Universidade Estadual Paulista
"Júlio de Mesquita Filho"
Faculdade de Ciências - Bauru

CIÊNCIAS DO MOVIMENTO
PROGRAMA DE PÓS-GRADUAÇÃO

GRADUATE PROGRAM IN MOVEMENT SCIENCES – INTERUNIT PROGRAM

KLARA KARIN BRIGITTE KNOBLAUCH

COMPARATIVE ANALYSIS BETWEEN VULNERABILITY BIOMARKERS OF AGING AND HEALTH BIOMARKERS IN MIDDLE-AGED AND OLDER FEMALE ADULTS

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Master's dissertation presented to the Graduate Program in Movement Sciences, São Paulo State University, as part of the requirements for obtaining the Master's degree

Supervisor: Prof Assoc Anderson Saranz Zago

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ABSTRACT

Biological aging is typically associated with greater physiological changes that predispose individuals to adverse outcomes. In this way, the evaluation of vulnerability biomarkers and their relationships with other health biomarkers could contribute to the promotion of interventions and the improvement of older adults' quality of life. Thereby, this study explores the vulnerability biomarkers Growth Differentiation Factor 15 (GDF-15), General Functional Fitness Index (GFFI), and frailty phenotype, and their influence on health markers (blood biochemistry, body composition, and hemodynamic variables) in middle-aged and older female adults. **Methods:** A cross-sectional observational study was conducted with community-dwelling females aged 54-84 with a history of physical training. Participants were categorized based on functional fitness, frailty phenotype, and GDF-15 quartiles. The General Functional Fitness Index (GFFI) was assessed using the AAHPERD test battery, while frailty phenotype was determined using Fried's criteria. GDF-15 concentration was measured through ELISA. **Results:** Higher training status (TS) was associated with better functional fitness and favourable biochemical profiles, including lower total cholesterol ($p=0.006$, $\eta^2p=0.253$), LDL-cholesterol ($p=0.001$, $\eta^2p=0.346$), triglycerides ($p=0.048$, $\eta^2p=0.195$), and systolic blood pressure ($p=0.001$, $\eta^2p=0.333$). Individuals classified as robust presented better physical performance scores, lower total cholesterol ($p=0.002$, $\eta^2p=0.306$), and LDL-cholesterol ($p=0.014$, $\eta^2p=0.216$) than their pre-frail and frail counterparts. GDF-15 did not show significant differences in physical performance, blood biochemistry, body composition, or blood pressure between quartiles. **Conclusion:** These findings suggest that GFFI may be considered a health biomarker for middle-aged and older adult females, while highlighting the need for further research on the role of biomarkers of vulnerability and healthy aging.

Keywords: Vulnerability; Biomarker; GDF-15; Frailty; Functional Fitness; Older Age.

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LIST OF ABBREVIATIONS

AAPHERD	American Alliance for Health, Physical Education, Recreation and Dance
ATF4	Activating Transcription Factor 4
BMI	Body Mass Index
BP	Blood Pressure
CHOP	C/EBP Homologous Protein
CKD	Chronic Kidney Disease
CRP	C-Reactive Protein
DAMPs	Damage-Associated Molecular Patterns
DBP	Diastolic Blood Pressure
DXA	Dual-Energy X-Ray Absorptiometry
ELISA	Enzyme-Linked Immunosorbent Assay
GDF-15	Growth Differentiation Factor 15
GFFI	General Functional Fitness Index
GDNF	Glial Cell-Derived Neurotrophic Factor
GFRAL	Glial Cell-Derived Neurotrophic Factor Receptor Alpha-Like
GL	Glucose
HALE	Health-Adjusted Life Expectancy
HDL-C	High-Density Lipoprotein Cholesterol
HRP	Horseradish Peroxidase
IL-6	Interleukin-6
ISR	Integrated Stress Response
LDL-C	Low-Density Lipoprotein Cholesterol
MAPK	Mitogen-Activated Protein Kinase
MIC-1	Macrophage Inhibitory Cytokine-1
NAG-1	NSAID-Activated Gene-1

NK	Natural Killer Cells
NSAID	Nonsteroidal Anti-Inflammatory Drug
NT-proBNP	N-terminal Pro–B-type Natriuretic Peptide
OECD	Organisation for Economic Co-operation and Development
PDF	Prostate-Derived Factor
PI3K	Phosphoinositide 3-Kinase
PLCγ	Phospholipase C Gamma
PLAB	Placental Bone Morphogenetic Protein
RET	Rearranged During Transfection (Receptor Tyrosine Kinase)
ROS	Reactive Oxygen Species
SASP	Senescence-Associated Secretory Phenotype
SBP	Systolic Blood Pressure
SD	Standard Deviation
SMAD	Small Mothers Against Decapentaplegic (Signaling Proteins)
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TCA	Trichloroacetic Acid
TC	Total Cholesterol
TG	Triglycerides
TGF-β	Transforming Growth Factor-Beta
TNF-α	Tumor Necrosis Factor-Alpha
TS	Training Status
VLDL	Very Low-Density Lipoprotein
WHO	World Health Organization
WHR	Waist-to-Hip Ratio
η^2p	Partial Eta Square

1. OVERVIEW

As global populations age, it becomes increasingly important to understand the biological and functional vulnerabilities of older adults. Aging is accompanied by physiological changes that increase susceptibility to stressors such as infections, injuries, or chronic conditions (Andrew et al., 2012).

Biological aging leads to a decline in the homeostatic and regenerative capacities of cells and tissues, aggravated by chronic inflammation, oxidative stress, and mitochondrial dysfunction (Guo et al., 2022; Li et al., 2023; Lima et al., 2022). These processes increase the risk of frailty—a clinical syndrome characterized by diminished physiological reserves, reduced mobility, and increased risk of adverse health outcomes, such as falls, hospitalization, and mortality (Corral-Pérez et al., 2023). Therefore, biological aging is typically associated with greater vulnerability, a multifaceted concept including physical, cognitive, and social dimensions, which predisposes individuals to adverse outcomes (Sanchini et al., 2022). In this way, the evaluation of vulnerability biomarkers and their relationships with other health biomarkers could contribute to promoting interventions to improve the quality of life of older adults.

The Growth Differentiation Factor 15 (GDF-15), a stress-responsive cytokine from the transforming growth factor-beta (TGF-β) superfamily (Bootcov et al., 1997; Wischhusen et al., 2020), has gained attention as a biomarker of vulnerability in aging research. Its expression increases in response to inflammation, oxidative damage, and mitochondrial dysfunction (Teng et al., 2021; Yatsuga et al., 2015) - hallmarks of aging. Elevated GDF-15 levels have been linked to frailty, functional decline, and mortality. Additionally, GDF-15 is implicated in sarcopenia, which is characterized by the loss of skeletal muscle mass and strength (Cruz-Jentoft et al., 2010; Walston, 2012). It also plays a role in modulating appetite and energy homeostasis (Wang et al., 2021), suggesting its involvement in malnutrition among frail older adults. Importantly, GDF-15 levels are modifiable through interventions targeting inflammation, oxidative stress (Tsai et al., 2016) and lifestyle factors, making it a promising tool for monitoring therapeutic effectiveness.

Furthermore, aging is generally associated with a decline in overall functional fitness (Caldas, 2003), which includes strength, endurance, balance,

and flexibility, which are essential components for maintaining independence and quality of life. Therefore, the assessment of General Functional Fitness Levels (GFFI) can also be considered as a functional marker of vulnerability. Assessing GFFI provides a comprehensive view of health and enables targeted interventions to enhance resilience.

Another condition of vulnerability commonly associated with aging is frailty (Soysal et al., 2016). It reflects a decline in physiological reserves across multiple systems, increasing susceptibility to stressors and adverse outcomes such as disability and mortality (Dent et al., 2019). Chronic inflammation, driven by elevated cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), underpins frailty progression (Alberro et al., 2021). Frailty is clinically assessed using instruments such as the Fried frailty phenotype (Fried et al., 2001), which evaluates unintentional weight loss, weakness, slowness, exhaustion, and low physical activity.

Addressing vulnerability through biomarkers, clinical assessments, and fitness measures is a critical priority in aging research. Investigating the interrelationships among GDF-15, frailty, and functional fitness offers opportunities to develop personalized strategies that strengthen resilience, promote healthy aging, and reduce age-related morbidity. However, it is still unknown what influence these vulnerability biomarkers have on other important health markers such as blood pressure, body composition, and blood biochemistry. Additionally, although GDF-15 is a biological biomarker, it is believed that both GFFI and the frailty phenotype can also be considered good health markers. In this way, it is important to define the influence each biomarker has on health variables, so that better management of health conditions for older adults can be planned.

2. INTRODUCTION

2.1 Global demographic aging

The aging of the population represents one of the most profound demographic transformations of the 21st century and poses major challenges to health and social systems worldwide (WHO, 2025). Currently, individuals aged 65 years and older represent approximately 10–11% of the global population, corresponding to an estimated 750–830 million people worldwide (World Bank, 2026). Projections suggest that this number may reach between 1.5 and 1.7 billion by 2050 (World Bank, 2026). Notably, approximately 80% of older adults are expected to live in low- and middle-income countries by 2050, reflecting a demographic transition that is occurring at an unprecedented pace (WHO, 2025).

Although improvements in life expectancy are widely regarded as a major societal achievement, increased longevity does not necessarily translate into prolonged healthy life. Health-adjusted life expectancy (HALE) estimates healthspan by weighting years of life according to health status (WHO, 2026; OECD, 2025). Across 183 Member States of the World Health Organization, the mean global healthspan–lifespan gap has been estimated at approximately 9.6 years (Garmany et al., 2024). Furthermore, this gap increased by 13% between 2000 and 2019, indicating that gains in survival have not been matched by equivalent improvements in healthy longevity (Garmany et al., 2024). Importantly, women tend to live longer than men but spend a greater proportion of later life in poor health. Globally, women exhibit a mean healthspan–lifespan gap approximately 2.4 years larger than that of men, largely attributable to a higher burden of noncommunicable diseases (Garmany et al., 2024).

These findings stress a critical shift in public health priorities from extending lifespan to preserving functional capacity and promoting resilience in older age. In rapidly aging middle-income countries such as Brazil, identifying early biological and functional indicators of vulnerability is essential to prevent functional decline, reduce morbidity, and improve quality of life among older women.

2.2 Biological Mechanisms of Aging and Vulnerability

Aging is characterized by the progressive decline of cellular and systemic homeostasis, caused by interconnected molecular and biochemical processes (Aunan et al., 2016). The hallmarks of aging model proposed by López-Otín et al. (2023) organizes these mechanisms into three interrelated categories: primary hallmarks (e.g., genomic instability, telomere attrition, loss of proteostasis), antagonistic hallmarks (e.g., deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence), and integrative hallmarks (e.g., stem cell exhaustion, altered intercellular communication, chronic inflammation). Together, these processes contribute to weakened stress responses and increased biological vulnerability.

