ELISA. Spearman's correlations were conducted to explore the correlations between MSTN and specific frailty criteria and physical function measures, while logistic regressions were performed to analyse the relationship between MSTN and frailty status. Receiver operating characteristic (ROC) curves were used to assess models' discrimination ability. Both absolute and relative MSTN levels were correlated with the low physical activity frailty criterion. Absolute MSTN was positively correlated with SPPB (r=0.28), BI (r=0.34), and knee extension strength/body weight (r=0.30), while relative MSTN was correlated with BI (r=0.26). Higher MSTN levels were associated with significantly lower odds of frailty (absolute MSTN OR = 0.41; BCa 95% CI: 0.18, 0.75; relative MSTN OR = 0.46; BCa 95% CI: 0.22, 0.80). ROC analyses showed moderate predictive ability of the models (AUC 0.70–0.76). These findings suggest MSTN levels are positively associated with physical function and inversely related to frailty in pre-frail and frail nursing home residents, potentially supporting the role of MSTN as a muscle chalone.

KEYWORDS: Myostatin, physical frailty, physical function, physical performance, basic activities of daily living

Introduction

Ageing is associated with morphological and functional changes in skeletal muscle, including motor unit loss, changes in myofibre type, muscle atrophy, and degeneration of the neuromuscular junction (Tieland et al., 2018). These age-related changes contribute to loss of muscle mass and function, which are features of sarcopenia and frailty syndrome (Fried et al., 2021; Sayer et al., 2024). The pathophysiology of frailty is complex and multifactorial, with skeletal muscle dysfunction playing a central role in its development and contributing to the distinctive signs, symptoms, and adverse outcomes associated with the syndrome (Fried et al., 2021). Currently, this condition is considered a major public health challenge associated with a decline in physical function, dependence in basic activities of daily living (BADL), disability and institutionalisation (Dent et al., 2025).

Myostatin (MSTN), a member of the transforming growth factor-beta (TGF-β) superfamily, acts as a negative regulator of muscle mass, inhibiting muscle growth and promoting atrophy (Lee et al., 2023). In addition, it impairs the proliferation and differentiation of satellite cells, thereby hindering skeletal muscle repair. Due to its central role in muscle regulation, MSTN has emerged as a promising biomarker and therapeutic target for conditions involving muscle loss. Previous evidence has shown that an upregulation of absolute MSTN levels is associated with muscle-wasting conditions, such as sarcopenia and frailty (Bergen et al., 2015; Chew et al., 2019). However, recent studies have demonstrated that higher absolute MSTN levels were associated with a lesser frailty status (Arrieta, Hervás, et al., 2018), better physical function (e.g physical performance and muscle strength) and muscle mass in older adults (Cawthon et al., 2023; Choi et al., 2021), sparking the debate about the role and relationship between MSTN, frailty and physical function. Some suggested that these inconsistencies could be attributed to confounding factors such as muscle mass and sexual dimorphism (Baczek et al., 2020; Bergen et al., 2015; Laurent et al., 2019).

To provide some insights into this topic, we investigated the relationship between circulating serum MSTN levels (e.g. absolute and relative to total body lean mass) and frailty status, as well as physical function outcomes, including physical performance, independence in BADL, and lower-limb strength, in pre-frail and frail older adults (mean age >80 years) residing in nursing homes.

Methods

Study design and data collection

This is a secondary, cross-sectional exploratory analysis. The sample size was determined by the availability of enzyme-linked immunosorbent assay (ELISA) kits for MSTN analysis. No formal a priori sample size calculation was performed. This exploratory cross-sectional analysis included baseline data from one previous trial [n=22;(Barros et al., 2024)] and additional participants from a separate trial [n=38;(Barros et al., 2025)], resulting in a combined sample of 60 participants.

A convenience sample of older adults residing in nursing homes aged 65 or older was recruited. The eligibility criteria included any resident who was able to walk with or without assistance and was classified as pre-frail or frail according to the frailty phenotype (Fried et al., 2001).

A concise description of the measures relevant to this manuscript is provided here. For a more thorough description of the methodology, see Barros et al. (2024). Assessments conducted at the nursing home included blood sample collection, physical performance tests, frailty evaluation, and anthropometric measurements. A separate assessment was carried out at the university laboratory, involving body composition analysis using Dual-energy X-ray Absorptiometry (DXA) and isokinetic knee strength testing. Additionally, nursing staff completed questionnaires based on participants' electronic medical records to collect sociodemographic information, health-related data, and levels of dependence in BADL.

This study was approved by the BLINDED FOR REVIEW Ethics Committee (BLINDED FOR REVIEW) and conducted in full compliance with the Declaration of Helsinki. After obtaining medical approval from the medical teams of each nursing home, all participants and their legal representatives provided written informed consent.

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement checklist for cross-sectional studies.

Physical frailty

Frailty status was defined based on five criteria according to a modified version of the frailty phenotype (Fried et al., 2001). Unintentional weight loss was assessed with the question, “In the last year, have you lost more than 4.5 kg unintentionally (i.e., not due to dieting or exercise)?” Weakness was determined by measuring dominant handgrip strength, adjusted for gender and body mass index (BMI). Slowness was evaluated by timing a 15-foot walk. Exhaustion was assessed using two self-reported questions from the previous week, based on the Center for Epidemiological Studies-Depression scale. The physical activity criterion was modified and evaluated using the question: “Do you engage in any of the following activities—dancing, intentional walking, exercise, or gardening?” A “no” response was classified as positive for this criterion (Alves et al., 2020). The original cut-off points were applied, and each criterion was dichotomised as “yes” or “no”. Participants were categorised as frail (met three or more criteria), pre-frail (met one or two criteria), and robust (met none) (Fried et al., 2001).

Physical performance

Physical performance was assessed using the Short Physical Performance Battery (SPPB), a widely used tool for measuring lower extremity functioning and predicting mobility disability, frailty, and risk of falls (Guralnik et al., 1994). It evaluates three components: balance, gait, and lower-limb strength. The total score ranges between 0 (disabled) and 12 (high functioning).

Independence in basic activities of daily living

Independence in basic activities of daily living was assessed using the Barthel Index (BI) (Mahoney & Barthel, 1965). The BI evaluates ten core daily activities: feeding, bathing, grooming, dressing, toileting, bowel and bladder control, transfers, mobility, and stair use. The total score ranges from 0 to 100, with lower scores indicating higher levels of dependency.

Lower-limb muscle strength

The dynamic concentric muscle strength of the knee flexors (KF) and extensors (KE) was measured on an isokinetic dynamometer (Biodex System 4 Pro; Biodex, Shirley, NY). Measurements were taken at an angular velocity of 60/s (1.05 rad/s), as per the manufacturer's guidelines. After a few repetitions for familiarisation, each participant performed three maximal repetitions at 60/s with their preferred leg. The dynamometer angle reading was calibrated to the anatomic joint angle measured by a goniometer. Before testing, subjects performed a 2-minute warm-up on a step platform. During the test, participants were encouraged verbally to exert maximal muscular force. Concentric, isokinetic peak torque (PT) for KE and KF was defined as the highest value of torque (Nm). Relative peak torque (PT) for KF and KE was calculated as PT divided by the body weight (BW) ratio (Nm/kg) and used in the analysis.

Serum myostatin levels

Resting blood samples were collected after an overnight fast (10–12 hours) and >48 hours after any exercise or assessment session. Venous blood samples were drawn from the antecubital fossa in the morning (8:30 a.m.–9:30 a.m.), followed by a 30-minute coagulation period and then centrifuged (3500 × g) for 15 minutes. The supernatant serum was pipetted into labelled aliquots and stored at –80°C until analysis.

According to the manufacturer's instructions, absolute serum myostatin levels (ng/ml) were assayed in duplicate by ELISA (R&D Systems and DLdevelop, respectively). Intra- and interassay coefficients of variation (CV) from data from trials 1 and 2 were 3.1% and 9.96%, respectively.

Relative MSTN levels were calculated by normalising the absolute MSTN levels to total body lean mass (ng/ml/kg). Both absolute and relative MSTN values were included in the statistical analysis.

Covariates

Sociodemographic information (age, sex, education, years living in nursing homes) and health-related data (e.g. daily medication) were collected from the participants' electronic medical records by the nursing team.

Height was measured to the nearest 0.1 cm with a portable stadiometer (Seca 206, Seca Ltd, Birmingham, UK) and body weight to the nearest 0.1 kg using a calibrated scale (InBody 120, InBody Co. Ltd, Seoul, Republic of Korea). Body mass index was calculated as body weight (kg) divided by height squared (m2). Measurements were taken with participants wearing light clothing and barefoot. DXA was used to assess the total body lean mass (kg) (Horizon DXA system, Hologic, Inc., Marlborough, MA, USA).

Statistical analysis

Sample characteristics were analysed using descriptive statistics, with continuous variables reported as medians and interquartile range (IQR), and categorical variables as frequency and percentages (%).

Group comparisons between frail and pre-frail individuals were performed using the Mann-Whitney U test for continuous variables and the chi-square or Fisher's exact test for categorical variables.

Absolute and relative MSTN were standardised to enable comparison of effect estimates across variables measured in different units.

Correlations between absolute and relative MSTN levels and frailty criteria (e.g. Slowness, unintentional weight loss, weakness, low physical activity and exhaustion) as well as with SPPB score, BI, KE and KF PT/BW, were assessed using Spearman's rank correlation coefficient. Associations between absolute and relative MSNT levels and frailty status were evaluated using a multivariate logistic regression. Due to the limited sample size, models were adjusted only for sex, given its established biological relevance to both the predictor and the outcomes. The multivariate analysis was conducted unadjusted (Model 1) and adjusted for sex (Model 2). Bootstrap resampling (5,000 iterations) was applied to enhance the robustness of estimates. Results were expressed as odds ratio (OR) with 95% Bias-corrected confidence intervals (BCa 95% CIs).

To evaluate the discriminative ability of absolute and relative MSTN levels in identifying frailty status, a receiver operating characteristic (ROC) curve analysis was performed, and the area under the curve (AUC) was calculated with 95% CI and compared using the DeLong test.

The statistical software used for analysis was IBM SPSS Statistics for Windows (version 29.0; IBM, Armonk, NY) and R software, Version 4.5.1 (R Core Team, 2024). The significance level was set at $\alpha=0.05$.

Results

Of the 60 assessed individuals, one was excluded due to a measured value exceeding the upper detection limit of the assay. The final sample comprised 59 older adults (median age 83 (IQR 13) years, female 66.1%) (Figure S1). Most individuals were classified as frail (74.6%). Overall sample characteristics are shown in Table 1. Significant differences were observed in MSTN concentrations, BI and SPPB scores between frail and pre-frail individuals, with frail individuals exhibiting lower absolute and relative MSTN levels, BI and SPPB scores.

Relationship between myostatin and frailty criteria and physical function

Among frailty criteria, absolute MSTN levels were negatively correlated with slowness (r = -0.32, BCa 95% CI: -0.53, -0.09) and low physical activity (r = -0.36, BCa 95% CI: - 0.57, -0.11), but not with exhaustion, weakness and unintentional weight loss. Relative MSTN levels were negatively correlated with low physical activity (r = -0.29, BCa 95% CI: -0.51, -0.05) (Fig. 1A). Absolute MSTN was positively correlated with SPPB score (r=0.28, BCa95% CI: 0.01, 0.53), BI (r= 0.34, BCa95% CI: 0.10, 0.56) and KE PT/BW (r= 0.30, BCa95% CI: 0.02, 0.55), while relative MSTN was positively associated with BI (r= 0.26, BCa95% CI: 0.01, 0.50) (Fig. 1B).

Relationship between myostatin and frailty status

In multivariate logistic regression, higher absolute MSTN levels were associated with lower odds of being frail. Each 1 standard deviation increase in absolute MSTN was linked to a 59% reduction in the odds of frailty compared to pre-frailty (OR = 0.41; BCa 95% CI: 0.18, 0.75). Similarly, higher relative MSTN was also associated with lower odds of being frail (OR = 0.46; BCa 95% CI: 0.22, 0.80), with each 1-unit increase corresponding to a 54% decrease in the odds of frailty (Table 2).

ROC analysis showed that absolute MSTN Model 2 had the highest area under the curve (AUC = 0.76; 95% CI: 0.64–0.88; p = 0.002), followed by relative MSTN Model 2 (AUC = 0.73), relative MSTN Model 1 (AUC = 0.71), and absolute MSTN Model 1 (AUC = 0.70; 95% CI: 0.55–0.85; p = 0.02) (Figure 2). Pairwise comparisons using DeLong's test showed no statistically significant differences between models (Table S1).

Discussion

The primary finding of this exploratory cross-sectional study is that higher MSTN levels, both absolute and relative, are associated with reduced odds of being frail in a pre-frail and frail population. No significant differences were found between absolute and relative MSTN levels in their ability to discriminate between frail and

pre-frail individuals. Furthermore, absolute MSTN levels were significantly positively correlated with physical function outcomes, including physical performance, independence in BADL, and isokinetic knee extension strength. In contrast, relative MSTN levels were only significantly positively correlated with independence in BADL.

Our findings align with previous studies demonstrating lower absolute levels of MSTN in frailer status (Arrieta, Hervás, et al., 2018; Echeverria et al., 2021). This reduction may represent a compensatory mechanism aimed at mitigating muscle atrophy associated with frailty (Mariot et al., 2017). As highlighted by Lee (2023), MSTN has chalone-like properties and functions within a complex regulatory network involving multiple inhibitory binding proteins that modulate the balance between muscle degradation and growth. In this context, MSTN and its antagonists (i.e., Follistatin, Follistatin-like 3 and Decorin) may play a critical role in attenuating muscle loss under catabolic conditions, while simultaneously preventing excessive skeletal muscle hypertrophy in response to anabolic stimuli, such as testosterone treatment (Peng et al., 2022). Further evidence supporting MSTN as a key regulator of muscle lies in its role as a myokine. Chronic exercise, particularly resistance training, has been shown to downregulate MSTN expression and circulating levels, thereby potentially promoting muscle growth, even in frail individuals (Barros et al., 2022; Barros et al., 2024). Variability in reported findings across the literature may reflect the confounding influence of muscle mass on circulating MSTN levels, as most studies report absolute concentrations without adjustment (Bergen et al., 2015; Laurent et al., 2019).

To date, limited evidence is available regarding the association between circulating MSTN and measures of physical function in older adults, as most existing studies have predominantly examined its relationship with muscle mass or handgrip strength, which restricts direct comparison with out findings. We found that higher absolute levels of MSTN were inversely associated with the low physical activity criterion of frailty phenotype, indicating that elevated MSTN levels were correlated with greater participation in physical activities. Similarly, Arrieta, Hervás, et al. (2018) reported a positive correlation between MSTN levels and daily step count, as well as light to moderate-to-vigorous

physical activity, in women residing in nursing homes. We also observed a positive relationship between absolute MSTN and physical function outcomes such as physical performance, dependence in BADL and isokinetic knee extension strength. The correlation with lower extremity function may reflect the predominant expression of MSTN in large muscle groups, particularly the quadriceps femoris, which plays a central role in mobility, knee extension, and daily functioning (Mizuno et al., 2021; Wearing et al., 2019).

To account for the potential confounding effect of muscle mass, MSTN concentrations were normalised to total body lean mass, as suggested by Bergen et al. (2015). We found that the association between MSTN and frailty status remained robust, even after controlling for sex. Moreover, normalisation to lean mass did not meaningfully alter MSTN's ability to discriminate between pre-frail and frail individuals. However, relative MSTN was no longer correlated with physical function, except for independence in BADL. This suggests that muscle mass may partially mediate the relationship between MSTN and physical function. It is also hypothesised that MSTN's predictive ability may vary according to the frailty status (de Jong et al., 2025), which could help explain the conflicting findings reported in the literature. For example, Cawthon et al. (2023) did not find a significant association between MSTN and isokinetic knee extension strength in community-dwelling adults. In such relatively healthy populations, with preserved muscle mass and function, circulating MSTN may have limited discriminatory value.

Based on our findings, MSTN shows promise as a component of a biomarker panel for identifying frailty status in nursing home residents (Arrieta et al., 2022; Chew et al., 2019), given its association with frailty and physical function, as well as its responsiveness to frailty interventions such as exercise. Future longitudinal studies with larger, prospectively powered cohorts that follow individuals through the transition from robust to frail states, while rigorously addressing potential confounding, are required to validate and extend these observations.

While our study has notable strengths, including its focus on an understudied population of pre-frail and frail nursing home residents and the use of multiple objective physical function measures, several limitations must be acknowledged. First, the study was not powered on the basis of a formal sample size calculation, and the sample size was constrained by assay availability. As such, the analysis is exploratory and may lack sufficient power to detect small-to-moderate associations, warranting cautious interpretation. Second, the highly specific target population and the exclusion of non-frail individuals limit the generalizability of our results to the broader older adult population. Additionally, circulating MSTN was measured using ELISA, which does not distinguish between biologically active and inactive forms, potentially limiting the interpretability of MSTN-related associations. Although we adjusted for sex and examined MSTN relative to lean mass, other potential confounding variables were not considered. Furthermore, DXA-derived lean mass is an indirect proxy that does not accurately reflect muscle mass and shows an inconsistent and weak association with functional outcomes (Kirk et al., 2024). Finally, the cross-sectional design of our study precludes any conclusions regarding causality between MSTN levels, muscle mass, and physical function.