2.2.1 Oxidative and mitochondrial stress

One of the earliest mechanistic explanations of aging was the free radical theory proposed by Denham Harman in 1956. This theory postulated that aging results from cumulative oxidative damage caused by free radicals generated during aerobic metabolism (Harman, 1956). Reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, and hydroxyl radicals, are natural byproducts of mitochondrial respiration. Under physiological conditions, ROS function as signalling molecules. However, excessive ROS production or insufficient antioxidant defences lead to oxidative stress, defined as an imbalance between pro-oxidant and antioxidant systems (Liguori et al., 2018).

Oxidative stress induces damage to lipids, proteins, and DNA, therefore compromising cellular integrity and promoting apoptosis (Liguori et al., 2018; Sun et al., 2016). Mitochondria play a central role in this process. Mitochondrial dysfunction, recognized as a key antagonistic hallmark of aging, leads to compromised oxidative phosphorylation, reduced ATP production, and increased ROS generation (López-Otín et al., 2023; Sun et al., 2016). This creates a chain reaction in which oxidative stress further damages mitochondrial DNA and respiratory chain components, increasing bioenergetic decline and disrupting cellular homeostasis. Such shifts have been strongly associated with age-related diseases and functional deterioration.

Mitochondrial stress activates adaptive signalling pathways for the integrated stress response, aimed at restoring metabolic balance. These include upregulation of antioxidant enzymes, activation of stress-response pathways, and increased production of circulating stress-responsive molecules. One such molecule is Growth Differentiation Factor 15 (GDF-15), a cytokine induced by mitochondrial dysfunction and oxidative stress (Johann et al., 2021). Elevated GDF-15 levels are considered part of the integrated stress response and may reflect systemic attempts to reduce cellular damage. However, chronic activation of these pathways may also signal constant biological stress and reduced physiological reserve.

Together, oxidative stress, mitochondrial dysfunction, and dysregulated stress signalling represent central molecular mechanisms linking biological aging to increased vulnerability.

2.2.2 Inflammaging and altered intercellular communication

Chronic low-grade systemic inflammation (“inflammaging”) is a central feature of biological aging. It is classified within the integrative hallmarks of aging as altered intercellular communication and chronic inflammation (López-Otín et al., 2023). The concept, as described by Franceschi et al. (2018), characterizes aging as a state of persistent, sterile, low-grade inflammatory activation in the absence of acute infection.

With advancing age, accumulated cellular damage, mitochondrial dysfunction, and oxidative stress promote the release of damage-associated molecular patterns (DAMPs), which activate innate immune pathways. Cellular senescence leads to the development of the senescence-associated secretory phenotype (SASP), characterized by the secretion of pro-inflammatory cytokines, chemokines, and matrix-remodeling enzymes (Li et al, 2023). This results in elevated circulating concentrations of cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP), which have been consistently associated with increased morbidity, disability, and mortality in older adults (Franceschi et al., 2018; Giovannini et al., 2011).

Chronic activation of these inflammatory pathways contributes to anabolic resistance, muscle protein degradation, and reduced regenerative capacity. Those mechanisms are linked to sarcopenia and frailty. Elevated inflammatory markers

have been identified as predictors of functional decline and adverse health outcomes in aging populations (Giovannini et al., 2011).

Chronic inflammation therefore links molecular mechanisms of aging to clinical vulnerability, as accumulated cellular damage leads to impairment of systemic function.

2.3 Frailty

Frailty is a geriatric syndrome characterized by the loss of the body's ability to maintain homeostasis due to cumulative declines in several physiological systems associated with a reduced ability to resist stressors. It increases vulnerability to adverse outcomes, including falls, hospitalization, disability, and mortality (Kim et al., 2024; Corral-Pérez et al., 2023).

Two conceptual models have been proposed to assess frailty. The phenotype model introduced by Fried et al. (2001) defines frailty by five clinical criteria: unintentional weight loss, weakness, exhaustion, slow walking speed, and low physical activity. This model was selected in the present study because it is one of the most widely validated and clinically applicable frailty measures, with clear operational criteria that focus on physical function and can be practically assessed in research and healthcare settings. Meeting three or more of these criteria classifies an individual as frail, one or two indicate a pre-frail state, and none suggest robustness (Allison et al., 2021). Therefore, the frailty phenotype primarily assesses frailty as a categorical construct by classifying individuals into robust, pre-frail, or frail groups. A limitation of the phenotype approach is that it mainly focuses on physical signs of frailty and may not fully capture cognitive, psychological, or social dimensions, potentially underestimating frailty in some individuals. The deficit accumulation model developed by Rockwood et al. (2007) conceptualizes frailty as the cumulative burden of health deficits, measured by a Frailty Index. While differing in methodology, both models describe a state of reduced physiological resilience.

At the biological level, frailty reflects the systemic consequences of the molecular mechanisms described in previous chapters. Elevated circulating levels of IL-6, TNF-α, and CRP have been consistently associated with frailty status and progression (Giovannini et al., 2011). Mitochondrial dysfunction and impaired energy metabolism further compromise muscle performance and endurance,

reducing functional reserve. Identifying biological and functional markers associated with frailty may therefore provide critical insight into early vulnerability and opportunities for targeted intervention.

2.4 Growth differentiation factor 15

2.4.1 Molecular Structure

Growth differentiation factor 15 (GDF-15), a divergent member of the transforming growth factor-beta (TGF-β) superfamily, is a stress-responsive cytokine with a broad range of biological activities (Wischhusen et al., 2020). Initially identified in 1997 as macrophage inhibitory cytokine-1 (MIC-1), GDF-15 has since been recognized under several alternative names, including nonsteroidal anti-inflammatory drug (NSAID)-activated gene-1 (NAG-1), prostate-derived factor (PDF), and placental bone morphogenetic protein (PLAB), among others (Bootcov et al., 1997). The TGF-β superfamily, to which GDF-15 belongs, encompasses over 40 ligands that regulate cellular proliferation, differentiation, and apoptosis across diverse cell types, including cardiomyocytes, macrophages, and adipocytes (Valdimarsdottir et al., 2005).

GDF-15 is synthesized as a precursor protein that undergoes proteolytic processing to generate a 22 kDa disulfide-linked homodimer, which represents the biologically active circulating form (Baek et al., 2019). The protein is secreted by various tissues, including the heart, placenta, liver, lungs, and kidneys. Under physiological conditions (in the absence of cellular stress, characterized by metabolic homeostasis, lack of inflammation, normoxia, intact mitochondrial function, and absence of acute physical or psychological stressors) circulating GDF15 levels are low, typically less than 1000 pg/mL (Cimino et al., 2022), but expression can be induced by stressors such as inflammation, oxidative stress, hypoxia and ischemia, endoplasmic stress, tissue injury, and mitochondrial dysfunction (Xu et al., 2006; Ost et al., 2020; Fujita et al., 2015). For example, in patients with advanced cancers GDF-15 can reach levels of 10,000–100,000 pg/mL (Cimino et al., 2022). Its circulating levels can be readily quantified in serum or plasma. The expression of GDF-15 in response to cellular stressors suggests a protective role in various pathophysiological conditions.

2.4.2 Transcriptional Regulation of GDF-15 Expression

The GDF-15 promoter contains two functional binding sites for the transcription factor p53, which are both necessary and sufficient for the induction of GDF-15 expression (Yang et al., 2003). Activation of p53 occurs in response to telomere erosion, DNA damage, oxidative stress, hypoxia, oncogene activation, and inflammatory signalling (Wollert et al., 2017). Because p53 responds not only to acute severe stress but also to chronic low-grade cellular stress, GDF-15 expression may reflect cumulative biological damage, linking it mechanistically to aging and frailty phenotypes (Vousden et al., 2007). Experimental data demonstrate that high glucose exposure, oxidative stress, and ischemic injury induce p53-mediated GDF-15 expression, suggesting a cytoprotective adaptive response (Li et al., 2013).

Beyond p53, the cellular integrated stress response (ISR) is a major upstream regulator of GDF-15 expression (Lockhart et al., 2020). The ISR is activated by ER stress (e.g., tunicamycin), amino acid deprivation, viral infection, and mitochondrial dysfunction (Lockhart et al., 2020; Myojin et al., 2026). ISR activation results in phosphorylation of eIF2α, which promotes preferential translation of ATF4 and therefore induces expression of C/EBP homologous protein (CHOP) (Pakos-Zebrucka et al., 2016; Miyake et al., 2021). CHOP directly binds to the GDF-15 promoter and promotes transcriptional upregulation (Chung et al., 2017). This mechanism is particularly relevant in mitochondrial diseases and metabolic stress states.

Circulating GDF-15 levels are elevated in patients with mitochondrial disorders and correlate with disease severity (Ji et al., 2017). Based on these insights, GDF-15 has been characterized as a mitokine (mitochondria-derived signaling molecule) and a mediator of mitohormesis, reflecting adaptive stress signaling (Sorrentino et al., 2018).

2.4.3 Glial Cell-derived Neurotrophic Factor Receptor Alpha-like

A breakthrough in understanding GDF-15 signaling was the identification of glial cell-derived neurotrophic factor receptor alpha-like (GFRAL) as its specific receptor (Yang et al., 2017). GFRAL expression is highly restricted to the area postrema and nucleus tractus solitarius in the hindbrain (Sabatini et al., 2021).

GDF-15 binding to GFRAL promotes recruitment of the transmembrane tyrosine kinase coreceptor RET, forming a GDF-15–GFRAL–RET signalling complex (Fichtner et al., 2024).

This complex initiate intracellular phosphorylation cascades analogous to glial cell-derived neurotrophic factor (GDNF) signalling, including RET autophosphorylation, activation of MAPK/ERK pathways, PI3K/AKT signalling, and PLCγ pathways (Takahashi et al., 2022). The activation of the GFRAL–RET Signalling Axis leads to the suppression of appetite, reduction of body weight, and the alteration of energy homeostasis (Ling et al., 2023). This mechanism explains the anorectic and weight-reducing effects observed in cancer cachexia, chronic disease, and metformin therapy (Filippini et al., 2025).

2.4.4 Downstream Intracellular Signalling Pathways

As previously described, GDF-15 activates several intracellular signalling cascades. In cardiomyocytes and vascular cells, GDF-15 activates SMAD1/5 signalling through ALK4/5/7 receptors, consistent with canonical TGF-β superfamily signalling (Adela et al., 2015). SMAD activation leads to transcription of genes involved in anti-hypertrophic signalling, anti-apoptotic pathways, and extracellular matrix modulation. Following ischemia/reperfusion injury, GDF-15 expression is highly induced in the infarct border zone, where it exerts cardioprotective effects via SMAD activation and upregulation of Bcl-xL and β-catenin (Savic-Radojevic et al., 2017; Rochette et al., 2021).

Furthermore, studies show that GDF-15 activates PI3K/AKT signalling, endothelial nitric oxide synthase (eNOS), and anti-apoptotic cascades (Adela et al., 2015). The protein also inhibits pro-apoptotic mediators, including JNK and Bcl-2–associated death promoter (Li et al., 2013). These effects collectively promote cell survival under ischemic and metabolic stress conditions.

2.4.5 Immunomodulatory Functions

GDF-15 modulates immune responses by affecting the functions of various immune cells (e.g. T cells, natural killer cells, macrophages). It can act as an immunosuppressive and immunostimulatory factor. Immunosuppressive actions include the inhibition of T-cell proliferation, promotion of regulatory T cell

differentiation, suppression of NK cell cytotoxicity, or the direct inhibition of myeloid cell recruitment after myocardial infarction (Wang et al., 2021; Guo et al., 2024; Reyes et al., 2023; Wollert et al., 2017). These effects suggest a role in limiting excessive inflammatory damage (Salminen et al., 2024). On the other hand, GDF-15 may enhance macrophage activation and pro-inflammatory cytokine expression in atherosclerosis models (Reyes et al., 2023). The dual immune functionality makes GDF-15 a potential target for immunomodulatory therapy, particularly in oncology and chronic inflammatory disease.

2.4.6. GDF-15 in Cardiometabolic and Systemic Diseases

Elevated circulating GDF-15 concentrations are consistently observed across a broad spectrum of cardiometabolic and systemic disorders, supporting its role as a general biomarker of cellular stress and disease burden (Kempf & Wollert, 2007).

Several clinical studies demonstrate a positive association between circulating GDF-15 levels and body mass index (BMI), with significantly higher concentrations observed in individuals with obesity class II (BMI 35.0–39.9 kg/m²) and class III (BMI ≥40 kg/m²) compared to normal-weight controls (Sarkar et al., 2020; Vila et al., 2011). In obese patients, GDF-15 levels correlate with markers of insulin resistance and endothelial dysfunction, suggesting that its elevation reflects metabolic strain and vascular dysfunction rather than adiposity alone (Lind et al., 2009).

A key translational insight comes from studies investigating metformin. Treatment with metformin significantly increases circulating GDF-15 concentrations (Gerstein et al., 2017). In both experimental and clinical settings, metformin-induced weight loss has been shown to depend on GDF-15 signalling through the GFRAL–RET pathway (Yang et al., 2017). In humans with type 2 diabetes, increases in GDF-15 concentrations correlate with appetite suppression and subsequent body weight reduction, supporting a causal role in metabolic regulation (Day et al., 2019). These findings position GDF-15 as a metabolic stress hormone linking peripheral mitochondrial perturbation to central appetite control.

Intense physical exercise, another metabolic stressor, also transiently elevates circulating GDF-15 levels (Hackney et al., 2006), further reinforcing its characterization as a systemic stress-responsive cytokine.

Plasma GDF-15 concentrations are markedly elevated in patients with heart failure, myocardial infarction, and atherosclerosis (Gaggin et al., 2013; Kempf et al., 2006; Wang et al., 2019). In heart failure cohorts, GDF-15 independently predicts mortality beyond established biomarkers such as NT-proBNP and troponins (Zhou et al., 2021). Elevated levels have also been associated with endothelial dysfunction and vascular remodelling, showing its integration into inflammatory and hemodynamic stress pathways (Wand et al., 2019; Ozdemir et al., 2024)

Beyond cardiovascular pathology, GDF-15 predicts renal outcomes. In community-based cohorts, elevated baseline GDF-15 levels independently predicted incident chronic kidney disease (CKD), even after adjustment for diabetes, hypertension, baseline proteinuria, and cystatin C (Ho et al., 2013). This indicates that GDF-15 reflects subclinical organ stress before overt renal dysfunction becomes clinically apparent.

2.4.7. GDF-15 as a Predictor of Frailty and Adverse Outcomes

As previously mentioned, frailty is a multidimensional state of increased vulnerability resulting from age-related decline across multiple physiological systems (Kim et al., 2024). Given the established links between GDF-15 and mitochondrial dysfunction, inflammation, and metabolic stress, growing attention has focused on its potential role as a biological predictor of frailty and adverse aging outcomes.

Longitudinal evidence supports this association. In older adults with cardiometabolic diseases, elevated serum GDF-15 levels independently predicted the incidence of frailty over follow-up, even after adjustment for confounders (Oba et al., 2024). These results suggest that GDF-15 may reflect early biological vulnerability preceding clinical frailty. Importantly, the authors proposed that GDF-15 could serve as a marker to identify individuals who may benefit from preventive interventions such as structured exercise and nutritional support.

Cross-sectional and longitudinal data from community-dwelling cohorts further reinforce the prognostic relevance of GDF-15. Plasma GDF-15 has been associated with lower-limb physical performance and cognitive decline, however its ability to predict clinically significant decline separately appears limited (He et al., 2022). These results indicate that GDF-15 may be better conceptualized as part of a multidimensional vulnerability profile rather than a single diagnostic tool.

Mortality prediction provides additional support for its clinical relevance. In a population-based sample of 1470 community-dwelling older adults, higher baseline GDF-15 concentrations were independently associated with 8-year all-cause mortality, even after adjustment for gait speed, NT-proBNP, and high-sensitivity troponin T (Rothenbacher et al., 2019). This independent association suggests that GDF-15 captures broader systemic stress and biological aging processes beyond those captured by established cardiovascular biomarkers.

In a recent study by Torrens-Mas et al. (2025), associations between circulating GDF-15 levels and epigenetic clocks, as well as with telomere shortening and inflammatory markers, have been demonstrated. Moreover, GDF-15 correlated negatively with grip strength, gait speed, and pulmonary function, reinforcing its relationship with declining physiological reserve. Notably, sex-specific differences have been reported, with stronger associations observed in men.

Clinical studies in hospitalized populations extend these findings. In acutely admitted older medical patients, elevated GDF-15 concentrations were strongly associated with both frailty and sarcopenia, even in fully adjusted models, and optimal cut-off values were proposed (Kamper et al., 2024). These results show its potential utility in early risk stratification.

Taken together, current evidence indicates that GDF-15 functions as a biomarker of systemic biological stress. Although its causal role remains to be established, its consistent association with frailty incidence, functional decline, and mortality supports its potential value as a predictor of adverse outcomes in aging populations.

2.4.8 GDF-15 and Sarcopenia

Sarcopenia, defined by the progressive loss of skeletal muscle mass and strength with aging, is a major contributor to disability, loss of independence, and mortality (Walston, 2012). Although diagnostic criteria continue to evolve, consensus increasingly emphasizes reduced muscle strength and physical performance as core components.

Sarcopenia is multifactorial, involving mitochondrial dysfunction, chronic inflammation, insulin resistance, hormonal changes, reduced physical activity, and nutritional deficits (Xue et al., 2011). Notably, many of these mechanisms overlap with pathways that regulate GDF-15 expression, including oxidative stress and activation of the integrated stress response.

In older patients with cardiometabolic disease, higher serum GDF-15 concentrations were associated with reduced muscle strength and poorer lower-extremity performance (Oba et al., 2020). These findings suggest that GDF-15 may serve as a biomarker of muscle weakness rather than only reflecting systemic disease burden.

Additionally, in prefrail older adults with diabetes, GDF-15 was associated with poor physical function independent of inflammatory markers (Merchant et al., 2023). This evidence suggests that GDF-15 may reflect mitochondrial or metabolic stress mechanisms not fully reflected by cytokine measurements alone.

In acutely admitted older patients, plasma GDF-15 concentrations were significantly higher among individuals with sarcopenia, and elevated levels remained strongly associated with sarcopenia after multivariable adjustment (Kamper et al., 2024).

Interestingly, while GDF-15 consistently correlates with grip strength and gait speed, associations with body composition measures are less robust after adjustment for age and sex (Torrens-Mas et al., 2025). This suggests that GDF-15 may be more strongly linked to muscle function than to absolute muscle mass, in line with the latest definitions of sarcopenia that stress strength over quantity.

Collectively, current evidence indicates that GDF-15 reflects biological processes underlying muscle dysfunction, including mitochondrial stress, anabolic resistance, and chronic low-grade inflammation. Although causality has not been established, elevated GDF-15 levels consistently identify older adults with poorer

physical performance and increased vulnerability. Further longitudinal studies are required to determine whether GDF-15 directly contributes to sarcopenic progression or serves primarily as a sensitive indicator of underlying systemic stress.

2.5 General Functional Fitness Index

Aging is consistently accompanied by a decline in functional capacity, arising from progressive changes in musculoskeletal, cardiovascular, metabolic, and neurological systems (Caldas, 2003). Objective assessment of functional fitness is, therefore, essential to translate biological vulnerability into clinically meaningful outcomes. Functional fitness refers to the physical capacities required to perform activities of daily living independently and safely, including muscular strength, aerobic endurance, flexibility, balance, coordination, and agility.

Reductions in these domains are strongly associated with increased risk of falls, disability, hospitalization, and mortality in community-dwelling older adults (Ho et al., 2021; Zhao et al., 2016; Dos Santos et al., 2020).

The General Functional Fitness Index (GFFI) provides a multidimensional evaluation of physical capacity by combining measures of upper- and lower-body strength, aerobic endurance, flexibility, and motor performance into a composite score (Schneider et al., 2025). Unlike isolated performance tests, composite indices capture cumulative functional impairment and may more accurately reflect overall physiological reserve. Importantly, functional decline often precedes frailty and may represent an earlier, potentially reversible stage of vulnerability.

Physical exercise is a key modifiable factor within this pathway, as regular participation in exercise is consistently associated with lower biological vulnerability and more favourable biomarker profiles, including reduced systemic inflammation, improved metabolic regulation, enhanced neuromuscular function, and better preservation of skeletal muscle mass.

Assessment of functional fitness is particularly relevant in community-dwelling older females, who not only exhibit a greater healthspan–lifespan gap but also show a higher prevalence of sarcopenia, osteoporosis, and frailty-related disability in advanced age (Garmany et al., 2024; Dos Santos et al., 2020). In this population, regular physical exercise may attenuate sex-specific risks such as

accelerated postmenopausal muscle and bone loss, while improving vulnerability-related biomarkers and preserving independence.

Because functional decline may occur even in the absence of clinically diagnosed disease, indices such as the GFFI may identify vulnerability before the progression to frailty. This multidimensional approach enables early detection of at-risk individuals, supporting the design of targeted interventions to maintain independence, prevent functional decline, and promote healthy aging.

3. AIM

Although several biomarkers have been proposed to characterize vulnerability in aging, their relationships with broader health indicators are not fully understood. In particular, it remains unclear which markers best reflect overall health status and how molecular markers interact with functional ones. This thesis aimed to identify early biological indicators to prevent functional decline in older female adults. Therefore, it investigated the relationship between vulnerability biomarkers and specific health biomarkers in middle-aged and older female adults engaged in community-based exercise programs, and analysed how different characteristics of physical exercise programs could improve these health biomarkers. To address these objectives, the thesis was structured into three complementary studies, each with a specific aim:

- Study 1: Compare each vulnerability biomarker (GDF-15, GFFI, and frailty phenotype) on each health biomarker variable, such as blood biochemistry, body composition, and hemodynamic variables, in middle-aged and older female adults.
- Study 2: Analyse the relationship between both vulnerability biomarkers, GDF-15 and GFFI.
- Study 3: Analyse the different characteristics of each physical exercise program and compare the differences in the health biomarkers according to each vulnerability biomarker in middle-aged and older female adults.

Based on current literature, the following expectations were formulated. First, it is anticipated that older female adults exhibit higher GDF-15 levels, lower GFFI scores, and a higher proportion of individuals classified as pre-frail or frail (frailty phenotype), in combination with less favourable health biomarker profiles (blood biochemistry, body composition, hemodynamic variables), compared to middle-aged female adults.