Conclusion

This study demonstrates that MSTN, a negative regulator of muscle mass, is associated with reduced odds of frailty among pre-frail and frail nursing home residents. Absolute MSTN concentrations were positively correlated with physical function, whereas relative MSTN was not, suggesting that muscle mass may partially mediate the relationship between MSTN and function. Although counterintuitive, these findings support the concept of MSTN as a skeletal muscle chalone and highlight its potential utility as a biomarker of frailty.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Table 1. Characteristics of the study participants

Characteristics	Frail (n=44)	Pre-frail (n=15)	p-value
Age, years	87 (13)	82 (14)	0.453
Female sex, *n (%)*	*27 (61.4)*	*12 (80)*	0.159
Years of education, years	4 (2.75)	4 (0)	0.051
Years living in NH, years	3 (4)	1 (1)	0.065
Daily medications, number	9.5 (7.75)	8 (3.0)	0.162
BMI, kg/m^2	28.55 (5.90)	26.6 (5)	0.158
Total lean mass, kg	37.05 (8.37)	36.40 (6.96)	0.702
HG strength, kgF	16.25 (6.4)	17.4 (6.6)	0.951
Absolute MSTN, ng/ml	1.83 (1.10)	2.25 (1.45)	0.024
Relative MSTN, ng/ml/kg	0.047 (0.03)	0.062 (0.04)	0.017
SPPB, score	6 (3)	11 (6)	<0.001
Barthel Index, score	85 (28.75)	95 (15)	0.026
Relative KE Peak Torque, Nm/kg	87.3 (34.30)	77.9 (35.20)	0.324
Relative KF Peak Torque, Nm/kg	33.10 (16.6)	39.20 (18)	0.505

Data expressed as medians (IQR) or numbers (frequencies). Abbreviations: BMI: body mass index; HG: handgrip; KE: knee extension; KF: knee flexion; MSTN: myostatin; NH: nursing home; SPPB: short physical performance battery. Differences between groups were tested using the Mann–Whitney U test or Fisher's exact test.

Table 2. Association between myostatin and frailty status

Independent variable	Model 1 OR (95%CI)	p-value	Model 2 OR (95%CI)	p-value
Absolute MSTN	0.45 (0.24, 0.77)	0.006	0.41 (0.18, 0.75)	0.008
Relative MSTN	0.44 (0.22, 0.73)	0.008	0.46 (0.22, 0.80)	0.011

Note: Model 1 is unadjusted, and Model 2 is adjusted for sex. Abbreviations: CI: confidence interval; MSTN: myostatin; OR: odds ratio

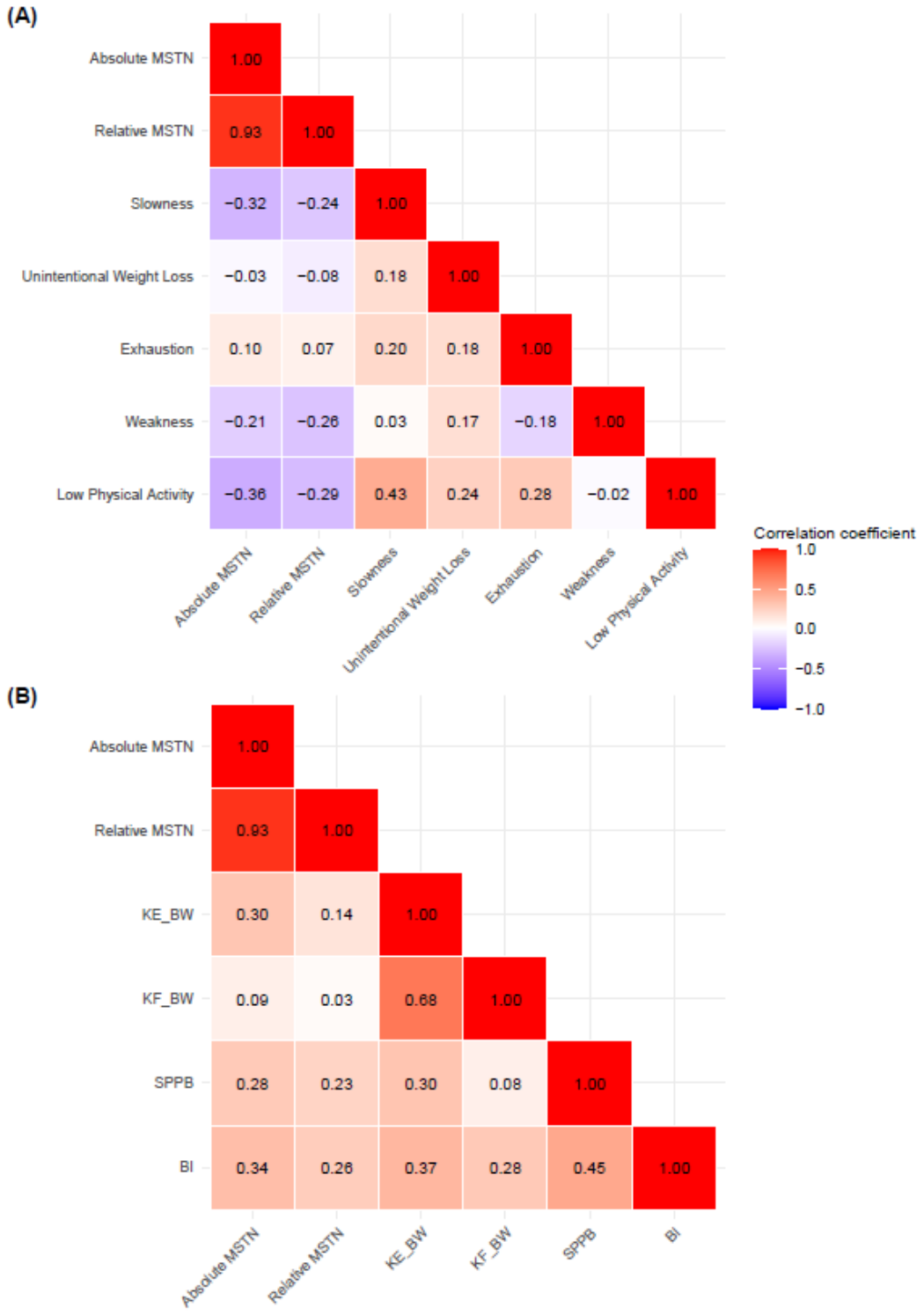

Fig. 1. Correlation matrix showing the relationships between absolute and relative MSTN and frailty criteria (A) and physical function measures (B).

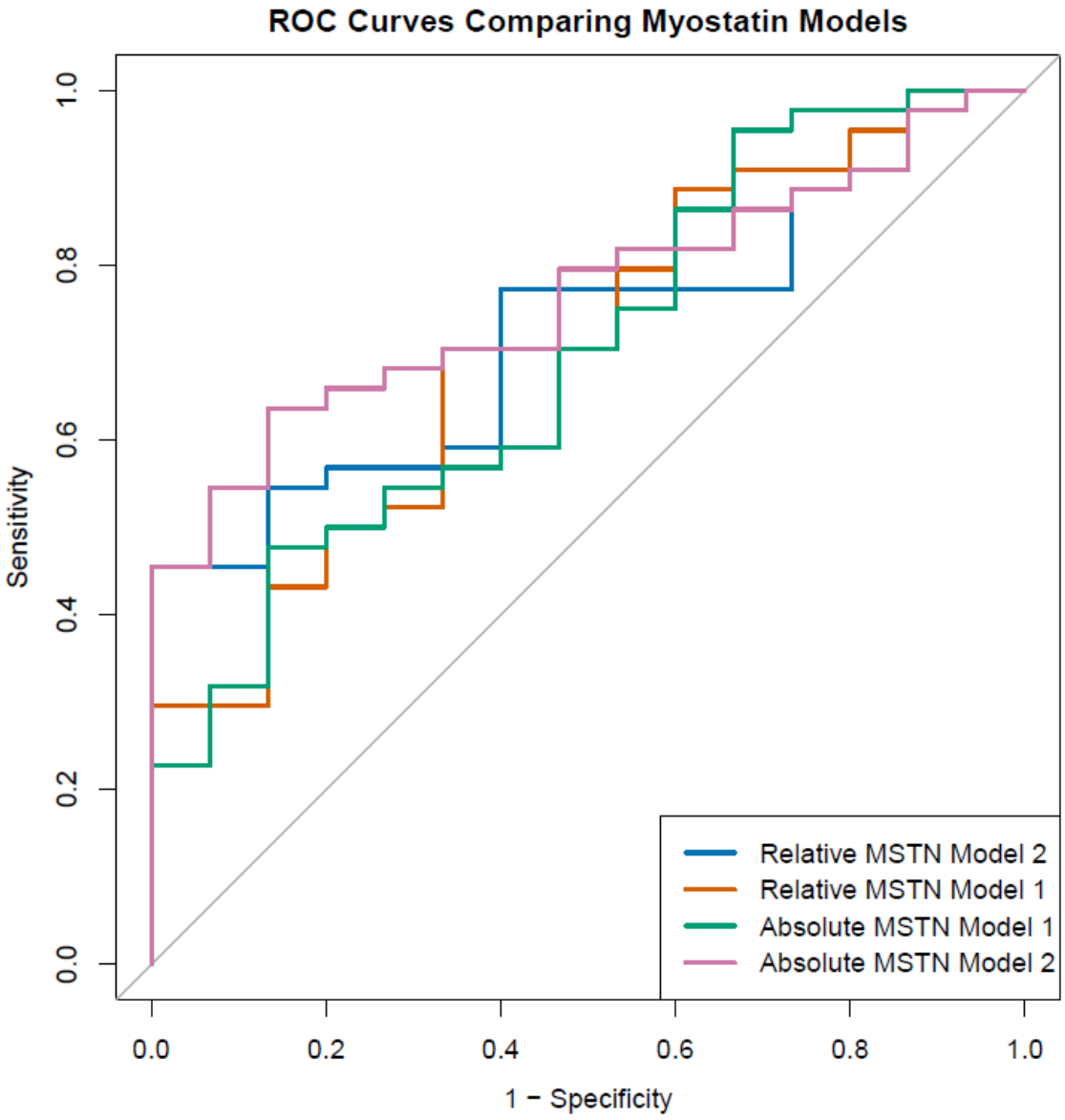

Fig 2. Receiver Operating Characteristic (ROC) curves from logistic regression models evaluating the association between MSTN levels and frailty status.

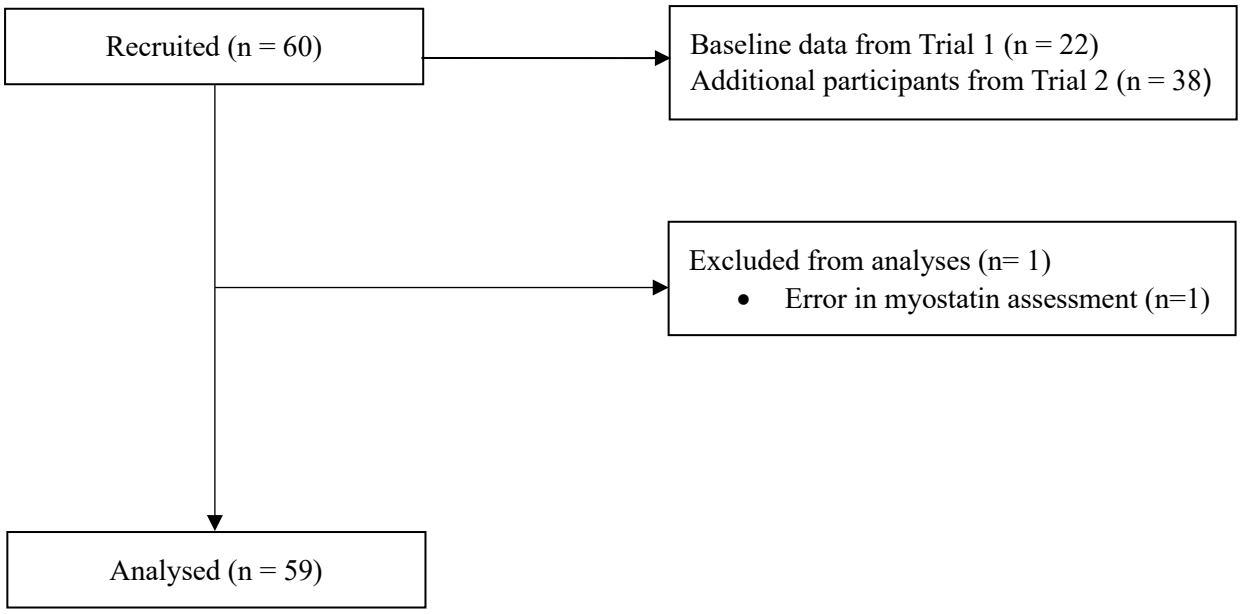

Fig S1. Flow of study participants

Table S1. Pairwise Comparison of ROC Curves between absolute and relative MSTN models

Comparison	AUC (first model)	AUC (second model)	p-value DeLong	Z statistic
Abs. MSTN M1 vs Abs. MSTN M2	0.70	0.76	0.12	-1.54
Abs. MSTN M1 vs Rel. MSTN M1	0.70	0.71	0.74	0.34
Abs. MSTN M1 vs Rel. MSTN M2	0.70	0.73	0.61	0.51
Abs. MSTN M2 vs Rel. MSTN M1	0.76	0.71	0.15	-1.43
Abs MSTN M2 vs Rel. MSTN M2	0.76	0.73	0.24	-1.18
Rel. MSTN M1 vs Rel. MSTN M2	0.71	0.73	0.60	0.53

Note: Abs.: Absolute, Rel.: relative, AUC: Area under the curve; MSTN: Myostatin; M1: Unadjusted model; M2: Model adjusted for sex.

4.2 Study III

Barros, D., Silva-Fernandes, A., Martins, S., Guerreiro, S., Magalhães, J., Carvalho, J., & Marques, E. A. (2024). Feasibility and Effectiveness of a 12-Week Concurrent Exercise Training on Physical Performance, Muscular Strength, and Myokines in Frail Individuals Living in Nursing Homes: A Cluster Randomized Crossover Trial. Journal of the American Medical Directors Association, 25(11), 105271. https://doi.org/10.1016/j.jamda.2024.105271

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Original Study

Feasibility and Effectiveness of a 12-Week Concurrent Exercise Training on Physical Performance, Muscular Strength, and Myokines in Frail Individuals Living in Nursing Homes: A Cluster Randomized Crossover Trial

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Exercise
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A B S T R A C T

Objective: To examine the feasibility and effects of a 12-week exercise intervention on physical performance, muscular strength, and circulating myokines in frail individuals living in nursing homes.
Design: A cluster randomized, 2-period, 2-intervention crossover trial.
Setting and Participants: Frail residents of 9 nursing homes were randomly assigned to either 12 weeks of concurrent exercise training (n = 5, 29 participants) or usual care (n = 4, 17 participants). The concurrent exercise training consisted of resistance and aerobic exercises (3 days/week). The usual care consisted of everyday routine and standard care. After a 4-week washout period, participants crossed to the other intervention.
Methods: The feasibility outcomes included recruitment rate, dropout rate and reasons, harms during the trial, adherence to exercise, and implementation cost. The primary endpoint was the change in physical performance measured by the Short Physical Performance Battery (SPPB). The secondary endpoints were changes in muscular strength (eg, handgrip strength, isokinetic knee extension, and flexion strength) and serum myokines concentration (myostatin and decorin).
Results: From the 46 participants enrolled (aged 70–99 years, 67.4% female), 34 completed the trial (26.1% dropout rate), the median adherence was 93.75%, and no adverse events occurred during the exercise sessions. The concurrent exercise training provided significant benefits over usual care on SPPB (B = 2.18; 95% CI, 1.35–3.00; $P < .001$), handgrip strength (B = 2.15; 95% CI, 1.00–3.30; $P < .001$), myostatin concentrations (B = −7.07; 95% CI, −13.48 to −0.66; $P = .031$) and myostatin-decorin ratio (B = −95.54; 95% CI, −158.30 to −32.78, $P = .004$). No significant between-group differences were found for the remaining secondary endpoints.

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Conclusions and Implications: This concurrent exercise training is feasible, well-tolerated, and effective in improving physical performance, handgrip strength, myostatin, and myostatin-decorin ratio concentrations in frail older adults residing in nursing homes. These data reinforce the relevance of integrating exercise interventions in long-term care settings.

Frailty is an age-related condition characterized by increased vulnerability to stress factors and decreased resilience that is becoming a public health concern with significant health and economic burdens.[1] This condition is highly prevalent among long-term care residents and significantly increases their risk of adverse health outcomes, including falls, disability, hospitalization, and mortality.[2,3]

Individuals with frailty often exhibit skeletal muscle function and structure dysregulation, physical decline, and diminished energy levels. Thus, frailty trajectories frequently overlap with low physical performance and dependency.[3] The decline in lower extremity function is an established negative outcome related to frailty that plays a crucial role in the disability process.[4] Lower scores in Short Physical Performance Battery (SPPB)—an objective tool for measuring lower extremity physical performance status—have been associated with decreased mobility, falls, loss of independence in activities of daily living (ADLs), and mortality.[5] Muscle strength, particularly in the lower extremities, seems to decline faster than muscle mass and also contributes to a decline in physical performance and, consequently, loss of independence in ADLs.[4,6] Thus, most frailty research and interventions are focused on preventing and reversing frailty and on more meaningful person-centered outcomes such as physical performance.

Resistance and aerobic training are recommended for older adults and frail individuals due to well-established health benefits.[7] Therefore, concurrent (the combination of resistance and aerobic training in the same program) or multicomponent (usually adding balance and/or flexibility) exercise programs are a cornerstone in frailty management,[8] not only by addressing its signs and symptoms and functional consequences but also by boosting physiological resilience.[9] The action mechanisms of exercise include stimulating an anti-inflammatory environment and reducing age-related muscle mass and function loss related to frailty, partially by releasing myokines, including decorin and myostatin.[10]

Previous limited intervention trials in frail institutionalized older adults were mostly based on resistance training alone or concurrent/multicomponent exercise.[11-19] Despite the growing body of evidence supporting the effectiveness of such interventions in long-term care settings, there is still high heterogeneity in exercise protocols and insufficient details on exercise characteristics reports (eg, exercises included, intensity, session duration, progression or work-to-rest ratio).[20] The inconsistent participant's inclusion in trials also adds to current limitations in the literature. For instance, many trials have included both pre-frail and frail individuals,[15,18] or frail individuals have been identified based on performance-based tests, which may not accurately assess frailty status.[19] Although most research is conducted in the early stages of frailty, older frail individuals with complex medical conditions and significant comorbidities are often excluded from trials.[21] Exercise trials examining the feasibility and safety of such interventions are urgently needed to improve the generalizability of findings to real-world populations and inform specific exercise recommendations and future implementation strategies in this population.

Therefore, the present trial primarily aimed to examine the feasibility and effects of a 12-week concurrent exercise training on physical performance, muscle strength, and circulating myokines in frail individuals living in nursing homes.

Methods

This was a cluster randomized crossover trial with 2 interventions and 2 periods. The recruitment phases occurred from October 1 to December 20, 2021, and from October 1 to December 15, 2022, in the Porto Metropolitan Area, Portugal. Initially, 10 nursing homes were contacted via email. Of those, 2 did not respond despite 2 follow-up attempts, and 1 declined participation due to ongoing COVID-19 concerns. After the initial screening, 2 more nursing homes were excluded for not having at least 4 residents who met the trial's inclusion, a minimum established for logistical convenience. Consequently, 6 nursing homes were successfully randomized in 2022. To reach the required sample size, 4 additional nursing homes were invited, and 3 agreed to participate.

This study was conducted according to the updated version of the CONSORT statement to report randomized crossover trials,[22] and was in full compliance with the Declaration of Helsinki. The study is registered at ClinicalTrial.Gov (NCT06380127) and was approved by the Faculty of Sports, University of Porto Ethics Committee (CEFADE 01 2021). After receiving medical approval from each nursing home medical team, all participants and their legal representatives signed an informed consent before enrollment.

Participants and Randomization

Older adults aged 65 or older living in 11 nursing homes in the Porto Metropolitan Area, Portugal, who could walk (with or without assistance) were invited to participate in the study, and the potential participants were screened. The eligibility criteria included 2 inclusion criteria: being classified as frail according to the Fried criteria (eg, frailty phenotype $\geq$ 3 criteria),[3] and not having participated in any exercise intervention for the past 3 months; and 3 exclusion criteria: any contraindication that could affect physical exercise performance or testing procedures, including terminal illness, uncontrolled disease, or other unstable medical condition, bone fracture in the past 3 months, inability to understand and follow the instructions of physical tests, and having an SPPB score $<$3. SPPB scores are commonly stratified into groups (0–3, 4–6, 7–9, 10–12), with a score of 10–12 as the best performance group. Thus, participants with an SPPB score between 0 and 3 have the worst physical performance reflecting higher odds of mobility-related disability.

Randomization was done at the cluster level (nursing homes). Clusters were randomly assigned to 1 of 2 sequence orders with a 1:1 allocation using a computer-generated block randomization schedule (Research Randomizer V.4). For safety, exercise sessions were capped at 6 participants. Clusters larger than 6 were split into 2 groups following the same sequence to prevent contamination.

Each participant was assigned an alphanumeric code to maintain anonymity. University and laboratory assessors were blinded to all participants' data, including the allocation sequence. However, the principal researcher was not blinded due to his role in testing and supervising some training sessions. After baseline assessments, the nursing homes were informed about their group allocation. Due to the intervention's nature, participants knew whether they were receiving exercise or usual care.

Trial Design

The trial followed a 28-week crossover design: 12 weeks for the first intervention period, a 4-week washout, and another 12 weeks for the second intervention period. Nursing homes (clusters) were randomized into sequences (AB or BA) in which intervention A involved concurrent exercise training, and B was usual care. A crossover design was chosen over a parallel design to reduce between-subjects variability and confounding, enhancing statistical efficiency, and allowing for a smaller sample size. A 4-week washout was deemed sufficient to mitigate carryover effects based on previous studies of detraining in older adults.[17]

Data collection occurred at 4 points: baseline, post-first intervention period, post-washout/pre-second intervention period and post-crossover, as shown in Supplementary Table 1. Assessments took place during 2 (~40-minute) sessions: the first in the nursing home and included blood collection, physical performance, and handgrip strength tests; and the second in the university lab for body composition and isokinetic knee strength assessments.

Participants and nursing home medical teams were instructed not to engage in other structured exercise programs or change their diets during the trial. Participants received standard care during the exercise intervention.

Interventions

The usual care intervention included standard medical and rehabilitation care as needed.

The exercise intervention, conducted at the nursing homes and in a group, consisted of 12 weeks of supervised concurrent exercise training, held 3 times weekly on nonconsecutive days to prevent overtraining and fatigue. Each session, lasting 50 to 60 minutes, included a warm-up, resistance and aerobic training, and a 5-minute cool-down. The warm-up involved 1 set of 10 repetitions of later resistance exercises performed without load. The resistance training comprised 2 to 3 sets of 10 to 15 repetitions with an external load equivalent to 40% to 70% of the 1-repetition maximum (1RM) for upper (ie, bicep curl) and lower body exercises (ie, seated knee extension). The sit-to-stand exercise was performed as fast as possible according to each participant's capacity without external load. The recovery between sets lasted 1 to 2 minutes. The aerobic training included walking exercises with changes in pace and direction, which progressed from 5- to 10-minute duration in the first weeks to 10 to 15 minutes. Intensity was monitored through observer-rated perceived exertion (eg, sweating, difficulty talking while walking, and respiratory rate) due to participants often presenting cognitive impairment. The sessions ended with stretching exercises. The same exercise professional led all sessions and was not involved in any assessment. A detailed description of the exercise protocol is provided in Supplementary Table 2.

Loads were tailored to individual strength and assessed every 3 weeks from the second week using a 10-repetition maximum (10RM) test for bicep curl and seated knee extension exercises. Participants warmed up with light loads before attempting the 10 RM test up to 3 times, with a 3-minute rest between the attempts. Resistance was increased if participants could exceed 10 repetitions with the proper technique.[11] The test concluded when participants could not perform more than 10 repetitions. Subsequently, the 1RM was calculated based on the Brzycki[23] formula.

Feasibility Outcomes

Feasibility outcomes included recruitment, dropout rates and reasons, and adherence to exercise intervention. Recruitment rate was defined as the number of people randomized over the total eligible people $\times$ 100. Dropout rate was calculated as the percentage of participants who did not complete the trial, with reasons recorded. Adherence to the exercise intervention was the percentage of sessions attended. Reasons for missing sessions were also noted.

Costs analysis assumed the nursing home (payer) perspective, excluding indirect costs like travel time. The analysis covered 12 weeks, factoring in human resources at 9.375€ per hour [based on a monthly (40 h/wk) gross salary of 1500€] and sports equipment costs. The costs of implementing this exercise intervention were calculated in euros based on the 2022 market reference (Supplementary Table 3). The incremental cost-effectiveness ratio (ICER) was estimated as the difference between the trial interventions in mean total costs per participant divided by the difference in the changes in the main outcome (SPPB, Supplementary Table 4).

Throughout the 28-week trial, harms such as falls, infections, acute events, or any disease were recorded by directly asking the participants and medical team (after an absence from 1 training session) or when spontaneously mentioned by the participant or medical team.

Endpoints

Primary endpoint

The primary endpoint was the change in physical performance, assessed by the SPPB score (0–12), evaluating balance, gait speed, and lower-limb strength. A 1-point change is considered a meaningful clinical change.[24]

Secondary endpoint

Muscular strength. The handgrip strength test was performed using a calibrated Jamar Plus Digital Hand Dynamometer (Sammons Preston Inc.). Participants were asked to sit in a chair without an armrest, with feet fully resting on the floor and the arms were adducted and neutrally rotated, the elbow flexed to 90°.[25] Test time was followed by a rest period of at least 15 seconds between trials in each hand. The best value with the dominant hand in kilogram-force (kgf) was used in the analysis.

The dynamic concentric muscle strength of the knee flexors and extensors was measured on an isokinetic dynamometer (Biodex System 4 Pro). Subjects were asked to be comfortably seated with the trunk and thigh restrained using chest, waist, and thigh straps. The measurements followed the manufacturer's instructions for knee extension/flexion at the angular velocity of 60/s (1.05 rad/s). After a few repetitions for familiarization, each participant performed 3 maximal repetitions at 60/s with their preferred leg. The dynamometer angle reading was calibrated to the anatomic joint angle measured by a goniometer. Before testing, subjects performed a 2-minute warm-up on a step. During the test, participants were encouraged verbally to exert maximal muscular force. The absolute (Nm) knee flexors and extensors peak torque (PT) were measured, and the relative (PT/body weight) peak torques (Nm/kg) were calculated.

Serum myostatin and decorin levels—Subgroup analysis. Resting blood samples were collected after an overnight fast (10–12 hours) and >48 hours after any exercise or assessment session. Venous blood samples were drawn from the antecubital fossa in the morning (8:30 AM–9:30 AM) followed by a 30-minute coagulation period and then centrifuged (3500*g*) for 15 minutes. The supernatant serum was pipetted into labeled aliquots and stored at −80 °C until analysis.

According to the manufacturer's instructions, serum myostatin and decorin levels (pg/mL) were assayed in duplicate by enzyme-linked immunosorbent assay (R&D Systems and DLdevelop, respectively). Intra- and interassay coefficients of variation were 3.1% and 9.96% for myostatin and 15.40% and 15.29% for decorin, respectively.

A pre-planned subgroup analysis based on the available budget was conducted on participants with higher adherence. Myostatin concentration adjusted for lean mass was performed, including 22 participants [median adherence to exercise: 95.84%; interquartile range (IQR): 6.95].[26] Similarly, a subgroup analysis in decorin included 15 participants (median adherence to exercise: 97.23%; IQR: 13.88). A sensitivity analysis was conducted with and without 2 extreme outliers' values. These outliers were excluded from the analysis because no quantitative or qualitative changes were found. Myostation-decorin ratio was analyzed in 15 participants with both measures (median adherence to exercise: 97.23%; IQR: 13.88).

Other Measures

Sociodemographic data such as age, sex, health-related data, and usual medication were gathered via questionnaires.

Anthropometric measures included height, which was measured to the nearest 0.1 cm with a portable stadiometer (Seca 206), and body mass, which was determined to the nearest 0.1 kg using a calibrated scale (Seca HV120). Body mass index (BMI) was calculated as body mass (kg) divided by height squared (m^2). Measurements were taken with participants wearing light clothing and barefoot.

Physical activity levels and time spent on sedentary behaviors (min/d) were measured using an ActiGraph GT9X Link wearable accelerometer (ActiGraph) at 2 time-points (during the initial assessments and the last week of the washout period) to determine whether participants maintained similar physical activity levels before starting a new intervention. Participants were asked to wear the device on their nondominant wrist for 7 consecutive days. Data were collected at 100 Hz. A valid day was defined as ≥10 hours wear time, and data between 8:00 and 22:00 were analyzed after manual inspection to exclude sleep time. Activity levels were captured in 60-second epochs with vector magnitude counts (VMC). Montoye, Clevenger, Pfeiffer, Nelson, Bock, Imboden, Kaminsky VMC-based cut points were used to classify daily time spent in sedentary behavior (<2860 VMC/min), light physical activity (LPA) (2860−3940 VMC/min), and moderate-to-vigorous physical activity (MVPA) (>3940 VMC/min).[27] Data were collected before participants started periods I and II. Of the 46 participants, 5 refused to wear the accelerometer, 5 did not wear the device because of the high risk of low compliance, and 4 did not wear it because the device was unavailable. Consequently, 32 participants wore the devices before starting period I. Of these, 3 had invalid data, resulting in 29 participants with valid data for period I and 22 participants with valid data for both periods.

Participants' nutritional status was determined by the Mini Nutritional Assessment−Short Form (MNA-SF).[28]

Global cognitive capacity was measured using the Mini-Mental State Examination, a commonly used cognitive screening test for identifying cognitive impairment.[29]

Dual-energy x-ray absorptiometry (DXA) was used to assess lean mass (kg) and body fat percentage (%). Appendicular lean mass (ALM) (ie, the sum of the lean tissue in the arms and legs) was derived from DXA scans and adjusted for height (ALM/Height2) (DR 4500/A, Hologic Explorer), and used to ascertain the prevalence of sarcopenia.[30]

Sample Size

The sample size, calculated to detect an effect size of 0.49 obtained from a previous study,[15] with a 0.7 correlation in repeated measures, an $\alpha = 0.05$ and 80% power, required 34 participants (GPower v. 3.1 software). Allowing for a 25% dropout rate, the targeted sample increased to 46 participants. The calculations were at participant level (not accounting for clustering effects) based on the following assumptions: (1) uniformity in exercise programs across clusters, conducted by the same professional; (2) all nursing homes were public and geographically close to each other; and (3) similar usual care during and after COVID-19 (no other external activities were allowed).

Statistical Analysis

Sample characteristics were analyzed using descriptive statistics and were reported as mean and SD or median and IQR and frequency and percentages (%) for continuous and categorical variables, respectively. Data distribution was checked for normality using the Shapiro-Wilks test and histograms.

Generalized linear mixed models (GLMMs) analyses were performed to assess the effects of experimental conditions on the primary and secondary outcomes, considering sequence (AB or BA), intervention (exercise or usual care), time (pre- and post-intervention), and interaction time by intervention as fixed effects and participants within clusters as random effects. Covariates that did not change throughout the trial or did not contribute significantly to the model were removed. Sex and baseline frailty remained as covariates in GLMMs. Estimated mean scores and 95% CIs were provided and Bonferroni post hoc tests were performed to explore differences between the mean of initial and final scores in each intervention.

In GLMM analysis, outcomes that met the assumption of normal distribution were treated with an identity link function. For positively skewed outcomes (eg, knee flexion strength), a gamma distribution and log link function were applied. Decorin was log-transformed before analysis.

The primary analysis was based on a per-protocol (PP) analysis, including participants who completed the full crossover trial design and had an exercise adherence ≥80%.

Sensitivity analysis included comparing intervention effects estimated by the intention-to-treat principle (ITT) (including all randomized participants) and comparing participants who completed the trial and those who dropped out. The *t* test, Mann-Whitney *U* test for continuous variables, and Fisher's exact test for categorical variables were used.

The statistical software used for analysis was IBM SPSS Statistics for Windows (version 29.0). The significance level was set at $\alpha = 0.05$.

Results

Participant Characteristics

Figure 1 shows the study flow diagram. Of 154 screened individuals, 42.21% were eligible, and 75.41% of those (46 of 61) consented to participate, forming 10 exercise groups across 9 clusters. Of the 46 participants starting period I of the trial (mean ± SD age: 82.87 ± 7.29 years, age range: 70−99), 67.4% were women, 69.6% were widowers, and nearly one-third (28.26%) had sarcopenia. The baseline characteristics of participants allocated to each intervention at period I were balanced (Table 1). There were no significant baseline differences between completers and dropouts ($P > .05$).

Participants (n = 22) showed no significant differences in sedentary time, LPA, and MVPA ($P = .592$, $P = .339$, $P = .149$, respectively) before starting a new intervention.

Feasibility

At the end of the 28-week trial, 34 participants (26.1% dropout rate) completed it, and from these, 28 who had an adherence ≥80% (median adherence 93.75%, IQR, 86.12−97.23) were included in the PP analysis. Nonattendance to exercise sessions was mainly due to acute health issues (eg, infection) or scheduling conflicts with daily routines at the nursing home (n = 3 from 1 cluster).