Second, vulnerability biomarkers (GDF-15, GFFI, and frailty phenotype) are expected to be inversely associated with health biomarker profiles, such that higher

vulnerability is related to adverse blood biochemical parameters, higher body fat composition, and impaired hemodynamic measures.

GDF-15 levels are expected to reflect both acute metabolic stress and chronic health status. While acute increases are anticipated following exercise, chronically elevated levels are expected to be associated with less favourable health biomarker profiles and increased cardiometabolic risk. Accordingly, associations between GDF-15 and GFFI are expected to be context-dependent rather than strictly linear.

Variability in biomarker profiles is expected within age groups, with some older participants demonstrating biomarker profiles comparable to those of middle-aged individuals.

Participation in structured physical exercise programs is expected to be associated with more favourable health biomarker profiles, with effects that vary according to program characteristics.

Baseline vulnerability status (GDF-15, GFFI, frailty phenotype) is expected to modulate exercise-related changes in health biomarkers. Furthermore, specific combinations of these vulnerability biomarkers will be associated with unfavourable health biomarker profiles, allowing the identification of individuals at increased risk of functional decline.

The knowledge gained from these results may suggest which biomarkers are associated with better health and reduced vulnerability, and it may be incorporated into clinical practice to improve the health management of older adults.

4. MATERIALS AND METHODS

4.1 Study Design and Participants

A cross-sectional observational study was conducted at the “Center for the Study of Noncommunicable Diseases, Aging and Physical Exercise (CEDEE)” of the School of Sciences of the São Paulo State University (UNESP), Bauru, São Paulo, Brazil. This study was conducted according to the “Strengthening the Reporting of Observational Studies in Epidemiology” (STROBE) statement guidelines (von Elm et al., 2008). By following these standards, the study aligns with established practices for observational research. Data collection was performed on thirty-eight community-dwelling females aged 54–84 with a history of physical training.

A cross-sectional observational study design was chosen because it allows for the efficient assessment of associations between variables within a specific population at a single point in time, without the need for intervention or manipulation. This approach is particularly suitable for the present study, which aimed to characterize outcomes in physically active older females under natural conditions.

Compared to experimental designs, such as randomized controlled trials, this method is more ethically and practically appropriate, as it avoids imposing interventions or altering participants’ habitual behaviours. Additionally, the cross-sectional design is less time- and resource-intensive than longitudinal studies, which require extended follow-up and are more susceptible to participant attrition, especially in aging populations. While the study design does not allow for causal inference, it provides a robust foundation for identifying relevant patterns that may inform future longitudinal or interventional studies.

For study inclusion, participants had to meet the following criteria: postmenopausal females, nonsmokers, have no medical, cardiovascular, orthopaedic, and/or musculoskeletal restrictions that could compromise test evaluation; have alcoholic beverage consumption up to two drinks daily; and have no use of any known medication that affects glucose metabolism or renal hemodynamic. Older adults diagnosed with dementia, psychiatric disorders, cognitive impairment, or stroke were excluded from this study.

This information was collected during participants’ interviews, in which a standard screening health questionnaire was used to assess eligibility criteria as

described above. Additionally, participants who did not complete all assessments were excluded from the data analysis. In fact, 57 participants were selected in the first screening, but 19 were excluded from the sample according to the aforementioned inclusion criteria. A total of 38 participants (age = 66.8±6.5 / BMI = 28.8±5.4) were included in this thesis, which was structured into three complementary studies, each developed with a specific aim. The overall sample was composed of individuals participating in three distinct community-based exercise programs, each characterized by different objectives and activity structures.

- Group 1 (G1 – Activity older adults) included participants (age = 67.3±5.1 years; BMI = 26.6±3.7 kg/m²) with the primary focus on the control of diabetes through supervised exercise practice (2x per week / 60 minutes per session) combined with dietary guidance.
- Group 2 (G2 – Cursilho) included participants (age = 65.4±8.2 years; BMI = 28.3±3.7 kg/m²) with aimed the promotion of quality of life through generalized activities, including music, painting, educational lectures, and structured exercise sessions (2x per week / 60 minutes per session).
- Group 3 (G3 – AELESAB) included participants (age = 68.4±4.3 years; BMI = 31.0±7.6 kg/m²) predominantly from low-income backgrounds or those in situations of social vulnerability. This group participated in social activities and general health-promotion activities, including regular exercise practice (2x per week / 60 minutes per session).

The inclusion of participants from programs with distinct social and behavioural characteristics provided a more comprehensive representation of physically active community-dwelling older females, thereby strengthening the ecological validity and applicability of the findings across different community contexts.

4.2 Ethics Approval and Informed Consent

Before the evaluations, the participants signed an informed consent form after the principal investigator clarified all their questions and concerns. All procedures were approved by the Research Ethics Committee of the Faculty of Sciences/UNESP-Bauru (protocol no. 323.427), which is in accordance with the Declaration of Helsinki.

4.3 Frailty Assessment

The Frailty Phenotype, introduced by Fried et al. (2001), is a widely adopted framework for assessing frailty in older adults. It identifies frailty based on five specific criteria: unintentional weight loss, self-reported exhaustion, weakness (grip strength), slow walking speed, and low physical activity (Fried et al., 2001). The frailty criteria were measured in the following way:

- Unintentional weight loss: Through patient interviews, it was determined whether the individual has experienced an unintentional loss of 10 pounds (4.5 kg) or more, or more than 5% of their body weight, in the past year.
- Exhaustion: Feelings of exhaustion are evaluated using specific questions from the Center for Epidemiological Studies Depression Scale (CES-D) (Siddaway et al., 2017).
- Weakness (grip strength): Grip strength was assessed using a hand dynamometer. The individual squeezes the dynamometer with maximum effort, and the best of three attempts is recorded. Thresholds for weakness are adjusted based on sex and body mass index (BMI).
- Slow gait (walking speed): The time to walk a short distance (4.57 meters) is measured with specific cut-off points for slowness based on sex and height.
- Low physical activity: The individual's weekly energy expenditure is estimated using the Paffenbarger questionnaire to determine the number of kilocalories expended per week (Paffenbarger et al., 1986). Falling below <2.000 kcal/week indicates low physical activity, while >2.000 kcal/week classifies an individual as active.

Meeting three or more of these criteria classifies an individual as frail, one or two indicate a pre-frail state, and none suggest robustness (Allison et al., 2021). The cut-off points were adjusted according to Boreskie et al. (2019) to classify frailty phenotype in non-institutionalized older adults, rather than those originally proposed by Fried et al. (2001), which were developed in a different population context and are less specific for community-dwelling older adults.

4.4 Physical Fitness Assessment

The battery of functional fitness assessments proposed by the American Alliance for Health, Physical Education, Recreation, and Dance (AAHPERD) was used to determine the participants' training status levels (TS) (Osness et al., 1990). The battery consists of five standardized motor tests conducted in the following order: flexibility, coordination, muscular strength and upper-arm muscular endurance, agility and dynamic balance, and endurance, as described below (Benedetti et al., 2007; Mazo et al., 2010; Zago et al., 2003).

- Flexibility: During a sit-and-reach test, trunk and lower-body flexibility, particularly the hamstrings and lower back, is measured. The individual sits on the floor with legs extended, reaching forward toward the toes. The distance reached beyond the toes (or the shortfall) is recorded.
- Coordination: The test evaluates hand-eye coordination and upper limb control. Three empty soda cans are placed in a specific arrangement. The individual is required to turn over each can in a prescribed order as quickly as possible. The total time taken is recorded.
- Arm Curl Test: To measure upper-body strength and muscular endurance, the individual performs bicep curls with a specified weight (3 kg for women) for 30 seconds while seated. The total number of repetitions is counted.
- Balance and Agility: The test's purpose is to assess agility and dynamic balance. The participant starts seated in a chair and, on command, stands up, circles a cone placed 1.5 meters away, returns to the chair, sits down, and repeats the movement on the opposite side to complete one full cycle. Each attempt consists of two cycles; the best time from the two attempts is recorded as the final result.
- Endurance: To assess aerobic endurance, the individual walks a distance of 804,67 meters as quickly as possible. The time taken to complete the walk is recorded. The test was performed on a flat athletic track to ensure greater reliability of the results.

Participants were classified according to normative values based on the sum of scores obtained in each test to calculate the General functional fitness index (GFFI). The total possible score was 500 points, with each test worth up to 100 points (Table 1). GFFI values were classified as weak TS (< 200 points), regular TS (200-300 points), and good TS (> 300 points). The AAHPERD battery was selected due to its strong reliability and validity in the older adult population, with test-retest reliability coefficients ranging from 0.80 to 0.99 for each component (Osness et al., 1990).

Table 1: Normative Classification of Percentile Scores from the American Alliance for Health, Physical Education, Recreation and Dance (AAHPERD) Functional Fitness Battery and Corresponding General Functional Fitness Index (GFFI) Categories in Older Adults.

Physical tests [percentile score]	Classification	GFFI [points]
0-19	Very weak	0 - 99
20-39	Weak	100 - 199
40-59	Regular	200 - 299
60-79	Good	300 - 399
80-100	Very good	400 - 500

4.5 Anthropometric Assessment

For the anthropometric assessment, a properly calibrated Tanita® digital electronic anthropometric scale (model VM-080) with a capacity of 150 kg and a variation of 100 g, a stadiometer (SECA® 206) with a capacity of 220 cm, and a Sanny® TR4010 anthropometric tape were used. Participants stood upright, arms close to their bodies, barefoot, and dressed in light clothing during measurements.

4.5.1 Body Mass Index

One factor used to evaluate participants' nutritional status was body mass index (BMI). The BMI was calculated by dividing body mass in kilograms by the square of height in meters. The cut-off points of the World Health Organization

(WHO) were used as guidelines: underweight is defined as BMI below 18.5 kg/m², normal weight as 18.5 to 24.9 kg/m², overweight as 25 to 29.9 kg/m², and obesity as 30 kg/m² or higher (WHO, 2026).

4.5.2 Waist-To-Hip-Ratio

Another factor in determining participants' nutritional status was the waist-to-hip ratio (WHR). The WHR was calculated as waist circumference divided by hip circumference, with central obesity defined as a WHR greater than 0.85 in females (Liu et al., 2024).

4.6 Dual-Energy X-Ray Absorptiometry Method

The dual-energy X-ray absorptiometry (DXA) method (Discovery, Wi-HOLOGIC INC, Bedford, MA, USA) was used to measure body composition. The measured variables were lean mass, fat mass, and bone mineral content. To sum up, the equipment generates X-rays at two different energies and uses the differential attenuation of the X-ray beam. These two energies were used to calculate the tissue composition in the scanned area (Laskey, 1996; Lukaski, 1993). Data was analyzed by Hologic APEX software.

4.6.1 Sarcopenia index

The sarcopenia index was assessed using dual-energy X-ray absorptiometry (DXA), a widely accepted method for evaluating body composition. DXA provides precise measurements of lean soft tissue, from which appendicular skeletal muscle mass (ASM) can be derived by summing the lean mass of the upper and lower limbs. The sarcopenia index was then calculated by normalizing ASM to height squared (ASM/height², kg/m²), allowing for the comparison of muscle mass relative to body size. This approach is commonly used in clinical and research settings due to its accuracy, reproducibility, and suitability for assessing sarcopenia in older populations.