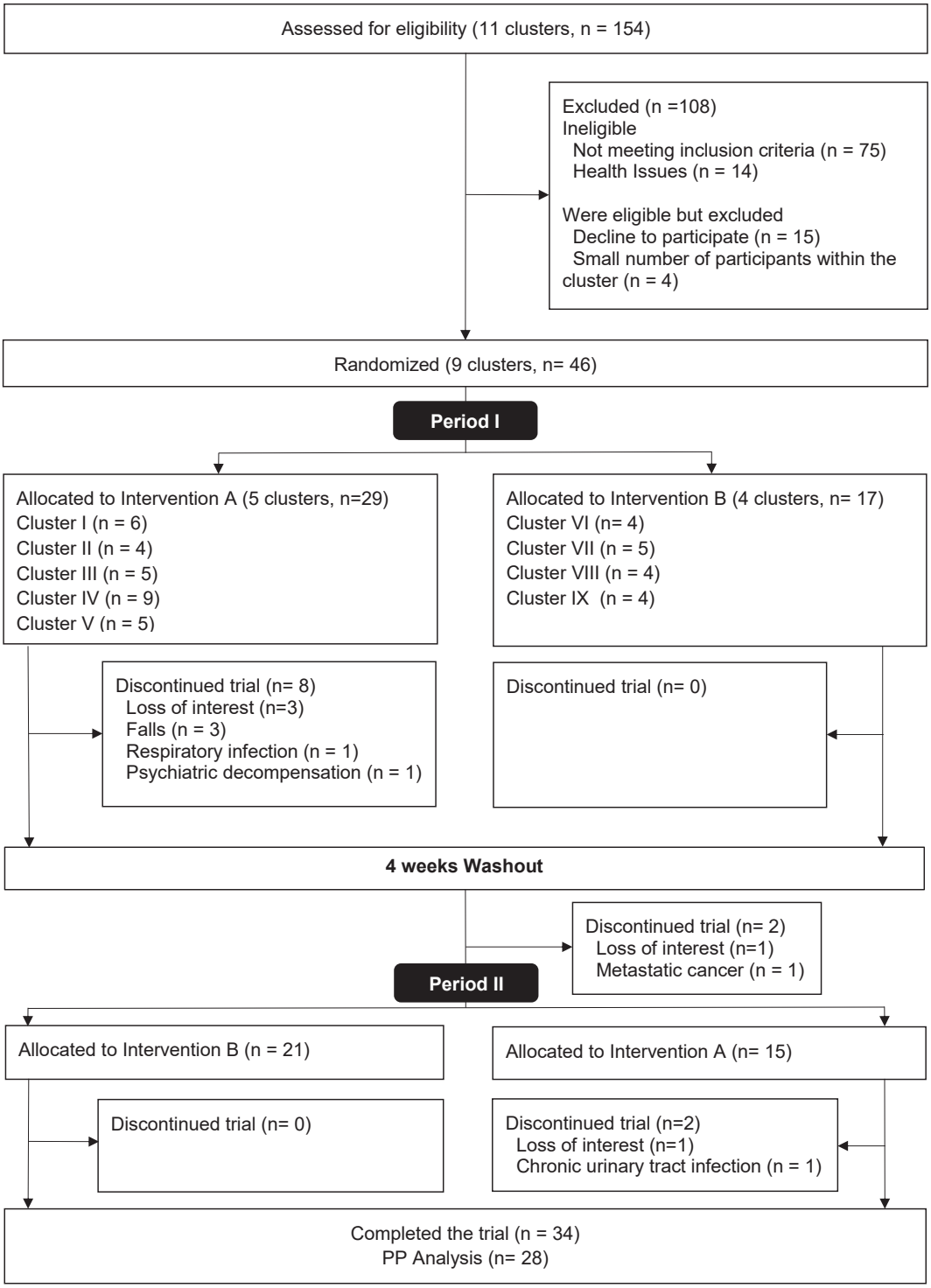

Fig. 1. Study flow diagram.

No adverse events occurred during the exercise or testing sessions. However, 16 participants experienced adverse events during the exercise period and 10 during the usual care period, mostly falls (exercise: n = 6, usual care: n = 8). During the exercise period, other adverse events included respiratory infections due to COVID-19 (n = 4), pneumonia (n = 1), urinary tract infection (n = 2), other infections (n = 2), and surgery (n = 1). Two hospitalizations occurred during the usual care period due to cholelithiasis and pneumonia.

Overall, the 12-week exercise intervention cost was 5829.32€, reflecting a cost per participant of 171.45€ (Supplementary Table 3). The ICER showed an intervention cost increase of 79.01€ per participant over 12 weeks with an incremental gain in SPPB score. Sensitivity analysis revealed that costs per SPPB score per participant

Table 1
Participants' Baseline Demographic and Clinical Characteristics by Allocation for Period I of the Crossover

Characteristics	A: Exercise (5 clusters, n = 29)	B: Usual Care (4 clusters, n = 17)
Age, y (SD)	82.93 (6.82)	82.76 (8.24)
BMI (kg/m^2)	29.82 (5.22)	27.83 (5.12)
Sex female, n (%)	20 (69)	11 (64.7)
Cardiovascular disease, n (%)	19 (65.5)	8 (47.1)
Diabetes, n (%)	13 (44.8)	9 (52.9)
Hypertension, n (%)	20 (69)	13 (76.5)
Dyslipidemia, n %	18 (62.1)	7 (41.2)
Sarcopenia, n %	8 (27.6)	5 (29.4)
Daily medications, n (SD)	10.38 (4.61)	8.76 (3.96)
MMSE, score	19.69 (7.2)	20.59 (5.68)
MNA-SF, score	10.34 (1.70)	11.29 (1.61)
Total lean mass, kg	37.70 (6.67)	36.08 (4.03)
Daily sedentary time, min/d	762 [65.5]	726 [106.5]
Daily LPA, min/d	45.20 [52.03]	25.30 [45.22]
Daily MVPA, min/d	1.14 [4.39]	0.71 [2.85]
Primary outcome		
SPPB Score	6.31 (2)	5.94 (1.71)
Secondary outcomes		
Handgrip strength, kgF	17.85 (5.29)	16.04 (5.45)
Relative knee extension PT, Nm/kg	86.14 (26.75)	75.62 (28.06)
Relative knee flexion PT, Nm/kg	32.65 [15.98]	31.50 [20.05]
Myostatin, pg/mL	1881.49 (1040.31)	1512.10 (412.71)
Decorin, pg/mL	9.29 [4.24]	16.05 [12.34]

MMSE, Mini-Mental State Examination.
Continuous variables are presented as mean (SD) or median IQR; categorical variables are n (%). Daily sedentary time, LPA, and MVPA (exercise n = 20), Usual Care (n = 9), Myostatin concentration (exercise n = 14) and usual care (n = 8), Decorin concentrations (exercise n = 11) and usual care (n = 4).

varied between €57.45 and €88.16 across different scenarios (Supplementary Table 4).

Exercise Effectiveness

Tables 2 and 3 present the estimated mean scores and 95% CIs from GLMMs adjusted for sex and baseline frailty for each outcome by intervention following a PP analysis.

The results showed a significant interaction effect (time by intervention) in the SPPB score (B = 2.18; 95% CI, 1.35–3.00; $P < .001$). Post hoc analyses demonstrated a significant increase in the exercise intervention (mean difference 1.75; 95% CI, 1.17–2.34; $P < .001$), whereas no significant changes were observed in the usual care ($P = .149$) after 12 weeks of intervention.

Regarding the secondary endpoints, handgrip strength results showed an interaction effect (time by intervention) (B = 2.15; 95% CI, 1.00–3.30; $P < .001$), supported by a significant increase in the exercise intervention (mean difference 0.94; 95% CI, 0.12–1.75; $P = .025$) and a significant decline in the usual care (mean difference −1.21; 95% CI, −2.03 to −0.40; $P = .004$). Results showed no significant interaction effect of time by intervention nor any main effect of intervention or time in maximal extension and flexion knee strength (all $P > .05$).

Table 3 shows GLMMs for subgroup analysis of the effect of intervention in myokine concentrations. There was a significant interaction effect of time by intervention in myostatin concentration (B = −7.07; 95% CI, −13.48 to −0.66; $P = .031$). Post hoc analyses demonstrated a significant decrease in the exercise intervention (mean difference −7.23; 95% CI, −11.71 to −2.75; $P = .002$), whereas no significant changes were observed in the usual care ($P = .944$) after 12 weeks of intervention. There was no significant interaction effect of time by intervention in decorin concentrations ($P = .100$). There was a significant time by intervention effect on the myostatin-decorin ratio in favor of exercise over usual care (B = −95.54; 95% CI, −158.30 to −32.78; $P = .004$), supported by a significant decrease in the exercise intervention (mean difference −52.51; 95% CI, −96.89 to −8.13; $P = .022$), whereas no significant changes were observed in the usual care ($P = .057$).

All ITT analyses' results were similar in direction and statistical significance to PP analyses (Supplementary Table 5). However, ITT analysis also showed significant between-intervention differences in knee flexion strength ($P = .023$).

Table 2
Effect of the Interventions on the Endpoints According to a PP Analysis

Variables	Exercise Intervention		Usual Care Intervention		*P* Time	*P* Intervention	*P* Interaction
	Pre-intervention n = 28	Post-intervention n = 28	Pre-intervention n = 28	Post-intervention n = 28			
Primary endpoint							
SPPB, score	5.91 (4.48–7.34)	7.66 (6.23–9.09)	6.62 (5.20–8.05)	6.19 (4.77–7.62)	.002*	.075	< .001*
Secondary endpoint							
Handgrip strength, kgf	17.34 (14.55–20.13)	18.27 (15.48–21.07)	17.64 (14.85–20.43)	16.42 (13.63–19.22)	.632	.009*	<.001*
Relative KE PT, Nm/kg	82.89 (64.90–100.88)	84.91 (66.92–102.91)	84.25 (66.24–102.26)	84.96 (66.93–102.99)	.416	.678	.697
Relative KF PT, Nm/kg	33.75 (25.13–45.34)	36.43 (27.11–48.95)	36.71 (27.31–49.34)	36.55 (27.18–49.15)	.310	.223	.254

KE, knee extension, KF, knee flexion.
Data are presented as mean (95% CI).
*P-value <.05.

Table 3
Effect of the Interventions on Myokine Concentration

Variables	Time	Interventions		*P* Time	*P* Intervention	*P* Interaction
		Exercise	Usual Care			
Myostatin (pg/mL/kg)	Pre (n = 22)	43.27 (31.09−55.45)	43.16 (30.96−55.36)	.024*	.039*	.031*
	Post (n = 22)	36.04 (23.86−48.22)	43.00 (30.80−55.20)			
LogDecorin (pg/mL)	Pre (n = 15)	1.07 (0.96−1.18)	1.23 (1.11−1.34)	.768	.024*	.100
	Post (n = 15)	1.15 (1.04−1.26)	1.17 (1.06−1.28)			
Myostatin-Decorin ratio (pg/mL)	Pre (n = 15)	163.19 (100.26−226.12)	91.10 (28.17−154.03)	.762	.125	.004*
	Post (n = 15)	110.68 (47.75−173.61)	134.13 (71.20−197.06)			

Data are presented as mean (95% CI).
*$P < .05$.

Discussion

This study found that concurrent exercise training significantly improved physical performance, handgrip strength, and serum myostatin and myostatin-decorin ratio concentrations compared with usual care in frail individuals aged 70 to 99 living in nursing homes. However, it did not significantly change lower-limb isokinetic strength and serum decorin levels. The training was feasible, with high adherence, low dropout rates, and no adverse events during sessions.

Current frailty management guidelines emphasize the importance of multicomponent exercise, particularly resistance training, to prevent, manage, and treat frailty.[8] Resistance training, alone or as a part of concurrent or multicomponent programs, can influence key signaling pathways, improve several frailty-related biomarkers, and enhance the function and the resilience of physiological systems.[31] For instance, resistance training is known to improve the metabolic capacity of skeletal muscle by enhancing muscle protein synthesis and anabolic response to nutritional stimuli, preventing intramuscular lipid accumulation, and increasing oxidative and glycolytic enzyme capacity, ultimately leading to better physical function, muscle strength, and neuromuscular performance.[7]

Although a few studies have investigated the effect of exercise on frail individuals living in long-term care settings, the feasibility outcomes have been understudied. For example, previous studies have not detailed their recruitment process.[16,32] In our trial, more than half of the screened individuals were ineligible, and 23.08% of eligible participants refused to participate. In contrast, studies by Arrieta et al and Caldo-Silva et al reported higher refusal rates than ineligibility, likely due to narrower screening criteria.[15,18] Also, the timing post-COVID-19's second wave may have contributed to greater acceptability and lower refusal rates.

No adverse events occurred during the assessments or exercise sessions. Our study achieved high adherence (93.75%) and a low dropout rate (26.1%), aligning with findings from previous research on frail residents in nursing homes.[13,15-17] This reinforces the notion that exercise interventions are feasible and well-received among this highly vulnerable clinical population. However, a few adverse events, such as falls and respiratory infections, occurred during the trial, highlighting the real-world challenges often underreported in studies involving this vulnerable population.

The ICER associated with our 12-week exercise intervention for frail nursing home residents revealed an incremental cost of +79.01€ per participant per point increase in the SPPB score. This result is in line with other studies conducted in the community and in long-term care that have demonstrated that multimodal interventions, including resistance training or multicomponent exercise programs, were cost-effective for improving physical performance and fall prevention and reducing health care costs.[33-35] The sensitivity analysis showed that the ICER remains stable across different scenarios, reinforcing the findings' reliability. Overall, the intervention seems to be a reasonable investment, especially given the potential clinical benefits associated with improvements in SPPB scores, which are associated with a reduced risk of hospitalization.[36] Comprehensive economic evaluations are recommended to validate these findings and assess the intervention's long-term value and sustainability in nursing homes.

Overall, our main endpoint results align with general findings from a recent meta-analysis, which examined the effects of different physical exercise protocols on physical function in older adults living in residential care.[20] The authors confirmed that concurrent/multicomponent exercise types improved several physical function−related outcomes (eg, SPPB and handgrip strength) and suggested that exercise benefits seem greater in individuals residing in nursing homes compared with other settings.[20] We found a statistically significant and clinically meaningful improvement in physical performance (change in SPPB score $\geq$ 1 point), which aligns with similar trials using multicomponent or RT-alone protocols in nursing homes.[15,18,19] Overall, the results seem consistent, independent of exercise frequency or the use of different exercise equipment. Notably, none of these previous studies included solely frail older adults (ranging from 60.0% to 85.7%,[15,18] identified according to Fried's criteria), or all participants were considered frail based on a performance test.[19] The clinical impact of the exercise intervention was evident in improved mobility; 1 participant transitioned from a wheelchair to using only a cane, and 2 others moved from using walkers to needing just a cane or no walking aid at all.

Findings from the present study indicated a significant improvement in handgrip strength (mean 2.15 Kgf) in the concurrent exercise training over the usual care, which is in agreement with previous evidence in frail institutionalized older adults.[12-14,16,17] Our data are particularly relevant as handgrip strength is a pivotal marker of sarcopenia and frailty and is associated with the ability to perform ADLs and mortality risk.[3,37] However, the isokinetic knee extension and flexion strength did not change after 12 weeks of training, similar to findings from the Vienna Active Ageing Study.[38] Others have found a significant increase in lower-limb strength assessed by 1-RM or by an isometric strength assessment in frail institutionalized older adults.[13,16,19] Therefore, protocol differences in lower-limb strength assessment (isometric vs isokinetic) may partly explain these divergent findings. A complex medical profile including high sedentary time (12.6 h/d on average), several comorbidities, a high number of daily medications including psychotropics and corticosteroids (82.1% and 10.7%, respectively), and a potentially higher inflammatory status may blunt the participants' adaptative response to exercise and influence lower-limb strength gains.[9,39]

Myokines such as myostatin and decorin have been proposed as potential orchestrators of exercise benefits by acting to modulate age-related loss of muscle mass and strength.[26] Myostatin is a negative regulator of muscle growth and strength regulated by extracellular binding proteins, such as follistatin and decorin.[26] In this sense, acknowledging the role of decorin in myostatin action,[10] we proposed using a new myostatin:decorin ratio to provide deeper and holistic insights into the physiological effects of exercise. Thus, the ratio

reflects the net anabolic or catabolic state of the muscle environment and may be more sensitive to changes than looking at the levels of myostatin and decorin separately. However, data are scarce and inconsistent in older adults, particularly in frail individuals.[10] Our study found a significant reduction in serum myostatin levels following exercise intervention compared with usual care, consistent with prior findings in healthier populations.[10] This suggests a comparable myostatin response in even the frailest individuals. Notably, only 1 prior study examined the effect of exercise on myostatin concentrations in a similar population.[40] Controversially, the study noted an increase in myostatin concentration in men post-exercise, potentially due to sex-related differences in myostatin and muscle mass.[10,26] In addition, our study is the first to assess the impact of exercise on decorin levels in frail individuals, which remained unchanged, although trends suggested a potential increase (expected direction after exercise).[41] On the other hand, the exercise regimen resulted in an anabolic net response, as evidenced by a decreased myostatin-decorin ratio, indicating potentially enhanced muscle growth. However, given our study's small size and potential limitations in statistical power, these results should be interpreted with caution.

Our study has some potential strengths that are worthy of mentioning. First, our sample comprised the most frail population, as it was selected using Fried's criteria to determine frailty, and all resided in nursing homes. Our targeted population often exhibits multimorbidity, complex medical conditions, and limited exercise tolerance, which increases the challenges of implementing an exercise trial. This may, in part, explain why frail older adults remain understudied. Second, our study design with participants acting as their own controls reduced the risk of confounding and the individual variability in response to exercise. Third, we examined the effect of concurrent exercise training on novel biomarkers among this population.

Some limitations need to be considered. First, despite the washout period and no significant sequence order effects observed in our analysis, the possibility of carryover effects from one intervention period to the other cannot be entirely ruled out. Second, aerobic training intensity was not objectively measured, as several participants took beta-blockers and had arrhythmias or pacemakers. Also, perceived exertion scales were tested but not implemented, as most participants failed to provide a valid report. Finally, the absence of clinical outcomes such as fall rates and the ability to perform the ADLs limits the understanding of the clinical benefits of the exercise intervention.