4.7 Blood Pressure Assessment

Blood pressure (BP) was measured using the auscultatory method after participants rested for five minutes in a quiet, calm environment, seated with their backs against the wall and legs uncrossed, following the VII Brazilian Guideline on

Hypertension (Barroso et al., 2021). Measurements were conducted using an aneroid sphygmomanometer (Welch Allyn DS44, Wan Med®, St. Skaneateles Falls, NY, USA) and a stethoscope (3MTM, Classic III, Littmann®, St. Paul, MN, USA) positioned on the brachial artery. Three measurements were performed on different days. The cuff was fitted to each participant's arm circumference and centered over the brachial artery, with the arm supported at heart level. Trained assessors inflated the cuff to 30 mmHg above the radial pulse reading, then deflated it at approximately 2 mmHg per heartbeat. Systolic blood pressure was determined by auscultation of the first sound (Korotkoff phase I), and diastolic blood pressure by the disappearance of the sounds (Korotkoff phase V).

4.8 Blood Serum Collection

Blood samples from participants were collected between 07:00 AM and 08:30 AM after a 12-hour fasting period. They were instructed to refrain from exercise on the day of testing. Participants rested for at least 10 minutes while sitting in a chair. A certified phlebotomist assisted in taking blood from each person. The blood was collected in 10 mL collection tubes (BD Vacutainer®, Serum). The tubes were centrifuged for 10 minutes at 10.000 rpm (Eppendorf 5415D). The supernatant was transferred to 1,5 mL tubes (Eppendorf Tubes®) and stored in 500 µL aliquots at -20°C and -80°C. Samples were assayed within one month.

4.8.1 Biochemical Assessment

Serum samples were used to determine the lipid and glycemic profile, including the parameters total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and glycemia (GL), by the spectrophotometry method using a biochemical analyzer with specific reagents (A-15, BioSystems SA, Barcelona, CAT, Spain). Additionally, the blood composition factors calcium arsenazo, creatinine, glucose, total protein, urea UV, and uric acid were analyzed.

4.8.2 Thiobarbituric Acid-Reactive Substance Assay

For thiobarbituric acid-reactive substance (TBARS) analysis, the protocol adapted from Buege et al. (1978) was used to quantify TBARS in the samples. 150

µL of human plasma sample, 150 µL of sodium decylsulphate (SDS), 300 µL of 10% (P/V) trichloroacetic acid (TCA), and 500 µL of 0,67% thiobarbituric acid (TBA) were added to a 15-mL Falcon tube. The TCA denatures the proteins present and acidifies the reaction medium, while the TBA reacts with lipid peroxidation products, forming a pink compound. The mixture was stirred and incubated at 100 °C for 30 minutes, then cooled on ice. Afterwards, the mixture was centrifuged at 4000 rpm for 5 minutes at room temperature, and 200 µL of the supernatant was removed. 200 µL of the supernatant was removed from the centrifuged solution and added to 96-well microplate wells to measure the absorbance at 535 nm on a spectrophotometer (Biospectro).

4.8.3 Growth Differentiation Factor 15 Determination

To quantitatively measure Human GDF-15 concentrations in vitro in serum samples from patients, the Elabscience® Human GDF-15 (Growth Differentiation Factor 15) ELISA (Enzyme-Linked Immunosorbent Assay) Kit, a sandwich ELISA assay, was used (Catalog No: E-EL-H0080, Elabscience Biotechnology Co., Ltd, Guangdong, China). The detection range of the kit is 23.44-1500pg/mL with a sensitivity of 14.06 pg/mL.

All samples and reagents were brought to room temperature before use. Necessary substances (wash buffer, standard working solution, Biotinylated Detection Ab working solution, HRP Conjugate working solution) were prepared according to the Elabscience® Human GDF-15 ELISA Kit. The standard working solution was diluted as a gradient in the following concentrations: 1500, 750, 375, 187.5, 93.75, 46.88, 23.44, 0 pg/mL. Blank, standard, and samples were run as duplicates.

To each well, 100 µL of standard working solution, blank, or sample was added; the plate was covered with a plate sealer and incubated for 90 min at 37℃. After that, the liquid was removed from the wells without washing. Then, 100 µL of Biotinylated Detection Ab working solution was added to each well and incubated for 1 h at 37℃ after covering the plate with a new plate sealer. The liquid was decanted after incubation, and the wells were washed 3 times using the wash buffer. Immediately after that, 100 µL HRP Conjugate working solution was added to the wells. The plate was covered with a new plate sealer and incubated for 30

min at 37 ℃. The liquid was removed, and the wells were washed 5 times with the wash buffer. Then, 90 µL of Substrate Reagent was added to each well, and the plate was covered with a new plate sealer and incubated in the dark at 37 ℃ for 15 min.

50 µL Stop Solution was added, and afterwards the optical density (OD) at 450 nm of each well was determined using a microplate reader (BioTek® PowerWave XS Microplate Reader). The microplate reader was set to the testing parameters in advance.

The protein concentration was determined through the linear standard curve of the measured standards in Excel (Microsoft® Excel® 2019 MSO). Therefore, the average OD for every standard was plotted against the Human GDF-15 concentration in pg/mL. The OD of each sample was blotted on the standard curve.

4.9 Statistical Analysis

All statistical analyses were conducted using *Jamovi* (version 2.6, The Jamovi Project, 2024). A two-tailed significance level of $\alpha = 0.05$ was applied throughout, and results are reported with 95% confidence intervals where appropriate. Continuous variables are presented as mean ± standard deviation (SD).

Before inferential testing, assumptions of normality and homogeneity of variances were evaluated using the Shapiro–Wilk test and Levene's test, respectively. In cases where assumptions were not met, results were interpreted with caution, given the relatively small sample sizes.

For Study 1, differences between groups were analysed using analysis of covariance (ANCOVA), adjusting for age and body mass index (BMI) as covariates. Health biomarkers (estimated training status, blood biochemistry variables, anthropometry and body composition variables, and blood pressure) were compared across vulnerability groups (GFFI classifications, frailty classifications, and GDF-15 quartiles. Each health variable was analysed concerning GFFI, frailty phenotype, and GDF-15 quartiles as independent variables. Post hoc comparisons were performed using Tukey's test when appropriate.

For Study 2, group differences were assessed using one-way analysis of variance (ANOVA). Furthermore, binary logistic regression analysis was

performed with frailty as the dependent variable, using GDF-15 concentrations and GFFI as independent variables and adjusting for age to evaluate the possible association between the markers (GFFI, GDF-15, and frailty phenotype).

In Study 3, multidimensional analysis, Homogeneity Analysis by Alternating Least Squares (HOMALS) was conducted to explore relationships between categorical variables and to identify underlying data structures. HOMALS was performed to spatially demonstrate the intensity of the associations among the categories included in the analysis (GFFI classification (good, regular, weak TS), frailty classification (robust or pre-frail/frail), exercise program group (G1, G2, G3)). Additionally, it was evaluated how specific exercise programs (G1, G2, G3) influence health biomarkers (total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides, glucose, total body mass, body mass index, body fat, sarcopenia index, systolic blood pressure, diastolic blood pressure).

Effect sizes were calculated to complement significance testing and to assess the magnitude of observed differences. Cohen's *d* and Hedges' *g* were reported for group comparisons, with Hedges' *g* used to account for unequal sample sizes. Effect sizes were interpreted according to conventional thresholds (small: 0.2, moderate: 0.5, large: 0.8). Furthermore, the common language effect size (CLES) was calculated to provide an intuitive measure of group differences. For example, in the comparison of GDF-15 levels between frailty groups, a moderate effect size was observed (Cohen's $d = 0.44$; Hedges' $g = 0.436$). However, the 95% confidence interval (−1.127 to 0.255) included zero, indicating that the difference did not reach statistical significance. The corresponding CLES value (0.621) suggests a 62.1% probability that a randomly selected individual from one group would exhibit a higher value than an individual from the comparison group.

Given the relatively small sample sizes, particularly in subgroup analyses, effect sizes and confidence intervals were emphasized to aid interpretation beyond sole reliance on *p*-values.

5. STUDY 1

5.1 Results

As aforementioned, the purpose of study 1 was to compare each vulnerability biomarker (GDF-15, GFFI, and frailty phenotype) on each health biomarker variable, including estimated training status (coordination, flexibility, muscular strength, agility and dynamic balance, cardiovascular endurance, general functional fitness index score), blood biochemistry variables (GDF-15, total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides, glucose), anthropometry and body composition variables (lean mass, fat mass, bone mineral content, sarcopenia index, body mass index), and hemodynamic variables (systolic blood pressure, diastolic blood pressure), in middle-aged and older female adults. A total of 38 participants (age = 66.8±6.5 / BMI = 28.8±5.4) were included in this study.

Table 2 highlights the differences in physical performance, biochemical markers, and body composition across estimated TS (weak, regular, and good). Significant improvements in physical performance metrics, including coordination ($p = 0.001$, $\eta^2p = 0.285$), flexibility ($p = 0.004$, $\eta^2p = 0.261$), muscular strength ($p = 0.001$, $\eta^2p = 0.613$), agility and dynamic balance ($p = 0.001$, $\eta^2p = 0.335$), and cardiovascular endurance ($p = 0.001$, $\eta^2p = 0.548$), were observed with increased TS. Participants with good TS had the highest GFFI scores (371.7 ± 44) compared to those with regular (224.1 ± 37) and weak TS (119.9 ± 42; $p < 0.001$, $\eta^2p = 0.861$). Biochemically, total cholesterol ($p = 0.006$, $\eta^2p = 0.253$), LDL cholesterol ($p = 0.001$, $\eta^2p = 0.346$), and triglycerides ($p = 0.048$, $\eta^2p = 0.195$) were significantly lower in participants with good TS than in those with weak and regular TS. As expected, HDL cholesterol was significantly higher in the good TS group (61.6 ± 7 mg/dL; $p = 0.002$, $\eta^2p = 0.296$). GDF-15 and glucose values did not differ significantly between the TS groups.

Body composition measures and blood pressure showed non-significant differences between groups, except for systolic blood pressure, which decreased significantly in good TS compared to weak TS ($p = 0.001$, $\eta^2p = 0.333$). The sarcopenia index showed a trend toward lower values in participants with good TS, although this difference was not statistically significant ($p = 0.055$, $\eta^2p = 0.153$).

Table 2: Physical Performance, Blood Biochemistry, Anthropometry, Body Composition, and Blood Pressure According to General Functional Fitness Index (GFFI)–Derived Training Status in Older Adults.