Conclusions and Implications

Our concurrent exercise training was feasible, well-tolerated, and effective in improving physical performance in frail nursing home residents. Improvements in handgrip strength and myostatin concentration also strengthen the positive impact of the exercise intervention on counteracting frailty, even in the frailest individuals. Despite the challenges of implementing such an intervention in nursing homes (particularly when targeting frail individuals), our findings contribute to the growing body of evidence supporting the benefits of physical exercise programs in long-term care settings.

Disclosure

The authors declare no conflicts of interest.

Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jamda.2024.105271.

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Supplementary Table 1. Data collection

Measures	**Screening**	**28-weeks trial duration**			
		T0 - before randomization (0 weeks)	T1 - after period I (12 week)	T2 - after washout period (16 week)	T4 - after period II (28 week)
Informed consent	X				
Sociodemographic questionnaire	X				
Frailty status	X	X	X	X	X
Physical performance	X	X	X	X	X
Handgrip strength	X	X	X	X	X
Isokinetic knee flexors and extensors strength		X	X	X	X
Serum myostatin and decorin concentrations		X	X	X	X
Body composition		X	X	X	X
Nutritional status		X		X	
Physical activity levels		X		X	

Supplementary Table 2. Detailed description of the exercise protocol

Session component	General description	Week 1-2	Week 3-5	Week 6-8	Week 9-12
Warm-up	1 set of 10 repetitions of the resistance exercises that they would be performing later but without any weight or by just lifting their own body weight	5-10 min	5-10 min	5-10 min	5-10 min
Resistance training	Progressive strengthening exercises were performed from the seated to standing positions. Exercises: biceps curl, knee extension, knee flexion, calves raise and sit and stand	~35min Non-weight bearing exercises 5 exercises: 1 upper limbs, 4 lower limbs; 2 series, 15 reps	~35 min 40-50% of 1-RM 5 exercises: 1 upper limbs, 4 lower limbs; 2 series, 10 reps	~35 min 55-60% of 1-RM 5 exercises: 1 upper limbs, 4 lower limbs; 2 series, 15 reps	~35 min 65-70% of 1-RM 5 exercises: 1 upper limbs, 4 lower limbs; 3 series, 10 reps
Aerobic training	Progressive sets of walking	~10 min 3 sets of 3 min	10 min 2 sets of 5 min	10 min 1 set of 10 min	15 min 1 set of 15min
Cool-down	Static and dynamic flexibility exercises	5 min	5 min	5 min	5 min

Supplementary Table 3. The estimated costs of implementing the 12-week exercise intervention delivered in 10 groups (total n=34)

	Units	Cost/unit	Total Cost
Human Resources			
Exercise professional (hours[1])	360	9.375€	3375.0€
Sports Equipment			
Pair of Dumbbells 1.5kg	34	11.52€	391.68€
Pair of Dumbbells 2kg	34	14.10€	479.4€
Pair of Dumbbells 3kg	34	18.80€	639.2€
Pair of Dumbbells 4kg	4	32.01€	128.04€
Pair of Ankle weight 2kg	34	24.0€	816€
Overall exercise intervention			5,829.32€

Sports equipment costs are dated to January 2022; VAT included; [1]3 hours/week x10 groups (9 clusters) x 12 weeks

Supplementary Table 4. Cost-effectiveness analysis

	∆Cost (€2022)
Base case	+79.01
Sensitivity Analysis	
+10% costs of equipment	+82.34
+20% costs of equipment	+85.66
+10% exercise professional costs	+83.59
+20% exercise professional costs	+88.16
Increasing the mean number of participants in exercise sessions to 6	+57.45

Base case (principal analysis including 34 participants) and sensitivity analysis according to different scenarios. [1]Material costs were adjusted for 6 participants in 10 groups. Intervention effectiveness was based on the 34 participants that fully completed the trial.

Supplementary Table 5. Effect of the interventions on the endpoints according to an intention-to-treat analysis

Variables	Exercise intervention		Usual care intervention		*P* Time	*P* Intervention	*P* interaction
	Pre-intervention n = 44	Post-Intervention n = 34	Pre-intervention n = 38	Post-Intervention n = 38			
Primary endpoint							
SPPB, score	5.58 (4.30 to 6.86)	7.41 (6.12 to 8.70)	6.40 (5.12 to 7.68)	6.00 (4.72 to 7.29)	<.001*	.119	< .001*
Secondary endpoint							
Handgrip strength, kgf	17.11 (14.06 to 20.21)	17.79 (14.69 to 20.90)	17.11 (14.06 to 20.26)	15.93 (12.83 to 19.03)	.303	<.001*	<.001*
Relative KE Peak Torque, Nm/kg	81.91 (65.39 to 98.43)	84.26 (67.68 to 100.83)	83.69 (67.14 to 100.25)	82.14 (65.56 to 98.71)	.788	.910	.186
Relative KF Peak Torque, Nm/kg	33.35 (26.11 to 42.59)	36.31 (28.38 to 46.46)	37.31 (29.18 to 47.71)	35.07 (27.40 to 44.87)	.717	.235	.023*

Data are presented as mean, CI (95% confidence interval). *P-value <0.05.

KE, Knee extension, KF, Knee flexion, SPPB, short physical performance battery

4.2 Study IV

5. **Barros, D.**, Johansson, J., Wilsgaard, T., Magalhães, J., Carvalho, J., & Marques, E. A. (2025). One-year concurrent training improves physical performance and handgrip strength in nursing home residents. *GeroScience*. Advance online publication. https://doi.org/10.1007/s11357-025-01770-y

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ORIGINAL ARTICLE

One-year concurrent training improves physical performance and handgrip strength in nursing home residents

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Abstract This parallel two-arm pragmatic controlled trial aimed to examine the effectiveness of a 12-month exercise intervention on physical performance, handgrip strength, independence in basic activities of daily living (BADL), and falls in real-world settings. Ninety-five older residents of nursing homes (mean age 81.9 ± 8.0 years) were allocated to either an exercise ($n = 43$) or usual care only ($n = 52$) group. The 12-month exercise training consisted of resistance and aerobic exercises (2 days/week), while the usual care consisted of everyday routine and standard care. The primary endpoint was the change in physical performance measured by the short physical performance battery (SPPB, score range 0–12). The secondary endpoints were changes in handgrip strength, independence in BADL measured by the Barthel index (BI), and the rate of falls. The exercise intervention significantly provided benefits over usual care on SPPB score by 2.59 points (95% CI: 1.75, 3.43) and handgrip strength by 1.85 kgf (95% CI: 0.56, 3.14). No significant between-group differences were observed for the BI or the rate of falls. However, within-group analysis revealed a significant decline in the BI score (− 11.8) and an increase in the rate of falls (+ 31.5 falls per 100 person-years) in the usual care group. Long-term concurrent exercise programme significantly improved physical performance and handgrip strength, but not independence in BADL and rate of falls in mostly frail nursing home residents. These findings support the integration of exercise physiologists and exercise programmes into standard care practices in nursing homes to help preserve physical function among residents.

Keywords Exercise · Falls · Long-term care · Physical function · Pragmatic trial

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Introduction

The global population is ageing rapidly, with individuals aged 80 and older representing the fastest-growing demographic segment [1]. This demographic

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shift is associated with a rising prevalence of chronic diseases and conditions such as dementia, sarcopenia, and frailty, which significantly contribute to functional impairment and increase the demand for long-term care (e.g. nursing homes) during the later stages of life [2]. Portugal is among the most aged countries in the world with 24.1% of its population aged 65 or older [3]. In 2023, nearly 4% of older adults resided in nursing homes, with a utilisation rate exceeding 90% of the available services [4], highlighting a significant public social and health challenge.

Functional impairment has been identified as a key predictor of nursing home admission [5]. Consequently, residents of nursing homes often exhibit high levels of frailty and dependency, which are exacerbated by the care environments that frequently promote sedentary behaviours and restrict opportunities for physical activity [6]. In addition, cognitive impairment is prevalent among this population [7] and increasing evidence suggests that physical and cognitive functions are closely interrelated [8]. The simultaneous decline of these functions poses a substantial challenge to preserving functionality in nursing home residents. Without proper targeted intervention, such as structured exercise programs, physical performance in this group typically deteriorates over time [9]. This decline leads to decreased mobility, increased dependence in basic activities of daily living (BADL), and increased risk of falls, which are frequently associated with serious adverse outcomes such as fractures, poor quality of life and death [10, 11].

Although exercise is a proven strategy for enhancing both physical and mental health across all age groups [12], evidence-based progressive exercise programmes are rarely implemented in nursing homes, and the majority are short-term (24 weeks or less) [13]. Particularly in Portugal, integrating such programmes into long-term care policies and real-world practices remains insufficient. The inclusion of exercise professionals or the promotion of exercise programmes is not mandatory under current care standards. Thus, physical activity promoted in nursing homes is often limited to seated stretching exercises with minimal health benefits [14].

Currently, the exercise guidelines for older adults are mostly based on evidence from highly explanatory controlled efficacy trials with selective inclusion criteria, often excluding medically complex multimorbid individuals [13, 15]. As a result, findings from these studies may have limited applicability and generalisability to real-world nursing home settings. There is a clear lack of trials evaluating the implementation of exercise interventions in everyday care. Pragmatic trials embedding exercise intervention in the daily routines of nursing homes are required to improve the generalisability of findings and support the development of evidence-based clinical and care policies in long-term care [16]. Therefore, this pragmatic trial aimed to examine the effectiveness of a 12-month concurrent exercise training program on physical performance (primary outcome), handgrip strength, independence in BADL, and rate of falls (secondary outcomes) in older adults living in nursing homes.

Methods

This is a pragmatic parallel two-arm controlled trial. The degree of pragmatism was retrospectively assessed by two raters (DB and EAM) using the PRECIS-2 tool [17] (Supplementary Table 1).

A formal a priori sample size calculation was not conducted for this study. Instead, a convenience sampling approach was used. Six public nursing homes participating in the prospective cohort study, "The Observatory of the Older Person" (ClinicalTrials.gov NCT06825026), were selected for this study based on their participation in follow-up assessments that coincided with the recruitment period (July to September 2023). All eligible residents from these institutions were invited to participate, resulting in a final sample of 103 participants (Fig. 1). This sample size is consistent with previous studies in similar populations and settings, which have demonstrated sufficient power to detect meaningful intervention effects on physical performance outcomes [18, 19].

This study was conducted according to the updated version of the CONSORT statement to report a pragmatic trial [20] (Supplementary File 2), and was in full compliance with the Declaration of Helsinki. The study was approved by the Faculty of Sports, University of Porto Ethics Committee (CEFADE 012021). All participants or their legal representatives signed an informed consent before enrolment.

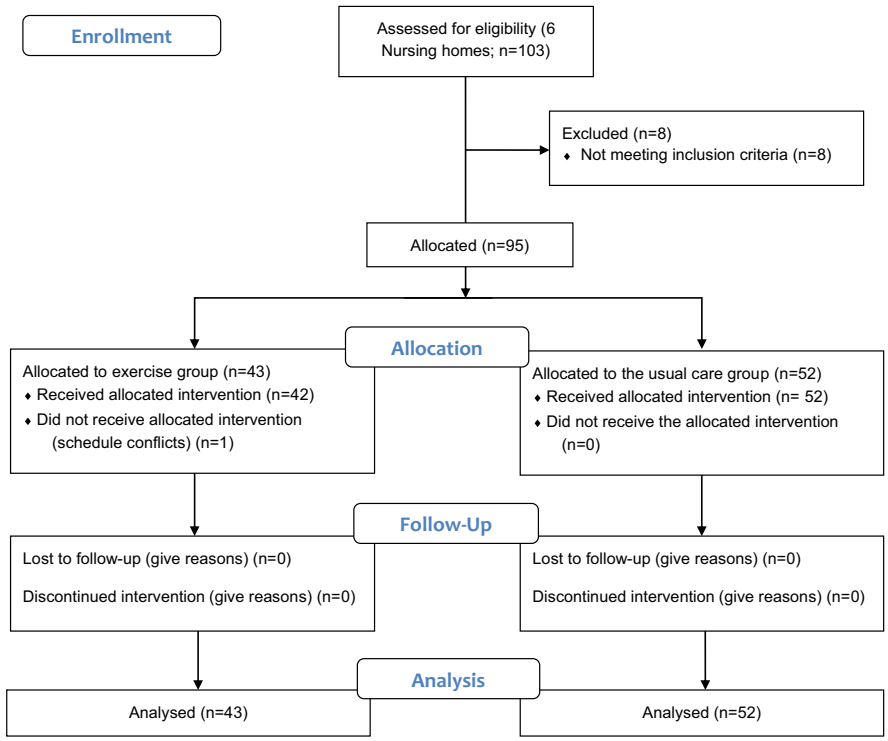

Fig. 1 Study flow diagram

Participants

This pragmatic trial was designed to reflect real-world implementation processes in nursing homes, where participation in activities is typically voluntary and determined by staff and resident preferences. Randomisation was not conducted to replicate real-world procedures, and due to ethical concerns about withholding a long-term supervised exercise programme from medically cleared residents who were likely to benefit. A list of potential participants who could walk independently or with assistance based on physical performance tests was extracted from the follow-up assessment data. This list was provided to the nursing home directors or activity coordinators, who then invited residents to participate in the exercise sessions after obtaining medical approval from the medical team.

Exclusion criteria included any contraindications where exercise could adversely affect the participant's health (e.g. uncontrolled or unstable medical conditions or a recent fracture) and physical disability as indicated by a short physical performance battery (SPPB) score ≤ 3.

Participants were not blinded to the nature of this intervention. However, outcome assessors from the nursing home staff were blinded to all group allocations. The principal researcher was not blinded due to involvement in data management.

Trial design

The trial followed a 12-month parallel design. The participants who agreed to take part in the exercise sessions were enrolled in a 12-month concurrent exercise intervention, while those who did not continued their usual care.

Baseline assessments were conducted in the nursing homes, with each participant completing two 25-min sessions spaced at least 2 days apart. In the first session, the principal researcher evaluated physical performance, frailty status, and cognitive function. In the second session, a blinded assessor measured anthropometric data and handgrip strength. Additionally, questionnaires covering sociodemographic characteristics, health-related information, independence in BADL, and fall history (i.e. number of falls in the past 12 months) were completed by experienced

nursing home staff using participants' electronic medical records. These questionnaires were filled out separately from the assessment sessions. Primary and secondary outcomes were reassessed after 12 months, through two 15-min evaluation sessions conducted at least 2 days apart, mirroring the baseline assessment protocol.

During the trial, participants were allowed to change their physical activity levels and diet, and those in the usual care group were allowed to switch to the exercise group to reflect real-world care decisions.

Interventions

The usual care intervention included unrestricted access to all nursing home activities, which may include some physical activities (i.e. stretching) but no formal exercise sessions and standard medical and rehabilitation care as needed. Participants in the exercise group received usual care plus a concurrent exercise intervention, similar to a previous intervention implemented in nursing homes [21], conducted for 12 months and held two times a week on nonconsecutive days to prevent overtraining and fatigue. A single exercise professional, independent of the nursing home staff and not involved in any assessments, conducted the group intervention across all six nursing homes. The session schedule was arranged with each nursing home's activities coordinator to avoid conflicts with other scheduled activities. The research team supplied the necessary equipment for each session, including dumbbells and ankle weights, to each nursing home.

Each session, lasting 60 min, included a warm-up, resistance and aerobic training, and a 5-min cooldown (Supplementary Table 2). The warm-up involved one set of ten repetitions of the same resistance exercises performed in the main session without load. Loads were only evaluated at the beginning of the trial using a ten-repetition maximum (10RM) test for bicep curl and seated knee extension exercises. Participants warmed up with light loads before attempting the 10RM test up to three times, with a 3-min rest between the attempts. Resistance was increased if participants could exceed ten repetitions with the proper technique [22]. The test concluded when participants could not perform more than ten repetitions and then, the one-repetition maximum (1RM) was calculated based on Brzycki formula [23].

During the first 2 weeks, participants began with one set of 15 repetitions at 30% of 1RM. Subsequently, the programme progressed to two–three sets of 10–15 repetitions, with an external load equivalent to 40–60% of 1RM for both upper-body exercises (e.g. bicep curls) and lower-body exercises (e.g. seated knee extension). Initial exercises included seated upper-body exercises such as bicep curls and lower-body exercises like seated knee extension/flexion, calf raises, and sit-to-stand movements. The sit-to-stand exercise was performed as fast as possible according to each participant's capacity. The recovery between sets lasted 2 min. Additional upper-body exercises, like front raises and Svend press, were introduced in the middle of the programme. The instructor recommended incremental increases in load for each participant once the current load became manageable during the intervention. The aerobic training consisted of walking exercises with variations in pace and direction. Initially, participants completed short 3-min walking sets, gradually progressing to a continuous 10-min set in the later months. The intensity of aerobic exercises (aimed at moderate intensity throughout the 12 months) was monitored through observer-rated perceived exertion, assessing signs and symptoms such as an accelerated respiratory rate, difficulty talking while walking, sweating, or facial expressions, as many participants exhibited cognitive impairments. The sessions ended with 5 min of stretching exercises.