	Weak TS	Regular TS	Good TS	p	η^2p
Estimated Training Status					
Coordination (seconds)	20.3±8.5	13.2±2.2	10.5±1.7[a]	0.001	0.285
Flexibility (cm)	48.6±13.2	61.8±8.5	63.3±6.8[a]	0.004	0.261
Muscular strength (repetition)	21.0±3.9	24.2±3.5	32.1±2.3[ab]	0.001	0.613
Agility and dynamic balance (seconds)	33.2±9.8	23.5±4.1[a]	20.7±2.0[a]	0.001	0.335
Cardiovascular Endurance (seconds)	655.3±92	547.6±25[a]	475.0±27[a]	0.001	0.548
General Functional Fitness Index (score)	119.9±42	224.1±37[a]	371.7±44[a]	0.000	0.861
Blood Biochemistry					
GDF-15 (pg·mL-1)	366.3±234	306.5±188	461.6±160	0.347	0.052
Total cholesterol (mg/dL)	199.0±40	219.0±19	154.7±37[ab]	0.006	0.253
HDL cholesterol (mg/dL)	44.3±16	32.0±10	61.6±7[ab]	0.002	0.296
LDL cholesterol (mg/dL)	140.3±44	166.0±29	80.0±32[ab]	0.001	0.346
VLDL cholesterol (mg/dL)	14.3±11	20.9±13	13.0±2	0.353	0.058
Triglycerides (mg/dL)	95.6±47	125.6±45	65.3±10[b]	0.048	0.195
Glucose (mg/dL)	115.8±41	102.1±8	95.2±13	0.310	0.065
Anthropometry and Body Composition					
Lean Mass (kg)	41.0±9	35.5±4	34.0±4	0.095	0.126
Lean Mass (%)	57.4±5	57.1±4	55.0±7	0.637	0.025
Fat Mass (kg)	29.2±9	25.0±5	27.0±9	0.540	0.035
Fat Mass (%)	39.8±5	39.8±5	41.9±7	0.706	0.020
Bone Mineral Content (kg)	1.9±0.4	1.8±0.2	1.8±0.3	0.935	0.004
Sarcopenia Index	6.8±1.4	6.1±0.7	5.6±0.6	0.055	0.153
Body Mass Index (kg/m^2)	29.9±6	27.4±3	26.3±3	0.220	0.083
Blood Pressure					
Systolic Blood Pressure (mmHg)	133.2±12	120.9±10	113.5±11[a]	0.001	0.333
Diastolic Blood Pressure (mmHg)	76.5±8	80.0±7	71.6±8	0.194	0.092

TS – training status; η^2p – partial eta square. [a]p<0.05 vs weak TS; [b]p<0.05 vs regular TS.

Differences in functional fitness, biochemistry, and body composition according to frailty classification are shown in Table 3. Robust participants exhibited significantly higher muscular strength (27.8 ± 5 repetitions; p = 0.050, η^2p = 0.321) and GFFI scores (262.7 ± 126; p = 0.009, η^2p = 0.237) compared to frail and pre-frail groups.

Table 3: Functional Fitness, Blood Biochemistry, Anthropometry, Body Composition, and Blood Pressure According to Frailty Classification in Older Adults.

	Frailty	Pre-frailty	Robust	P	η^2p
Estimated Training Status					
Coordination (seconds)	24.5±9	17.6±8	14.3±5	0.240	0.110
Flexibility (cm)	54.3±18	50.7±13	59.6±11	0.249	0.092
Muscular strength (repetition)	16.3±7	22.8±4	27.8±5 [ab]	0.050	0.321
Agility and dynamic balance (seconds)	33.9±8	30.6±10	24.9±5	0.266	0.093
Cardiovascular Endurance (seconds)	577.0±100	616.0±107	564.0±106	0.981	0.049
General Functional Fitness Index (score)	93.6±50	163.6±86	262.7±126[ab]	0.009	0.237
Blood Biochemistry					
GDF-15 (pg·mL-1)	558.2±302	370.5±173	354.1±256	0.621	0.069
Total cholesterol (mg/dL)	238.0±44	203.1±28	161.8±46[ab]	0.002	0.306
HDL cholesterol (mg/dL)	36.9±15	47.0±17	46.5±16	0.634	0.026
LDL cholesterol (mg/dL)	172.5±42	142.3±39	101.0±52[ab]	0.014	0.216
VLDL cholesterol (mg/dL)	28.5±22	13.8±10	14.2±7	0.078	0.135
Triglycerides (mg/dL)	142.6±110	93.7±31	77.4±29	0.074	0.170
Glucose (mg/dL)	135.6±61	105.4±31	110.2±34	0.376	0.054
Anthropometry and Body Composition					
Lean Mass (kg)	40.0±6	37.3±6	40.8±12	0.525	0.036
Lean Mass (%)	57.4±5	56.7±6	56.9±5	0.984	0.001
Fat Mass (kg)	27.4±2	27.8±9	28.7±8	0.946	0.003
Fat Mass (%)	39.6±5	40.4±6	40.1±5	0.974	0.002
Bone Mineral Content (kg)	2.0±0.4	1.8±0.3	2.0±0.3	0.230	0.081
Sarcopenia Index	6.8±0.9	6.4±1.2	6.4±1.6	0.928	0.004
Body Mass Index (kg/m^2)	29.2±1.7	28.4±5.0	29.3±6.7	0.883	0.007
Blood Pressure					
Systolic Blood Pressure (mmHg)	139.3±14	126.8±14	124.1±14	0.284	0.071
Diastolic Blood Pressure (mmHg)	78.3±5.5	77.8±9.9	72.1±6.0	0.181	0.096

TS – training status; η^2p –partial eta square. [a]p<0.05 vs frailty; [b]p<0.05 vs pre-frailty.

Regarding blood biochemistry, robust participants had significantly lower LDL cholesterol levels (101.0 ± 52 mg/dL; p = 0.014, η^2p = 0.216) and total cholesterol levels (161.8 ± 46 mg/dL; p = 0.002, η^2p = 0.306) compared to people living with frailty and pre-frailty. Other biochemical markers, including GDF-15 and glucose, showed non-significant trends across frailty classifications.

Body composition parameters and blood pressure did not reach statistical significance across the frailty groups. Systolic blood pressure was highest in frail individuals (139.3 ± 14 mmHg); however, this difference was not statistically significant compared to the other groups ($p = 0.284$, $\eta^2p = 0.071$).

Table 4 presents the analysis of GDF-15 quartiles related to physical and biochemical measures.

Table 4: Physical Performance, Blood Biochemistry, Anthropometry, Body Composition, and Blood Pressure According to Growth Differentiation Factor-15 (GDF-15) Quartiles.

	1st	2st	3st	4st	P	η^2p
Estimated Training Status						
Coordination (sec)	19.3±12	16.2±4	19.35±8	14.6±4	0.439	0.066
Flexibility (cm)	56.0±14	54.6±13	44.9±13	58.2±12	0.219	0.141
Muscular strength (repetition)	24.4±4	22.0±6	21.3±6	27.3±4	0.121	0.170
Agility and dynamic balance (sec)	29.4±9	29.1±7	30.1±10	27.4±13	0.977	0.010
Cardiovascular Endurance (sec)	640.9±106	604.6±116	573.5±93	541.7±118	0.401	0.119
GFFI (score)	183.6±95	169.8±109	153.4±120	247.5±122	0.325	0.101
Blood Biochemistry						
GDF-15 (pg·mL-1)	163.4±46	288.8±60[a]	422.4±34a[b]	686.8±168[abc]	0.001	0.833
Total cholesterol (mg/dL)	175.7±41	202.4±37	210.8±35	177.2±50	0.204	0.132
HDL cholesterol (mg/dL)	44.6±14	46.4±10	53.8±22	43.7±18	0.587	0.058
LDL cholesterol (mg/dL)	117.3±48	142.2±39	137.8±45	120.2±62	0.643	0.050
VLDL cholesterol (mg/dL)	13.8±9	13.8±9	19.1±14	13.2±9	0.654	0.049
Triglycerides (mg/dL)	89.1±33	89.0±35	107.8±69	85.2±34	0.779	0.042
Glucose (mg/dL)	111.7±38	110.5±49	112.2±36	104.4±17	0.968	0.008
Anthropometry and Body Composition						
Lean Mass (kg)	43.2±14.0	35.8±2.8	40.6±6.8	35.2±4.9	0.173	0.142
Lean Mass (%)	56.4±4	58.4±7	56.2±5	56.9±7	0.882	0.020
Fat Mass (kg)	31.2±9	25.0±9	30.0±7	25.7±8	0.349	0.096
Fat Mass (%)	41.0±4	38.6±7	41.0±5	39.9±7	0.845	0.025
Bone Mineral Content (kg)	1.9±0.4	1.7±0.2	1.9±0.4	1.9±0.3	0.682	0.045
Sarcopenia Index	7.1±1.9	5.9±0.5	6.8±1.0	6.1±1.3	0.186	0.138
Body Mass Index (kg/m^2)	32.4±7.8	25.9±3.9	29.9±3.2	27.0±4.7	0.059	0.224
Blood Pressure						
Systolic Blood Pressure (mmHg)	124.7±17	130.0±11	132.9±14	118.5±13	0.201	0.137
Diastolic Blood Pressure (mmHg)	77.0±8	75.9±7	76.5±10	72.1±9	0.683	0.046

GFFI – General functional fitness index, η^2p – partial eta square. [a]$p<0.05$ vs 1st quartile; [b]$p<0.05$ vs 2st quartile; [c]$p<0.05$ vs 3st quartile.

GDF-15 levels increased significantly across quartiles, from the first quartile (163.4 ± 46 pg/mL) to the fourth quartile (686.8 ± 168 pg/mL; $p = 0.001$, $\eta^2p = 0.833$). However, physical performance metrics such as coordination, flexibility, muscular strength, agility, and cardiovascular endurance did not demonstrate statistically significant differences across quartiles. Although participants in the fourth quartile exhibited the highest GFFI values (247.5 ± 122), no difference was found in the GFFI score between groups ($p = 0.325$, $\eta^2p = 0.101$).

Biochemical analysis showed no statistically significant differences in any variable across quartiles. Similarly, body composition metrics and blood pressure also displayed no significant differences across quartiles.

5.2 Discussion

Concisely, the results of this study suggested that GFFI presented a stronger influence on health markers than the frailty phenotype and GDF-15 values, highlighting the potential of this vulnerability biomarker to be incorporated into clinical practice to improve the health management of older adults.

Biomarkers like GDF-15, frailty, and GFFI levels are interrelated and play crucial roles in assessing vulnerability in older adults. Elevated GDF-15 levels may signal early biological changes predisposing individuals to frailty and functional decline, while frailty and reduced fitness provoke systemic stress, potentially increasing GDF-15 levels. Understanding these dynamics is essential for identifying at-risk populations and designing effective interventions. However, our results did not reveal the expected relationships. It was expected that individuals with lower GFFI and classified as frail would present high GDF-15 values, but this result was not confirmed.

Recently, GDF-15 has been strongly associated with aging and longevity (Lehallier et al., 2019; Liu et al., 2021; Tanaka et al., 2018). The authors observed that, in healthy, young individuals, circulating GDF-15 concentrations tend to remain lower. However, these concentrations increase in older adults, especially in the presence of pathological conditions (Conte et al., 2019; Fujita et al., 2016). Notably, the literature indicates that physical exercise can also stimulate GDF-15 secretion. Kleinert et al. (2018) observed a 34% increase in plasma GDF-15 levels after a single session of physical exercise. In addition, Zhang et al. (2019) showed

that the acute increase in GDF-15 induced by physical exercise was associated with improved energy metabolism in obese older adults. It is believed that the increase in GDF-15 levels may represent a compensatory metabolic response to mechanical and metabolic stress induced by physical exercise (Conte et al., 2019; Tchou et al., 2009), aiding in muscle repair and regeneration after acute exercise (De Sousa et al., 2021). However, it is important to note that these results were observed following a single acute physical exercise session, and, in the current study, the training status (chronic effect) of older adults was evaluated.

In this case, the chronic effect still requires further studies, especially given that both GFFI and the frailty classification reflect a chronic condition of the individual. In the literature, the chronic effects of exercise on GDF-15 concentrations remain inconclusive, as some studies have shown an increase (Zhang et al., 2019), a decrease (Seo et al., 2021), or no effect (Chang et al., 2021). As an example, Torrens-Mas et al. (2025) and Tavenier et al. (2021) demonstrated the potential role of GDF-15 as a biomarker of aging, as high concentrations of GDF-15 were associated with lower nutritional status, lower functional performance, frailty, and higher levels of inflammatory and glycemic markers. However, Seo et al. (2021) did not observe a difference in GDF-15 concentration after 16 weeks of resistance training in older adults. Although GDF-15 has been considered a marker of vulnerability in older adults, further studies are still needed to reach a definitive conclusion.

To assess the individual relationship of each vulnerability biomarker with health variables, all participants were stratified by GFFI classification, frailty phenotype, and GDF-15 quartile. Overall, our results showed that higher TS was strongly associated with better functional fitness and more favourable biochemical and hemodynamic profiles, whereas the frailty classification was associated only with improvements in some biochemical profile variables. GDF-15 levels did not reach statistical significance related to biochemical, anthropometric, and hemodynamic variables. Interestingly, participants in the higher GDF-15 quartiles tended to exhibit greater functional fitness and a higher likelihood of frailty, which partially supports previous findings linking elevated GDF-15 to adverse health outcomes in aging populations (Teng et al., 2021; Yatsuga et al., 2015). However, the lack of significance in this study may reflect the relatively small sample size or

the inclusion of physically active older adults (chronic effect of exercise), which could have attenuated the expected relationships.

Participants with better TS (Table 2) exhibited more favorable biochemical profiles, including lower LDL cholesterol and triglyceride levels, higher HDL cholesterol levels, and lower systolic blood pressure, reinforcing the role of exercise in promoting metabolic health (Smart et al., 2024). Good TS was also significantly associated with improved physical performance metrics, including coordination, flexibility, muscular strength, agility, and cardiovascular endurance. Studies developed by our group have also found these relationships. For example, Da Silva et al. (2021) found better results for total cholesterol, HDL-C, TG, GL, body mass index, body fat percentage, and systolic and diastolic blood pressure in participants with a higher GFFI score (>300 points). These findings align with previous studies highlighting the role of physical fitness in mitigating aging-related vulnerabilities (Cruz-Jentoft et al., 2010; Caldas, 2003).

Frailty classification revealed significant differences in physical performance and biochemical markers (Table 3). Participants classified as robust exhibited higher GFFI scores and lower LDL and total cholesterol levels compared to their frail counterparts. These results are consistent with existing literature, which identifies frailty as a multidimensional construct associated with reduced physiological reserves and increased susceptibility to adverse health outcomes (Sanchini et al., 2022; Dent et al., 2019).

This study contributes to the growing body of evidence supporting the integration of biomarkers, such as GDF-15, frailty classification, and functional fitness assessments into clinical practice for older adults. While GDF-15 has been extensively studied as a marker of inflammation, oxidative stress, and mitochondrial dysfunction (Guo et al., 2022; Lima et al., 2022), its potential as a predictor of health markers requires further exploration. However, the frailty phenotype, and especially the GFFI score, have been shown to be good markers for health management in middle-aged and older female adults.

Future studies should address the limitations of the present study, including its cross-sectional design and relatively small sample size. Longitudinal studies are needed to establish causal relationships between GDF-15, frailty, functional fitness, and other health markers. Moreover, the potential modulatory effects of exercise and

lifestyle interventions on the vulnerability biomarkers warrant further investigation, as these could inform personalized strategies to enhance resilience and reduce age-related morbidity. It is essential to consider lifestyle and biological markers to fully understand and address frailty in older adults. Exploring the influence of GDF-15, frailty phenotype, and GFFI score on specific health variables such as blood pressure, body composition, and biochemistry in larger, more diverse populations would also be valuable. Understanding the mechanisms underlying these relationships could provide new insights into the biological processes driving aging and vulnerability, though further investigation is needed.

5.3 Conclusion

Overall, higher training status and lower frailty were strongly related to improved functional fitness and more favourable biochemical and hemodynamic profiles. These findings emphasize that high GFFI values were associated with better health outcomes in middle-aged and older adult women, highlighting the need for further research on the role of vulnerability biomarkers in healthy aging. Although GFFI is not yet widely recognized in the current geriatric research literature as a vulnerability biomarker in older adults, the results of the current study suggest that this index has great potential to be incorporated into clinical practice, given its favourable effects on health parameters.

Although GDF-15 levels are also considered biological biomarkers of vulnerability, no relationship with health markers was found in this study; however, further studies are needed to confirm these results.

6. STUDY 2

6.1 Results

Although study 1 found interesting results for health parameters when the vulnerability biomarkers were treated as independent variables, there was a need to study the relationships among these biomarkers. Therefore, study 2 aimed to analyse the relationship between both vulnerability biomarkers.

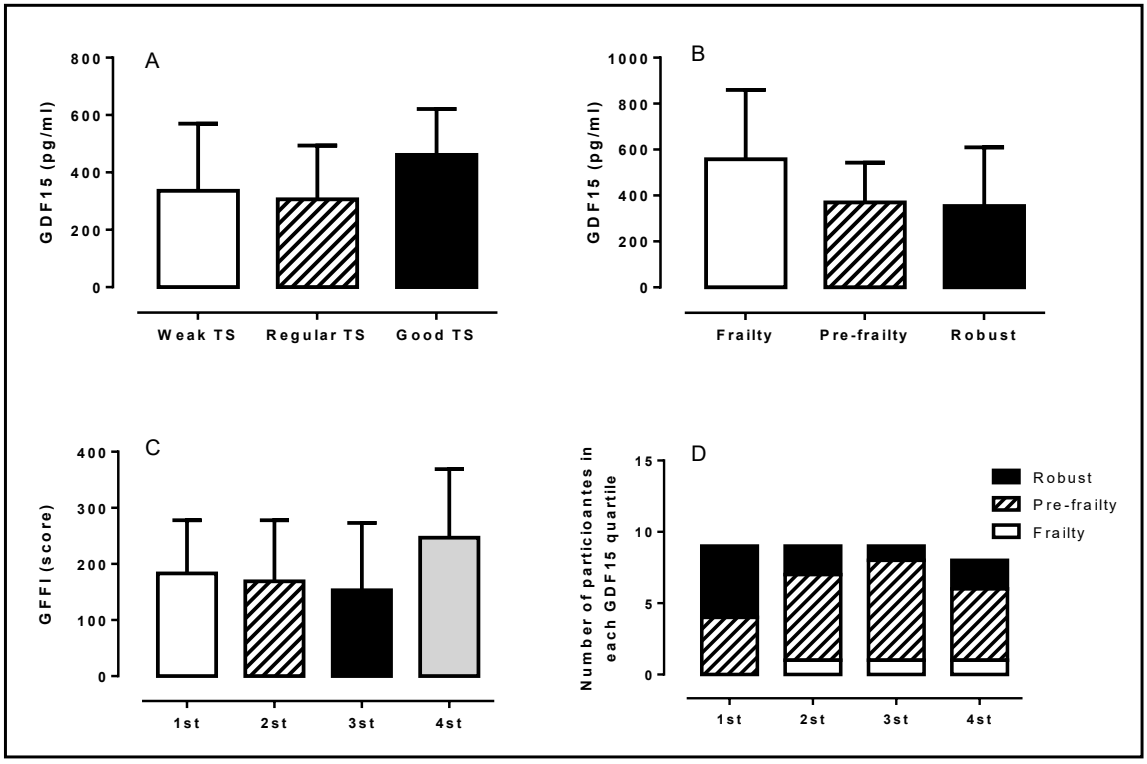

Figure 1. Growth differentiation factor 15 (GDF-15) according to different levels of training status - TS (Panel A) and frailty classification (Panel B). General functional fitness index (GFFI) according to GDF-15 quartiles (Panel C) and number of participants in each quartile according to frailty classification (Panel D).

A non-significant variation in GDF-15 concentration was observed across different TS levels ($p = 0.347$, $\eta^2p = 0.052$) (Figure 1A) and across different classifications of frailty ($p = 0.621$, $\eta^2p = 0.069$) (Figure 1B). Likewise, the General Functional Fitness Index (GFFI) did not reach statistical significance ($p = 0.325$, $\eta^2p = 0.101$) according to GDF-15 quartiles (Figure 1C).

Figure 1D showed that participants in the higher GDF-15 quartiles (2nd, 3rd, and 4th) were more likely to be classified as frail, suggesting a potential link between GDF-15 levels and frailty. No participant with low GDF-15 levels was classified as frail.

Therefore, it became relevant to perform a logistic regression analysis with frailty as the dependent variable, using GFFI and GDF-15 concentrations as independent variables and adjusting for age, to evaluate the possible association between TS and GDF-15 and frailty classifications. Significance level was set at $p < 0,05$.

Figure 2 shows the results of the logistic regression analysis, revealing that the GFFI ($p=0.001$, $\eta^2p=0.322$) and GDF-15 concentration ($p=0.002$, $\eta^2p=0.2470$) are significantly associated with frailty classifications. More specifically, based on the Exp(B) values for GFFI classifications, having a weak TS makes an individual 70.04 times more likely to be classified as frail/pre-frail than having a good TS ($p=0.016$). This result was obtained by inverting the reference category to generate an Exp(B) greater than 1 (Figure 2).

Omnibus tests

	X^2	df	p	η^2
GFFI	13.04	2	0.001	0.3220
GDF-15	10.00	1	0.002	0.2470
Age	3.37	1	0.067	0.0831

Parameter Estimates (Coefficients)

					Exp(B) 95% Confidence Intervals			
Name	Effect	Estimate	SE	Exp(B)	Lower	Upper	z	p
(Intercept)	(Intercept)	1.1526	0.67608	3.16650	0.8416	11.914	1.705	0.088
GFFI1	Regular TS - Weak TS	0.5345	1.58021	1.70665	0.0771	37.776	0.338	0.735
GFFI2	Good TS - Weak TS	−5.8242	2.42123	0.00296	2.57e−5	0.340	−2.405	0.016
GDF-15	GDF-15	0.0151	0.00667	1.01519	1.0020	1.029	2.261	0.024
Age	Age	0.1906	0.11789	1.21003	0.9604	1.525	1.617	0.106

Figure 2. Results of the Logistic Regression with Frailty as the dependent variable, and GFFI, GDF-15 concentration, and age as the independent variables.

In addition, the GDF-15 results indicate that for each unit increase in GDF-15 concentration, the likelihood of frailty increases by 1.5% ($p=0.024$). These results are

represented in Figure 3. This graph visualizes the synergistic pattern: as GDF-15 rises and GFFI declines, predicted frailty probability increases sharply.