Any adverse events that occurred during exercise sessions were recorded by the exercise professional.

Endpoints

Primary endpoint

The primary endpoint was the change in physical performance assessed by the SPPB score (0–12), which includes standing balance, usual gait speed over 4 m, and lower-limb strength test (five repeated chair stands). A one-point change is considered a meaningful clinical change [24]. A higher score reflects a better overall physical performance. Changes in each subcomponent were examined. Balance was defined as the sum of scores from three balance tests: standing in a side-by-side position, a semi-tandem position,

and a full-tandem position, with a total score ranging from 0 to 4. Walking speed and the five-chair stand test scores were categorised into three groups based on tertiles. Those who were unable to perform the test were categorised in the lowest tertile.

Secondary endpoints

The handgrip strength test was performed using a calibrated Jamar Plus Digital Hand Dynamometer (Sammons Preston Inc., Bolingbrook, IL, USA). Participants were asked to sit in a chair with the arms adducted and neutrally rotated, the elbow flexed to 90°, and to squeeze the dynamometer as forcefully as possible [25]. The test was performed three times for each hand, alternating between right and left. A rest period of at least 15 s was provided between trials in each hand. The mean value of the best trial from both hands (measured in kilogram-force, kgf) was used for analysis. In cases where data were available for only one side, the best value from that side was used for the analysis.

Independence in BADL was assessed by the nursing teams using the Barthel index (BI) [26]. The BI evaluates ten core daily activities: feeding, bathing, grooming, dressing, toileting, bowel and bladder control, transfers, mobility, and stair use. The total score ranges from 0 to 100, with higher scores indicating higher levels of independence.

For the rate of falls, we calculated the group rate by dividing the total number of falls by the number of participants at each time point.

Baseline covariates

Baseline covariates, including sociodemographic information, frailty status, cognitive capacity, anthropometric data, and comprehensive medical history, were assessed solely during initial enrolment to establish population characteristics and to control for potential confounding adjustment.

Sociodemographic information (age, sex, education, years living in nursing homes) and health-related data (history of chronic diseases and medication use) were collected from the participants' electronic medical records by the nursing team.

Anthropometric measures included measuring the height to the nearest 0.1 cm with a portable stadiometer (Seca 206, Seca Ltd, Birmingham, UK) and body weight to the nearest 0.1 kg using a calibrated scale (InBody 120, InBody Co. Ltd, Seoul, Republic of Korea). Body mass index (BMI) was calculated as body weight (kg) divided by height squared (m^2). Measurements were taken with participants wearing light clothing and barefoot.

Frailty status was assessed using the Fried criteria, which include unintentional weight loss, self-reported exhaustion, slowness, weakness, and physical inactivity [27]. The physical activity criterion was modified and evaluated using the question: "Do you engage in any of the following activities—dancing, intentional walking, exercise, or gardening?" A "no" response was classified as positive for this criterion [28]. Individuals were categorised as "frail" if they met three or more criteria, "pre-frail" if they met one or two criteria, and "robust" if they met none.

Global cognitive capacity was measured using the mini-mental state examination, a widely used screening test for identifying cognitive impairment [29].

Statistical analysis

Sample characteristics were analysed using descriptive statistics and were reported as mean and standard deviation (SD) or median and interquartile range (IQR) and frequency and percentages (%) for continuous and categorical variables, respectively.

Generalised linear mixed models (GLMMs) with the appropriate distribution were used to estimate the differences in outcome variables between groups at 12 months, considering intervention (exercise or usual care), time (pre- and post-intervention), and interaction as the cross-product between time and intervention as fixed effects and participants as random effects. A normal distribution was used for SPPB and handgrip strength. A negative binomial distribution with a log link function was applied to the BI scores. A negative binomial distribution model was chosen over the Poisson model, as it provided a better fit to the data and more effectively accounted for overdispersion [30]. Estimated mean scores and 95% confidence interval (CI) were provided. The rate of falls was expressed as cases per 100 person-years. Incidence rate ratios (IRR) with 95% CI were estimated using generalised estimating equations (GEE) with a negative binomial distribution. Bonferroni corrections were applied for within-group comparisons

to account for multiple tests. Potential confounding baseline variables, including age, sex, and BMI, were evaluated for inclusion in the models but were not retained in the final model, as their addition did not significantly improve model fit or alter the effect estimates. Sensitivity analyses adjusting for baseline variables that significantly differed between groups (e.g., frailty status, SPPB score) were performed, when applicable, to assess the robustness of the findings.

A secondary analysis was conducted to estimate the differences in SPPB subcomponents between groups at 12 months using a multinomial logistic regression model with a cumulative logit link function.

The analysis was based on an intention-to-treat analysis, including all participants according to their group assignment. For normal distribution GLMM, the assumption of normality of residuals was confirmed by visual inspection of Q-Q (quantile–quantile) plots.

The statistical software used for analysis was IBM SPSS Statistics for Windows (version 29.0; IBM, Armonk, NY). The significance level was set at $\alpha = 0.05$.

Results

Participants characteristics

Out of 103 screened individuals, eight were excluded due to SPPB ≤ 3, leaving 95 participants (Fig. 1). The majority were frail individuals ($n = 62$, 65.3%), women ($n = 63$, 66.3%), and non-fallers (81.1%) (with a mean age of 81.9 years (SD 8.0 years, range 65–100). Out of the 95 invited participants, 43 (45.26%) agreed to partake in the exercise programme. The baseline characteristics were not different between groups, except the exercise group had a higher SPPB score and a lower frailty status (Table 1). One participant, unable to attend exercise sessions due to a permanent scheduling conflict, switched from the exercise group to the usual care group.

All participants in the exercise group demonstrated an adherence rate of $\geq 80\%$. The primary reasons for missing exercise sessions were acute illness or scheduling conflicts with medical consultations. Only one mild adverse event of hypoglycaemia in a type-1 diabetes participant occurred during an exercise session and was promptly resolved with the assistance of a nurse.

Table 1 Clinical and demographic characteristics of participants at baseline

Characteristics	Usual care ($n = 52$)	Exercise ($n = 43$)
Age, years	82.1 (7.62)	81.6 (8.54)
Sex female, *n* (%)	35 (67.3)	28 (65.1)
BMI, kg/m^2	28.1 (5.72)	28.4 (4.15)
Years of education, years	4 [1]	4 [1]
Years living in NH, years	4.74 (5.69)	3.84 (4.20)
Hypertension, *n* (%)	32 (61.5)	30 (69.8)
Diabetes, *n* (%)	17 (32.7)	17 (39.5)
Dementia, *n* (%)	17 (32.7)	17 (39.5)
Daily medications, number	9.65 (4.16)	8.37 (3.88)
MMSE, score	22.5 [9]	23 [8]
Frailty categories, *n* (%)		
Robust	1 (1.9)	2 (4.7)
Pre-frail	11 (21.2)	19 (44.2)
Frail	40 (76.9)	22 (51.2)
Fallers, *n* (%)	8 (15.4)	9 (21.4)[a]
N. of falls over 12 months, *n* (%)		
1	4 (7.7)	6 (14.3)[a]
2	4 (7.7)	3 (7.1)[a]
Primary outcome		
SPPB, score	6 [3]	8 [5]
Secondary outcomes		
Handgrip strength, kgf	17.3 (5.27)	17.1 (5.48)
Barthel Index, score	90 [20]	90 [25]
Rate of falls, 100 falls per person-year	23.1	28.6[a]

Continuous variables are presented as mean (SD) or median and interquartile range [IQR]; Categorical variables are *n* (%). *BMI*, body mass index; *MNA-SF*, Mini Nutritional Assessment-Short Form; *MMSE*, Mini-Mental State Examination; *NH*, nursing home; *SPPB*, short physical performance battery. Data distribution was checked for normality using the Shapiro–Wilks test and histograms. [a]One missing participant data

Primary endpoint

After 12 months, the exercise group showed a significant increase in SPPB scores, with an improvement of 2.59 points (95% CI: 1.75 to 3.43), compared to the usual care group ($p < 0.001$; Table 2). The secondary analysis revealed that the exercise group significantly improved in every single subcomponent of SPPB (Supplementary Table 3).

Secondary endpoints

In the exercise group, handgrip strength increased significantly by 1.85 kgf (95% CI: 0.56, 3.14) relative to the usual care group ($p=0.005$), as shown in Table 2.

No significant between-group differences in BI changes over time were observed ($p=0.071$). However, within-group analysis revealed a significant decline in the usual care group (−11.84 points; 95% CI: −19.40, −4.28, $p=0.002$), whereas no significant change was observed in the exercise group ($p=0.686$).

Over the 12-month trial period, 33.3% of participants in the usual care group and 23.3% in the intervention group experienced falls compared to a history of 15.4% and 21.4%, respectively. Although the usual care group experienced a significant increase in falls over time (+31.5 falls per 100 person-year (95% CI: 4.63, 58.37, $p=0.022$), there were no significant differences between groups in the change in rate of falls ($p=0.111$).

Pre- and post-intervention values for primary and secondary outcomes in each group are presented in Fig. 2.

Sensitivity analyses showed that findings remained consistent after adjusting for baseline differences in frailty status and SPPB score, suggesting robustness of the primary analyses (Supplementary Table 4).

Discussion

Significant improvements in physical performance and handgrip strength were achieved among older adults residing in nursing homes through the integration of long-term concurrent twice-weekly exercise training into usual care. No significant between-group differences were found for BI or the rate of falls. To our knowledge, this is one of the few long-term, pragmatic exercise interventions conducted in nursing homes.

Current guidelines emphasise the role of progressive resistance training, whether as a single intervention or within a concurrent or multicomponent exercise programme, in improving functional capacity, especially for frail individuals or those living in nursing homes [12]. However, the considerable heterogeneity among exercise interventions, insufficient reporting on exercise characteristics, and the paucity of long-term studies make it challenging to draw meaningful comparisons between interventions [13]. Among 147 studies included in a recent systematic review, concurrent exercise interventions were considered within the multicomponent interventions, of which only nearly 5% lasted 12 months or longer. In this pragmatic trial, we implemented a 12-month concurrent exercise intervention tailored to the needs of primarily frail individuals and logistical constraints (e.g. 1-h sessions twice weekly). To enhance applicability in most nursing home settings, the programme was designed using low-cost equipment (e.g. free weights) and did not require a dedicated training room or commute to outside facilities. In addition, to prioritise improvements in physical performance, the programme emphasised the resistance training component (the longest segment of the training programme).

Table 2 Results of endpoints by group

Endpoints	Δ usual care	Δ exercise	Between-group differences	*p* between groups
Primary outcome				
SPPB, score	−0.94 (−1.51, −0.37)	1.65 (1.03, 2.27)	2.59 (1.75, 3.43)	<0.001
Secondary outcomes				
Handgrip strength, kgf	−1.58 (−2.45, −0.700)	0.27 (−0.68, 1.22)	1.85 (0.56, 3.14)	0.005
BI, score	−11.84 (−19.40, −4.28)	−1.80 (−10.56, 6.97)	1.14 (0.99, 1.31)	0.071
Rate of falls, falls per 100 person-year	31.5 (4.63, 58.37)	4.8 (−11.22, 20.87)	0.49 (0.20, 1.17)	0.111

Changes are from pre-baseline to post-intervention and are presented on the original scale of each variable with 95% confidence intervals. The between-group differences are presented as absolute differences for the short physical performance battery (SPPB) and handgrip strength, and as incident rate ratios (IRR) for the Barthel index (BI) score and rate of falls

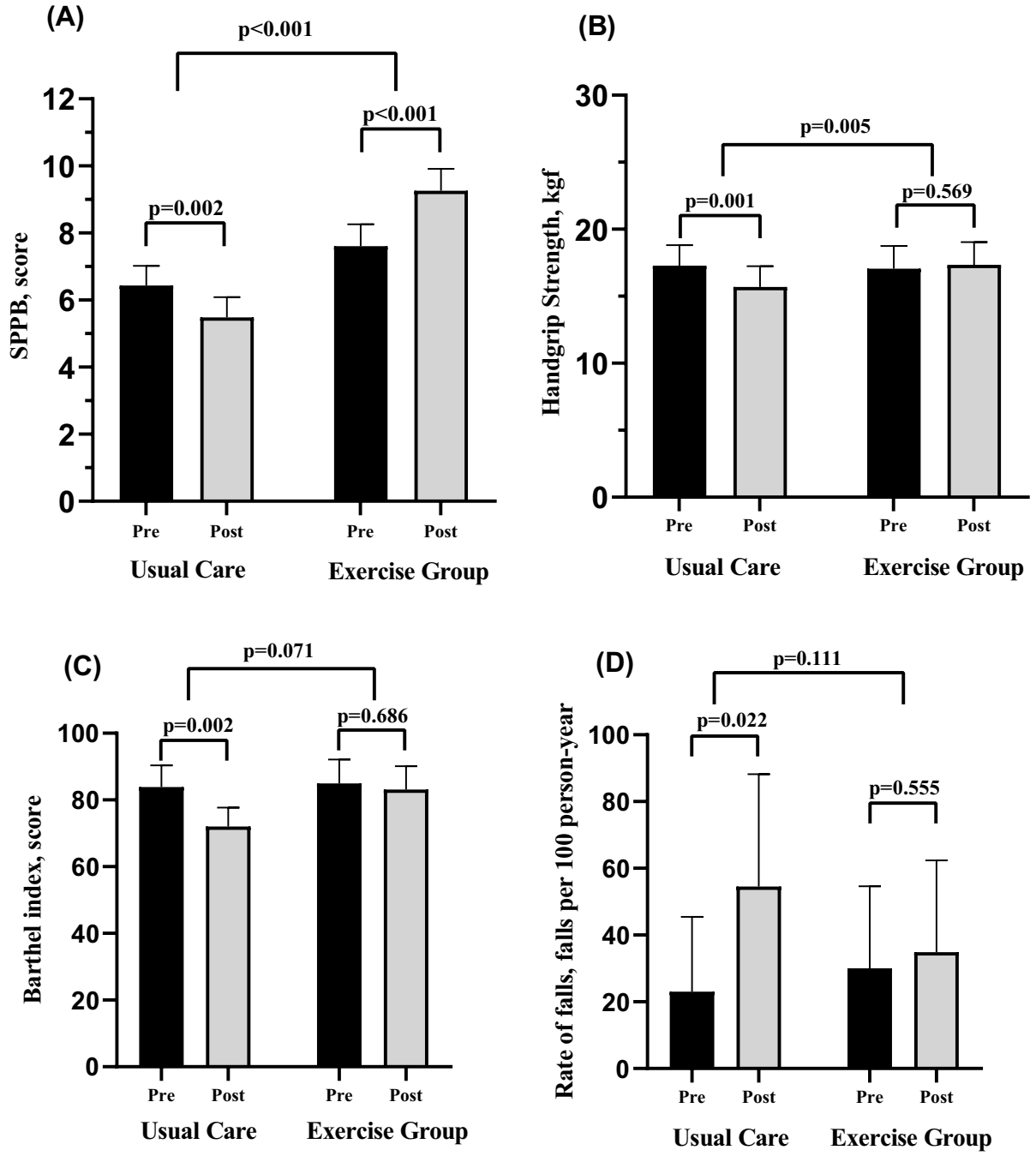

Fig. 2 Pre- and post-intervention outcomes values by group and within and between group comparisons. (A) Short Physical Performance Battery, (B) Handgrip Strength, (C) Barthel Index, (D) Rate of falls

This trial closely mirrored the expected real-life implementation of exercise sessions for this population, reinforcing its practicality and long-term sustainability in care practices. The fact that meaningful improvements were observed despite the inherent variability of a less strict trial strengthens the relevance of these findings for clinical practice and policymakers.

Our concurrent intervention effectively improved physical performance in nursing home residents. Specifically, participants in the exercise group showed a mean increase of 2.59 points in SPPB score compared to the usual care group. This improvement exceeds the established threshold for clinically meaningful changes, which is considered to be a change of one point or more on the SPPB [24]. Notably, these gains were observed across all SPBB subcomponents, including balance, walking speed, and chair stand tests. The magnitude of improvement in our study (+2.58 points) is also comparable to that reported in previous meta-analyses of resistance training interventions in similar settings, which typically demonstrate improvements of approximately + 2 points [13, 31]. Also, our overall results were consistent with those of a prior long-term pilot multicomponent intervention that incorporated moderate resistance and aerobic training with comparable exercise characteristics [32]. In addition, these findings align with previous studies with shorter interventions (i.e. 3–6 months), including either concurrent training [21] or multicomponent training [18, 19] with similar intensity, type of exercises and exercise equipment, conducted either twice or three times a week. Collectively, these data suggest that progressive concurrent exercise, including moderate-intensity resistance and aerobic training incorporated into their daily routine and setting, can counteract the natural trajectory of physical decline in frail and pre-frail nursing home residents [9].

Muscle weakness is a key determinant of ageing trajectory, a clinical marker of both sarcopenia and frailty [27] and is associated with dependency on BADL [10]. Our results showed a clinically significant increase in handgrip strength of 1.85 kgf in the exercise group compared to the usual care group, which is substantial given that an increase of 1 kgf is associated with a 9% reduction in all-cause mortality risk in older adults [33]. Another long-term multicomponent exercise programme that incorporated similar intensity and progression in the resistance component, twice a week, also observed a significant increase in handgrip strength in the oldest old population living in nursing homes [32]. These significant positive benefits were similar in magnitude to a previous short-term concurrent intervention [21]. In this trial, we followed an individualised approach to exercise intensity progression as participants were encouraged to increase the load when it became manageable. This could have led to a suboptimal intensity for some and reduced the strength gains. However, it reflects the real-world challenges in nursing homes, where ensuring adherence and engagement often outweighs achieving optimal training loads.

Changes in BI scores did not significantly differ between groups. In our study, the relatively high baseline independence levels among participants may have created a ceiling effect, limiting the sensitivity of BADL measures to detect small yet meaningful changes over time. Nevertheless, the usual care group experienced a significant decline in the BI score, which is in line with findings from two previous trials [32, 34]. Although a significant improvement in the BI score after a similar exercise programme has been reported [32], this finding is not consensual [34]. Furthermore, despite the benefits of our intervention on physical performance and muscular strength, factors such as staff perceptions, care routines, and safety protocols also influence BADL outcomes. Nonetheless, maintaining high levels of independence over an extended follow-up period is considered a clinically relevant effect for this population [34].

Exercise programmes combining resistance and balance training are key to fall prevention in older adults [35]. However, our intervention did not significantly reduce the rate of falls in nursing home residents compared to usual care. Several factors may explain this finding. First, the intervention did not specifically include balance exercises, which are essential for reducing falls [36]. Second, the rate of falls was assessed during the intervention period rather than during a follow-up period after the intervention ended, which might not fully capture the intervention's long-term effects. Third, the relatively small proportion of individuals experiencing falls likely limited the statistical power of the analysis, making it more challenging to detect significant differences between groups. It should be noted that the usual care group experienced a significant increase in the rate of falls during the trial, which may reflect a progressive decline in physical and functional status over time. This likely contributed to the increased susceptibility to falls in this frail, predominantly inactive population without targeted intervention. Comparing our findings with previous trials is challenging due to the complex nature of fall risk factors, which makes it difficult to isolate the impact of exercise as a single contributing factor [35].

Moreover, the variability in trial characteristics, such as differences in study design (primarily explanatory), interventions, and populations, further complicates direct comparisons. Future research should explore whether integrating complex, multimodal interventions—such as environment modification, cognitive training combined with exercise—designed and implemented in a pragmatic manner can enhance fall prevention through mediated pathways in frail, institutionalised older adults.

Our study has several key strengths. Firstly, its pragmatic design enabled the evaluation of the exercise programme's effectiveness in real-world nursing home settings, where it was integrated into daily routines. This approach, which did not require strict randomisation, is particularly valuable because it mirrors the complexities of actual care practice, thereby enhancing the generalisability of our findings. Secondly, as it focuses primarily on frail and pre-frail individuals—a vulnerable population often underrepresented in research—our study significantly increases the clinical relevance of its results.

This study has several limitations. First, the lack of randomisation and blinding may have introduced selection, performance, and detection biases. Although we performed a sensitivity analysis adjusting for baseline differences in frailty status and SPPB score, and findings remained consistent, the effect of unmeasured confounding cannot be excluded. Second, participants were recruited through convenience sampling in a limited number of nursing homes, which may restrict the generalisability of our findings. Third, the sample size was not based on a formal power calculation, potentially reducing the statistical power to detect differences in secondary outcomes such as falls. Fourth, the assessor of the SPPB was not blinded to group allocation, which may have introduced observer bias in the assessment of physical performance. Finally, the intervention addressed some physical aspect of fall prevention, without incorporating environmental, cognitive, or medication-related components recommended by current guidelines.

Conclusion

This study demonstrates that embedding structured concurrent exercise training into nursing home daily routines is effective in improving physical performance and handgrip strength in predominantly frail older residents. Additionally, the intervention helped mitigate the natural decline in independence in BADL and the rate of falls. Importantly, the pragmatic trial design, which reflects the real-world care environment, reinforces the effectiveness and potential for broader implementation of such exercise programmes in routine care. Future efforts should focus on exploring strategies to integrate exercise physiologists and individualised exercise programmes into standard nursing home practices to further improve exercise-related outcomes in this population.

Author contribution Conceptualisation: DB, EAM, JM, and JC; methodology: DB, EAM, and JJ; formal analysis and investigation: DB, JJ, and TW; results interpretation: DB, JJ, and TW; writing—original draft preparation: DB and EAM. Funding acquisition: JC; supervision: EAM; writing—review and editing: All authors contributed to the revision of the manuscript. All authors have approved the final version of the submitted manuscript for publication.

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Declarations

Ethics approval This study was approved by the Faculty of Sports, University of Porto Ethics Committee (CEFADE 012021), adhering to the principles of the 1964 Helsinki Declaration and its subsequent revisions or equivalent ethical standards.

Consent to participate All participants provided informed consent prior to their inclusion in the study.

Competing interests The authors declare no competing interests.

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Supplementary Table 1 PRECIS-2 scores for trial domains

	Domain	Score	Rationale
1	Eligibility Criteria	4	Participants included those who could walk with or without assistance. Exclusion criteria comprised conditions where exercise could negatively impact health, such as uncontrolled or unstable medical conditions, recent fractures, or severe physical disability, defined by a Short Physical Performance Battery (SPPB) score of ≤3.
2	Recruitment Path	5	We asked the nursing home director and activities coordinators to recruit participants, following the same procedures typically used for routine activities in the nursing home.
3	Setting	5	The settings were the same as those used for usual care.
4	Organization intervention	2	Exercise sessions led by an exercise professional are not part of standard nursing home care practices. However, since the sessions were conducted by external staff, they did not impose any additional burden on the nursing home staff's routines.
5	Flexibility of experimental intervention – Delivery	5	Exercise sessions were incorporated into daily routines to prevent conflicts with other activities. No strict measures were enforced to enhance compliance, and participants had the flexibility to switch between the intervention and usual care groups as desired. The exercise protocol was adaptable, with progression primarily guided by participants' willingness. In terms of flexibility, this intervention mirrored other usual care practices, such as medication intake or rehabilitation when necessary.
6	Flexibility of experimental intervention – Adherence	5	Only usual encouragement was provided during the sessions, with no additional strategies implemented to enhance adherence or compliance.
7	Follow up	4	Follow-up was conducted after one year, aligning with standard procedures in usual care. However, usual care does not typically include these specific assessments.
8	Outcome	5	The primary outcome was physical performance, which is closely linked to independence in BADL and fall risk. As such, it is considered a person-centered outcome.
9	Analysis	5	ITT with all available data

Supplementary Table 2 Detailed description of the exercise protocol

Session component	General description	Week 1-2	Week 2-3months	Months 3-6	Months 6-9	Months 9-12
Warm-up	1 set of 10 repetitions of the resistance exercises that they would be performing later but without any weight or by just lifting their own body weight	5-10 min	5-10 min	5-10 min	5 min	5min
Resistance training	Exercises were performed from the seated to standing (3 months onwards) positions. Initial exercises: biceps curl, knee extension, knee flexion, calf raise and sit and stand. Mid-program new exercises: front raises and svend press	~35min 30% of 1-RM 5 exercises: 1 upper limbs, 4 lower limbs; 1 set, 15 reps	~35 min 40-50% of 1-RM 5 exercises: 1 upper limbs, 4 lower limbs; 2 sets, 10 reps	~40 min 50-60% of 1-RM 5 exercises: 1 upper limbs, 4 lower limbs; 2 sets, 15 reps	~45 min 50-60% of 1-RM 7 exercises: 3 upper limbs, 4 lower limbs; 2 sets, 15 reps	~45 min 50-60% of 1-RM 7 exercises: 3 upper limbs, 4 lower limbs; 3 sets, 15 reps
Aerobic training	Progressive sets of walking	~10 min 3 sets of 3 min	10 min 2 sets of 5 min	10 min 1 set of 10 min	10 min 1 set of 10 min	10 min 1 set of 10 min
Cool-down	Stretching exercises	5 min	5 min	5 min	5 min	5 min

Supplementary Table 3 Secondary analysis of the effect of exercise intervention on SPPB subcomponents

Subcomponent Outcome	OR	95%CI	p-value
Balance	0.17	0.05, 0.53	0.004
Walking speed	0.25	0.08, 0.82	0.023
5-chair stand test	0.11	0.03, 0.37	<0.001

Note: Results from the multinomial logistic regression model are presented. The odds ratio (OR) for balance represents the odds of participants in the exercise group being in lower categories than the best score category compared to the usual care group after 12 months. For walking speed and the 5-chair stand test, the OR represents the odds of participants in the exercise group being in Tertile 2 or 3 compared to Tertile 1 (the best score), relative to the usual care group after 12 months.

Supplementary Table 4 Sensitivity Analyses Adjusting for Baseline Frailty Status and SPPB Score

Endpoints	Δ usual care	Δ exercise	Between-group differences	p between groups
Primary outcome				
SPPB, score	-0.94 (-1.50, -0.37)	1.65 (1.03, 2.27)	2.59 (1.75, 3.42)	<0.001
Secondary outcomes				
Handgrip Strength, KgF	-1.57 (-2.44, - 0.70)	0.27 (-0.67, 1.22)	1.84 (0.56, 3.13)	0.006
BI, score	-12.39 (-19.96, -4.82)	-1.92 (-10.27, 6.43)	1.14 (0.99, 1.30)	0.060
Rate of falls, falls per 100 person-year	25.4 (0.53, 50.2)	3.3 (-8.2,14.9)	0.48 (0.20, 1.14)	0.095

Note: Changes represent differences from pre-baseline to post-intervention and are presented on the original scale for each variable with 95% confidence intervals. Between-group differences are reported as absolute differences for Short Physical Performance Battery (SPPB) score and handgrip strength; and as Incident Rate Ratios (IRR) for Barthel Index (BI) score and rate of falls.

Chapter V – General discussion

5. General Discussion

Frailty is a highly prevalent and complex clinical syndrome among older adults living in NH, posing significant challenges to both individual health and the sustainability of healthcare systems worldwide. Characterised by a progressive loss of physiological reserves across multiple systems, frailty increases vulnerability to stressors, accelerates physical and functional decline, and diminishes quality of life. Although a growing body of evidence highlights the beneficial effects of exercise in mitigating frailty, substantial gaps remain in our understanding of the underlying biological mechanisms, particularly in the most vulnerable populations, such as NH residents, who have been largely underrepresented in research.

Therefore, this thesis aimed to address these gaps by exploring how exercise training can improve physical function and frailty in NH residents, thus contributing to the expanding body of literature on exercise as a strategy in frailty management. The four studies included herein were designed to advance our understanding of exercise as a therapeutic strategy for frailty, combining a comprehensive narrative review, a cross-sectional analysis, and two intervention trials. Collectively, our data consistently support the benefits of implementing concurrent training (including resistance and aerobic exercises) in NH to enhance physical function outcomes in frail older adults.

The narrative review (Study I) provides critical insights into the biological mechanisms of frailty, particularly the role of skeletal muscle dysfunction in energy dysregulation and how myokines can modulate key physiological pathways, thus mediating the positive effects of exercise on frailty and physical function. This theoretical framework served as the foundation and starting point for the subsequent studies. Our cross-sectional Study II suggests that myostatin (MSTN), a myokine, is associated with lower odds of being frail and positively correlates with physical function outcomes such as physical performance, BADL independence, and isokinetic knee extension strength. Study III demonstrated that a short-term (12 weeks) concurrent exercise intervention is both feasible and effective in frail residents, leading to significant improvements in physical

performance, handgrip strength, and MSTN levels, but not in isokinetic knee strength. This study showed that MSTN levels were responsive to exercise training, with reductions that may reflect favourable physiological adaptations. In contrast, the intervention did not result in significant changes in Decorin concentrations. Building on these findings, Study IV evaluated a longer, one-year intervention and confirmed similar improvements in physical function. However, this extended intervention did not lead to significant improvements in BADL independence or a reduction in the rate of falls.

This research underscores the feasibility and effectiveness of concurrent exercise interventions, not only in improving physical function but also in improving biological markers of skeletal muscle and frailty. Taken together, this thesis contributes to the limited evidence on exercise interventions for frail NH residents, highlighting the importance of incorporating exercise programmes into standard care practices within this setting.

We next discuss and summarise the main results from the four studies included in this thesis and their practical implications.

Relationship between myokines and frailty

The pathophysiology of frailty remains an active area of investigation. While numerous studies have attempted to identify its subcellular and cellular mechanisms, such as chronic inflammation, cellular senescence, mitochondrial dysfunction, and dysregulated nutrient sensing, the precise pathways by which these factors contribute to dysfunction across multiple, interconnected physiological systems and ultimately lead to the clinical manifestations of frailty remain unclear (Kim & Rockwood, 2024). Our narrative review (Study I) integrates current evidence suggesting that dysregulation in energy metabolism, particularly in the transport and utilisation of energy substrates, may play a central role in the onset and progression of frailty (Fried, 2016; Fried et al., 2021; Shaulson et al., 2024), which have been now highlighted by others (Fountain et al., 2024; Mishra et al., 2024).

Skeletal muscle is a key regulator of metabolic health and is profoundly compromised in frailty (Fried et al., 2021). It plays a vital role in inter-organ communication, especially in response to exercise, which enhances metabolic function and supports physiological resilience (Izquierdo & Fiatarone Singh, 2023). Skeletal muscle acts as an endocrine organ by releasing myokines such as MSTN, Decorin, IL-6, Irisin, IL-15, Meteorin-like protein, and Beta-aminoisobutyric acid, among many others (Severinsen & Pedersen, 2020). These molecules exert systemic effects that promote adaptive responses and improve the function of many physiological systems. In response to exercise, these myokines may contribute to improvements in frailty and physical function. In contrast, physical inactivity and ageing can impair myokine signalling, potentially contributing to the development of chronic diseases and metabolic dysfunctions, including frailty (Pedersen & Febbraio, 2012). Thus, these molecules may serve not only as potential biomarkers of frailty onset and progression (Coelho-Junior et al., 2019), but also as responsive biomarkers related to lifestyle interventions and health outcomes, particularly relevant in the era of personalised medicine (Jin et al., 2024).

Despite the compelling theoretical framework, our review revealed that very little research has been conducted on the acute and chronic effects of exercise on myokine secretion in the older population. This knowledge gap provided the rationale for our empirical investigations into the role of specific myokines, particularly MSTN, in frailty status and exercise adaptation (Studies II and III).

MSTN is a well-known myokine that acts as a negative regulator of skeletal muscle growth. In study II, we found higher levels of circulating MSTN in less frail individuals [pre-frail: median 2.25 (IQR: 1.45) ng/ml and frail: median 1.83 (IQR: 1.10) ng/ml], which was also previously observed in a similar population (Arrieta, Hervás, et al., 2018). Contrary to the prevailing view that elevated MSTN levels are solely detrimental, our findings revealed a paradoxical association in which higher MSTN concentrations, even when normalised to total body lean mass, were associated with lower odds of frailty (OR = 0.46). We also found that absolute MSTN levels were positively associated with physical function outcomes, including physical performance, independence in BADL and knee

strength in nursing home residents, which aligns with a previous study in frail older adults (Arrieta, Hervás, et al., 2018). However, relative levels of MSTN were only correlated with independence in BADL, thus suggesting a mediating effect of muscle mass.

Potential methodological and mechanistic factors may, to some extent, explain these findings. Emerging evidence suggests that MSTN act as a chalone, which is a circulating signalling molecule that regulates tissue growth through negative feedback regulation (Lee, 2023). In this sense, this myokine and its antagonists may be potentially responsible for minimising muscle loss in response to muscle-wasting conditions while restraining skeletal muscle overgrowth in response to anabolic stimuli (Cawthon et al., 2023; Lee, 2023; Peng et al., 2022). Previous data from large cohort studies provide some support for this theory. They found that higher MSTN levels were associated with better physical performance, greater muscle cross-sectional area, and higher handgrip strength (Cawthon et al., 2023). Conversely, higher levels of MSTN inhibitors, including Follistatin-like 3 (FSTL-3) and follistatin, were associated with persistent mobility limitations and lower muscle mass (Cawthon et al., 2023; Semba et al., 2018).

Results from our Study III indicate that basal MSTN concentrations significantly decreased as a chronic response to a short-term concurrent exercise training in frail individuals, aligning with the findings from our narrative review (Study I). This downregulation of MSTN likely represents a favourable physiological adaptation that promotes muscle hypertrophy and enhances muscle function, while also playing a role in energy homeostasis. MSTN has been shown to inhibit 5' adenosine monophosphate-activated protein kinase activity and reduce Glucose transporter type 4 translocation to the cell membrane, thereby decreasing glucose uptake and insulin sensitivity (Wetzlich et al., 2025). Consequently, exercise-induced reductions in MSTN may positively influence energy metabolism, leading to improved glycemic control and reduced insulin resistance (Khalafi et al., 2023).

Interestingly, although MSTN levels declined post-intervention, the expected compensatory increase in one of its antagonists, Decorin, was not statistically significant, despite a trend in the anticipated direction. This contrasts with more

robust findings in younger populations (Arabzadeh et al., 2023; Kanzleiter et al., 2014; Willoughby et al., 2022) and underscores the limited evidence on Decorin response to exercise in older adults. It is plausible that other exercise-responsive proteins, such as follistatin, FSTL-3, and growth and differentiation factor–associated serum protein-1, may play a more dominant role in mediating MSTN suppression. Indeed, prior studies have shown that reductions in MSTN following concurrent or resistance training in sarcopenic older adults are often accompanied by significant increases in circulating follistatin (Bagheri et al., 2020; Mafi et al., 2018; Negaresh et al., 2019). However, the literature remains inconclusive, likely due to methodological variability in MSTN quantification, failure to control for important confounders such as muscle mass and sex-specific differences.

Overall, emerging evidence points to a complex regulatory network in which MSTN and its counter-regulators interact to modulate muscle mass and function (Cawthon et al., 2023) with potential implications in energy metabolism. Our findings contribute to this growing body of research by highlighting the potential of MSTN as a biomarker of frailty (El Assar et al., 2024), given its association with frailty status, responsiveness to exercise interventions, and correlations with physical function outcomes. However, this hypothesis remains preliminary due to our study's limitations, and future research is warranted to further investigate MSTN clinical utility, its interactions with other myokines, and its role in the pathophysiology of frailty.

Feasibility of exercise training programmes in NH

Although an increasing body of evidence shows the positive effects of exercise interventions in NH residents (Valenzuela et al., 2023), the translation of research into evidence-based care practices remains a gap. Such interventions include highly heterogeneous populations in terms of frailty status, with many including both pre-frail and frail individuals (Valenzuela et al., 2023). Thus, concerns arise about the generalizability of findings and the appropriateness of exercise interventions for the most frail residents.

Our main results from Study III addressed this gap, as this study demonstrated that a 12-week concurrent training (three sessions per week) was feasible in an exclusively frail population, supported by the lack of adverse events and high adherence (93.75%). Attendance rates were comparable to those reported in previous NH-based interventions (Arrieta, Rezola-Pardo, et al., 2018; Fiatarone et al., 1994) and were notably higher than those typically observed in community settings, where adherence rates range from 68% to 89% (Casas-Herrero et al., 2022; da Silva Capanema et al., 2024; Dun et al., 2022).

Conducting sessions within the NH setting likely minimised environmental barriers, which are frequently cited in community-based programs (Kilgour et al., 2024). Additionally, the structure of the sessions and a gradual progression from seated to standing exercises likely contributed to improved self-efficacy, motivation and reduced fatigue (Andrews et al., 2024). In this sense, our results challenge the prevailing misconception that progressive exercise training is harmful for frail individuals, as well as the common practice of promoting only light-intensity stretching exercises in NH residents that offer minimal health benefits (Hurst et al., 2023; Izquierdo et al., 2025).

In both Study III (12 weeks) and Study IV (the long-term 12-month intervention), concurrent exercise training proved safe and feasible. Although one minor, non-serious adverse event (e.g., hypoglycaemia in a type 1 diabetes participant) occurred in Study IV, it was swiftly managed by the nursing team, reinforcing the importance and effectiveness of multidisciplinary collaboration in delivering exercise interventions to medically complex populations in NH.

In Study III, we also provided a simplistic cost analysis showing that 12 weeks of concurrent training using free weights cost €171.45 per participant, equivalent to approximately €57.15 per participant per month. The incremental cost-effectiveness ratio indicated that this intervention was cost-effective in improving physical performance, as measured by the SPPB, compared to usual care. Due to the lack of studies conducting economic analyses in a similar context, both in terms of intervention and population, and because our analysis did not include a standard metric such as quality-adjusted life years, we were unable to compare

our results with others. However, given that frailty is associated with substantial health and social care costs (Han et al., 2019), and early studies suggest that exercise may positively impact these outcomes (Bays-Moneo et al., 2023), future research should incorporate comprehensive economic evaluations to facilitate comparisons, support investment decisions, and guide policy development.

Effectiveness of exercise interventions in NH

Both Study III and IV showed that short and long-term concurrent exercise programmes significantly improved physical performance, particularly in lower-extremity function (e.g. SPPB score 2.18 and 2.59, respectively). The improvements observed in both of our studies likely reflect gains in lower-body strength and functional capacity, specifically the ability to stand and walk. These functional improvements have important implications for maintaining autonomy and independence in BADL among NH residents, potentially reducing dependence on staff for everyday care. Indirectly, enhanced physical function may also help mitigate psychological and social issues such as depression and isolation, by enabling residents to move more freely within the facility or even engage in social interactions and visits outside the NH setting (Resnick, 2024).

Study III failed to observe any significant changes in isokinetic relative peak torque for knee flexors and extensors, which is consistent with a previous study in a similar population (Oesen et al., 2015). These results contrast with the observed improvements in lower-extremity physical performance and may suggest that isokinetic testing does not adequately reflect functional everyday movements. In contrast, other muscle strength assessments, such as isometric knee strength tests, have demonstrated significant exercise-related improvements in several studies (Cadore et al., 2014; Valenzuela et al., 2023; Weng et al., 2022). This may be because isometric testing is less influenced by factors such as cognitive impairment, coordination, or complex motor control, making it potentially more suitable for frail older adults (Steffl & Stastny, 2020; Swales et al., 2023).

Studies III and IV found a positive effect of short and long-term concurrent exercise interventions on handgrip strength (2.15 and 1.85kgF, respectively), similar to previous studies. These improvements may be a reflection of the overall benefits of exercise in improving conditions such as sarcopenia and frailty, since handgrip is a key marker of such conditions (Fried et al., 2001; Kirk et al., 2024). A potential mechanistic explanation for these results is likely due to neural adaptations, even in the absence of significant initial changes in muscle morphology in older adults. Previous studies support the effects of resistance training on increased central nervous system activation and increased amplitude of maximal electromyogram activity, leading to improved rate of force development, increased motor unit recruitment and firing frequency, and decreases in muscle antagonist coactivation (Aagaard et al., 2010; Fragala et al., 2019; Walker, 2021). Additionally, the observed decreases in MSTN concentration in Study III may suggest a favourable net effect on muscle repair and hypertrophy, further aligning with existing evidence. Indeed, progressive resistance training has been shown to effectively increase the cross-sectional area of the quadriceps femoris and knee flexor muscles in frail residents (Cadore et al., 2014; Fiatarone et al., 1994).

The long-term concurrent exercise intervention (Study IV) did not result in statistically significant improvements in independence in BADL. However, several contextual and methodological factors may help interpret these findings beyond the intervention itself. Notably, while the usual care group experienced a significant decline in BADL performance (a reduction of more than 10% in score), the exercise group showed only a mild, non-significant decrease, suggesting that exercise may have mitigated the expected functional deterioration. These findings align with previous studies (Arrieta et al., 2019; Bays-Moneo et al., 2023), which also highlight the role of exercise programmes, including progressive resistance and aerobic training, in preserving functional independence over time. The maintenance of BADL independence is particularly important in nursing home settings, as it is closely related to residents' quality of life and well-being (Barile et al., 2012; Chan et al., 2015) and may also reduce caregiver burden. The staff population is often insufficient to meet the needs of highly dependent

residents, and the physical and psychosocial demands of care contribute to staff strain (Kunkle et al., 2021). Thus, promoting functional independence through exercise may have benefits that extend beyond individual health, potentially supporting more sustainable care delivery systems.

Falls are a complex major health concern due to their strong association with adverse outcomes, including functional decline, hospitalisation, decreased quality of life, and increased mortality (Shao et al., 2023). Given that our intervention was not specifically designed for fall prevention, as it consisted solely of an exercise program without specific components such as balance training, the non-significant differences in fall rates between groups in Study IV were not unexpected (Dawson et al., 2024). However, the intervention group showed higher odds of achieving better scores in key physical performance measures, such as balance, gait speed, and lower-limb strength (e.g., SPPB), compared to the usual care group. This suggests that although exercise enhanced the physical performance of lower extremities, it was insufficient on its own to translate into a meaningful reduction in the rate of falls, further highlighting the multidimensional nature of fall risk.

Our results contrast with those of previous trials specifically designed to prevent falls, which have demonstrated significant reductions in the rate of falls through multicomponent exercise interventions alone (Hewitt et al., 2018; Mak et al., 2022). Therefore, exercise programs should be carefully tailored to individual characteristics, such as frailty status, since frailer individuals may require higher frequency, greater volume, or longer intervention durations to achieve a meaningful effect on fall prevention (Suen et al., 2024). While a recent meta-analysis indicated that exercise interventions alone may be effective in preventing falls among NH residents, the evidence remains limited and inconsistent across studies (Dyer et al., 2023). Consequently, current guidelines recommend incorporating exercise into a multimodal approach to fall prevention, especially for medically complex populations such as frail older adults (Montero-Odasso et al., 2022).

Overall, comparable improvements were observed in both short- and long-term interventions that employed similar exercise protocols but differed in frequency, intensity, and duration. The absence of greater gains in the longer intervention highlights the importance of a well-designed, tailored exercise program that systematically adheres to fundamental training principles, as these critically determine its effectiveness (American College of Sports Medicine, 2022). In particular, the principles of progression and individualisation are central to inducing meaningful adaptations in older adults (Izquierdo et al., 2025). In Study IV, the absence of a controlled and individualised progression likely resulted in an insufficient training stimulus, thereby limiting gains in handgrip strength. This contrasts with Study III, in which progression was rigorously monitored and adjusted. A further distinction between the interventions was training frequency, with participants from Study III undertaking three sessions per week compared with two sessions per week in Study IV. Previous evidence suggests that while two sessions are generally sufficient to elicit improvements in physical performance, three sessions per week may optimise outcomes in pre-frail and frail populations (Nagata et al., 2023). This consideration is particularly relevant for highly sedentary populations, such as nursing home residents, in whom inconsistent or infrequent exercise participation can accelerate the reversibility of training-induced gains (American College of Sports Medicine, 2022).

Taken together, the differences between interventions highlight that both the characteristics of exercise prescription (e.g., training frequency) and its tailoring (e.g., progression and individualisation) are essential to maximise functional outcomes, particularly in vulnerable older populations. However, in this population, it is equally important to design interventions that are feasible and sustainable within real-world settings, accommodating NH routines, respecting participants' preferences, and ultimately maximising adherence and effectiveness.

In sum, both short- and long-term exercise interventions are feasible and effective in enhancing the physical function of NH residents. A meta-analysis by Valenzuela et al. (2023) found that the greatest effect of improvements in physical function occurred in pre-frail and frail subgroups, which supports the notion that

these populations, although often excluded from or underserved by exercise programs, may benefit the most from such interventions.

5.1 Future directions

One of the important future challenges is to unravel the complexity of frailty pathophysiology and to clarify how exercise can simultaneously modulate multiple interconnected physiological systems. Future studies specifically designed to examine multiple exerkines at the same time as a primary outcome, using novel technologies, such as omics, are needed to clarify the complex interplay between these molecules and the onset and progression of frailty. Moreover, further research is required to explore both the acute and chronic responses of key molecules to different types of exercise in older populations, including frail NH residents. Large-scale initiatives, such as the $170 million Molecular Transducers of Physical Activity Consortium, are expected to provide valuable insights into these topics shortly (Sanford et al., 2020).

A critical challenge moving forward is the incorporation of exercise programs as an integral part of standard care practices in NH. This will require more evidence from pragmatic trials that include representative populations (e.g. both pre-frail and frail individuals) and replicate recruitment and participation processes reflective of routine nursing home workflow, thereby reinforcing the active role of NH staff. Further research is also needed to optimise intervention characteristics such as frequency, volume, and exercise selection, to maximise functional benefits for frail residents while ensuring adherence and long-term sustainability. Particular attention should be given to improving recruitment strategies for the most frail individuals, who are often underrepresented in research. This may be facilitated by educating both staff and participants to improve motivation and promote adherence.

In parallel, comprehensive economic evaluations of exercise trials, including cost-effectiveness, healthcare resource utilisation, care costs, and caregiver burden, are essential for translating evidence into care policies, practices, and

governmental health programs. Although preliminary findings suggest that tailored exercise programs may offer substantial long-term health and economic benefits (Bays-Moneo et al., 2023), current data on their cost-effectiveness in this setting remain limited. As healthcare systems face growing pressures from ageing populations, especially those who are frailer, there is an urgent need to translate research findings into real-world practices.

5.2 Limitations and Strengths

Several methodological constraints surfaced during the implementation of the included studies and should be discussed to guide future research.

The COVID-19 pandemic posed a significant constraint, restricting access and raising safety concerns that hindered NH participation in external interventions, thereby reducing the sample size in Study III. To overcome this, we expanded recruitment in the following year by involving additional NH, which enabled us to reach the target sample size.

In Studies II and III, difficulties occurred in the myokines analysis using the Human Myokine Panel (HMYOMAG-56K-03). Despite strict adherence to the manufacturer's protocol, we observed little to no detectable levels of several target myokines, including IL-6, irisin, MSTN, and follistatin-like protein 1. This could be attributable to the lower sensitivity of the multiplex platform, lack of an acid activation step to release latent MSTN, and potential matrix effects inherent to samples from older adults. Consequently, we revised our approach to focus solely on MSTN measurement using a single-analyte Enzyme-Linked Immunosorbent Assay (ELISA), which successfully detected the target analyte. However, this approach was based on the available budget, precluding the analysis of other relevant myokines simultaneously and leading to a small sample size. This limitation warrants cautious interpretation of the results in Studies II and III. Nevertheless, our preliminary findings are novel and provide valuable insights into this field and in this understudied population.

The ELISA kit (DGDF80) used in studies II and III also has some limitations. First, the assay measures total MSTN, encompassing precursor, latent complex, and mature forms, rather than specifically detecting the biologically active MSTN. Since most circulating MSTN is bound to inhibitors such as follistatin and other antagonists, measured levels may overestimate MSTN concentration without reflecting its true functional activity. Additionally, although the ELISA kits are designed to specifically detect MSTN, some degree of cross-reactivity with Growth Differentiation Factor 11 (GDF-11) may occur due to their high sequence homology, potentially leading to slight inaccuracies in MSTN quantification; however, this cross-reactivity is generally minimal (Peng et al., 2022). In an ideal scenario, advanced techniques such as Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) would have been employed, as they offer more specific and accurate quantification of MSTN in serum (Cawthon et al., 2023; Peng et al., 2022). However, the high associated costs made this analysis unfeasible for this thesis.

To strengthen our studies, we also employed objective methods such as DXA and an isokinetic dynamometer.

A limitation of using DXA (Study II-III) to assess body composition in frail individuals is its reduced accuracy in estimating lean mass, particularly in those with significant muscle loss (Fragala et al., 2015). Furthermore, DXA has demonstrated a limited association with more direct muscle mass measures, such as MRI (Basty et al., 2024), and functional outcomes (Kirk et al., 2024). However, despite these limitations, DXA remains a widely used and non-invasive tool that provides a quick, accessible, and relatively low-cost method for assessing overall body composition, including fat mass, with minimal radiation exposure (Buckinx et al., 2018).

To strengthen our studies, we employed objective methods to assess physical function, such as isokinetic strength testing using the isokinetic dynamometer. However, a limitation of the test in frail individuals is that it measures isolated, controlled movements, which may not accurately represent the muscle performance needed for everyday functional activities (Zheng et al., 2024).

Additionally, the tests involve a degree of cognitive demand, which could impact performance, particularly in frail individuals with cognitive impairment (de Oliveira et al., 2022). Furthermore, isokinetic strength assessments may not fully capture muscle imbalances or neuromuscular deficits commonly present in frail individuals. Nonetheless, a key strength of isokinetic testing is that it follows a standardised protocol, allowing for consistent, objective measurements over time that can be compared across participants.

Despite these limitations, several measures were taken to minimise bias and enhance the reliability of the interventions. For instance, the exercise protocol was standardised and delivered by the same exercise professional to all participants, while the same evaluators consistently conducted the outcome assessments. These methodological choices strengthened the internal validity of the findings.

Each study's specific limitations and strengths are described in their respective section within each article.

Chapter VI – General conclusions

6. General Conclusions

Based on the included studies in this thesis, we reached the following conclusions:

Study I: There is growing evidence linking energy metabolism dysregulation to the onset and progression of frailty, with skeletal muscle playing a crucial role. Myokines such as IL-6, Irisin, and MSTN have emerged as potential mediators of the benefits associated with exercise, facilitating crosstalk between organs and systems and potentially improving the multisystem dysregulation underlying frailty. However, empirical studies examining the effects of both chronic and acute exercise on myokine concentrations in older adults remain limited.

Study II: Higher serum MSTN levels were associated with lower odds of being frail and higher physical performance, knee extension strength and greater independence in BADL.

Study III: A 12-week moderate intensity concurrent training was feasible and effective in improving physical performance, handgrip strength and serum MSTN levels in frail residents of NH. However, exercise intervention failed to have a significant impact on Decorin concentrations and isokinetic knee strength.

Study IV: A 12-month pragmatic moderate intensity concurrent intervention effectively improved physical performance and handgrip strength in NH residents. Although it did not significantly alter BADL or the rate of falls, the intervention appears to have attenuated the expected functional decline typically observed under usual care conditions.

In conclusion, this thesis contributes to the growing body of literature on the biological and physiological mechanisms underlying the onset and progression of frailty and reinforces the role of exercise as a therapeutic intervention capable of mitigating or even reversing its progression. Special emphasis should be placed on the endocrine role of skeletal muscle and its systemic influence via myokine secretion. Our results clearly show that concurrent exercise is both

feasible and effective, even among the most frail NH residents. Therefore, exercise interventions should be integrated as a mandatory component of care in NH settings.

Chapter VII - References

7. References

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