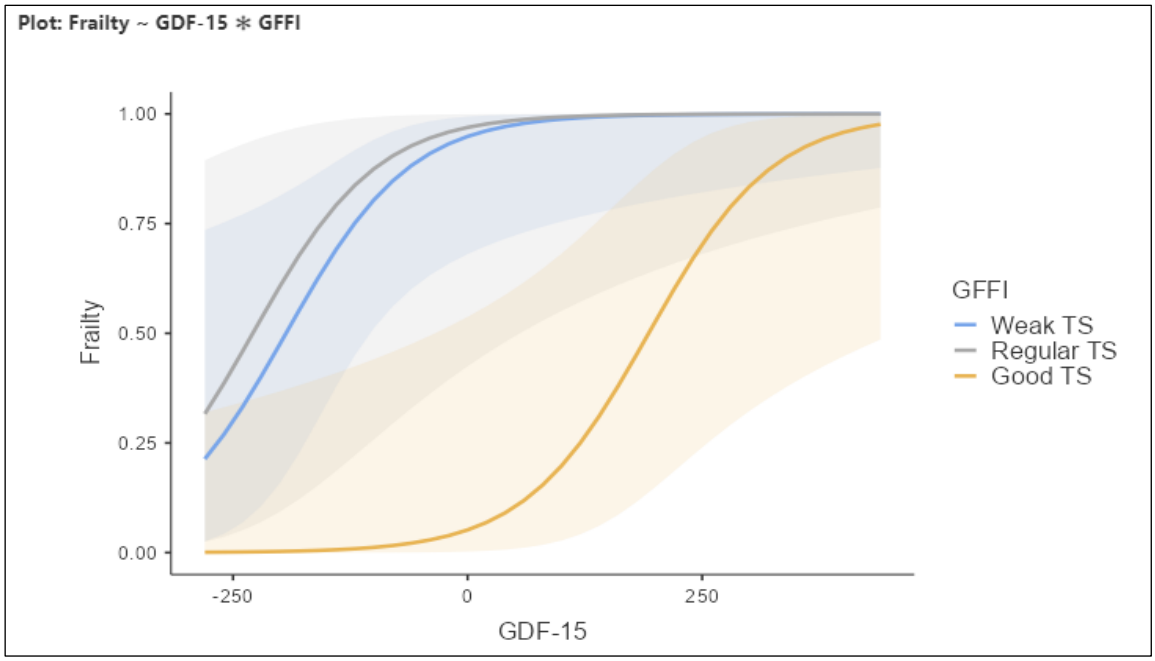

Figure 3. Graphical representation of the model; X-axis: GDF-15 concentration scale shown as mean and ± standard deviation; Y-axis: predicted probability of Frailty, with 1 representing Frail/Pre-Frail and 0 representing Robust; the legend showing the different GFFI categories.

6.2 Discussion

The objective of Study 2 was to explore the relationship between biological and functional vulnerability biomarkers, specifically GDF-15, General Functional Fitness Index (GFFI), and frailty classification, in middle-aged and older female adults. While Study 1 analysed these markers independently in relation to health parameters, the present study focused on their interdependence.

The findings revealed that although no significant mean differences in GDF-15 concentrations were observed across frailty categories in the ANOVA models (Figures 1A–C), logistic regression analysis identified significant associations between both GDF-15 and GFFI with frailty status. This discrepancy can be explained by the distinct questions each statistical approach addresses. The ANOVA model tests whether mean levels differ across categorical groups (frail, pre-frail, robust), answering the

question “Is GDF-15 a factor associated with belonging to the pre-frail/frail group?” Meanwhile, logistic regression evaluates the probability of belonging to a frailty group based on continuous predictors (Is there an effect of GDF-15 on frailty classification?).

Furthermore, in the ANOVA model, frailty was divided into three groups: frail, pre-frail, and robust, while in the logistic analysis, frail and pre-frail individuals were combined into a single group. This increased statistical power, and the model was adjusted for age, enhancing sensitivity to subtle trends. Consequently, the logistic approach detected gradual, continuous associations between GDF-15 and frailty that were not visible when comparing group means alone. This finding emphasizes that the relationship of GDF-15 and frailty is probabilistic rather than categorical. Small progressive increases in GDF-15 may reflect early biological stress or mitochondrial dysfunction that predisposes individuals to frailty, even before clear group differences emerge.

The combined significance of GFFI and GDF-15 reinforces the concept of frailty as a multidimensional construct, influenced by both functional reserve and biological stress (El Assar et al., 2024; Ferreira de Campos et al., 2025). GFFI represents a measure of functional capacity, encompassing strength, coordination, flexibility, and endurance (Antes et al., 2012), while GDF-15 reflects mitochondrial and inflammatory stress pathways (Merchant et al., 2022). Including training status as an independent variable in the regression model, alongside GDF-15, substantially improved the model’s explanatory capacity ($R^2 = 0.39$) and reduced its error, enabling the detection of more nuanced associations, such as the subtle effect of GDF-15 on frailty classification. Together, these two biomarkers explained a meaningful portion of the variability in frailty, supporting a biopsychological model of aging in which physical and molecular domains interact dynamically (Suganuma et al., 2024; Sepúlveda et al., 2022).

Supporting this idea is a study by Damanti et al. (2025) that found associations between elevated GDF-15 levels and frailty, reduced physical activity, and increased risk of sarcopenia. Physiological stress signals such as GDF-15 may damage neuromuscular and metabolic function (Alcazar et al., 2021; Conte et al., 2020), while declining physical fitness may further enhance GDF-15 secretion through chronic low-grade inflammation or oxidative stress (Schwarz et al., 2023). Thus, creating a potential feedback loop that increases vulnerability. In conclusion, GDF-15 and GFFI can be

seen as complementary indicators (one molecular and one functional) that together represent the same underlying pattern of aging.

GDF-15 has been described as a mitokine, secreted in response to mitochondrial stress, inflammation, and tissue damage (Zhang et al., 2024), and its concentration rises with advancing age and in the presence of chronic diseases (Conte et al., 2021). Indeed, older adults with frailty have shown elevated GDF-15 in association with oxidative stress and mitochondrial dysfunction (Arauna et al., 2020). Elevated GDF-15 levels are associated with frailty, reduced physical performance, and increased risk of mortality (Merchant et al., 2022).

The finding that even small increases in GDF-15 correspond to higher frailty probability in this cohort supports these previous observations. In parallel, lower GFFI values, as evidenced by poor performance in strength and endurance tests, have been repeatedly associated with frailty status and higher risk of adverse outcomes (Jeoung, 2015; Molari et al., 2021; Huang et al., 2021). Thus, the present results of study 2 indicate that biochemical and physical markers interact to capture distinct but complementary dimensions of vulnerability. This reinforces the concept that frailty arises from both molecular dysregulation and functional decline.

From a clinical perspective, these findings could have important implications. The combination of GDF-15 and GFFI suggests a model for frailty detection and management. While GFFI provides a practical, low-cost assessment of functional resilience, GDF-15 provides molecular-level insight into the biological aging processes underlying stress and metabolic imbalance. Incorporating both measures into clinical or community screening (e.g., geriatric evaluations) could improve early identification of at-risk individuals, allowing preventive actions (e.g., exercise programs or nutritional strategies) to be taken before frailty becomes apparent.

However, several questions remain open. For instance, is GDF-15 merely a marker of stress and frailty, or does it play a causal role in driving the physiological changes associated with vulnerability? Could reductions in GDF-15 through exercise or pharmacological interventions transform into measurable improvements in function and resilience? Additionally, to what extent might acute versus chronic changes in GDF-15 differ in their physiological implications for older adults? Addressing these questions through longitudinal studies would deepen the understanding of GDF-15's role in the aging process.

6.3 Conclusion

In summary, the findings of Study 2 indicate that GDF-15 and GFFI are complementary indicators of frailty, each representing distinct but interconnected dimensions of vulnerability: molecular and functional. Their combined use accounts for a substantial proportion of frailty variability and shows the importance of considering both biological and performance-based markers in aging research. The integration of these biomarkers provides a broader understanding of health status in aging and may increase early identification and prevention strategies aimed at maintaining functional independence and promoting healthy aging in older adults.

7. STUDY 3

7.1 Results

The third study aimed to analyse the types of activities offered across different community-based physical exercise programs for older adults and to examine how these program characteristics relate to variations in health biomarkers. Specifically, this study compared middle-aged and older female participants enrolled in three distinct programs:

G1 - Group focused on controlling diabetes through exercise and diet

G2 - Group focused on quality of life through generalized activities such as music, painting, lectures, and exercise

G3 - Group composed of low-income population or in a situation of social vulnerability, who carry out generalized activities, including exercise

By evaluating differences in metabolic, cardiovascular, functional, and psychosocial biomarkers across these programs, the study sought to identify how varying program emphases and participant vulnerability profiles influence health outcomes.

For the profiling, HOMALS (Homogeneity Analysis by Alternating Least Squares) was used to spatially demonstrate the intensity of the associations among the categories included in the analysis (van der Burg et al., 1988), namely: origin group (G1, G2, or G3), GFFI (good TS, regular TS, or weak TS), and frailty (robust or frail/pre-frail). The HOMALS analysis revealed two distinct participant profiles based on the spatial distribution of categories in the multidimensional solution (Figure 4). Profile 1 (P1) was characterized by the clustering of participants from G1, who exhibited good training status (TS) and were classified as robust. Profile 2 (P2) grouped participants from G2 and G3, who predominantly presented regular or weak TS and were more frequently categorized as frail or pre-frail. These associations demonstrate an alignment between program type, functional status, and frailty-related biomarkers.

Figure 4. Representation of the categories in a Cartesian plane using HOMALS, highlighting P1 (Profile 1), which groups the categories Good TS, G1, and Robust, and P2 (Profile 2), which groups the categories G2 and G3, Regular TS and Weak TS, and Frail/Pre-Frail.

Table 5 presents the observed and expected frequencies for frailty classifications (frail, pre-frail, robust) within the physical exercise program groups G1, G2, and G3. The observed values are actual survey data, while the expected values are based on statistical expectations. A higher-than-expected number of robust participants was observed in G1 (7 observed vs. 3.79 expected). G2 showed an overrepresentation of pre-frail individuals (13 observed vs. 9.39 expected), whereas G3 displayed a relatively even distribution, though with slightly more robust individuals than expected (6 observed vs. 5.05 expected). These findings reflect heterogeneity in participants' health status across programs, with G1 exhibiting the most favourable frailty profile.

Table 5: Observed and Expected Frequencies of Frailty Classifications (Frail, Pre-Frail, Robust) Across Physical Exercise Program Groups (G1, G2, and G3).

		Frailty Classification			
		Frailty	**Pre-frailty**	**Robust**	**Total**
G1	Observed	0	2	7	9
	Expected	0.237	4.97	3.79	9.00
G2	Observed	1	13	3	17
	Expected	0.447	9.39	7.16	17.00
G3	Observed	0	6	6	12
	Expected	0.316	6.63	5.05	12.0
Total	Observed	1	21	16	38
	Expected	1.000	21.00	16.00	38.00

Table 6 shows the observed and expected frequency of individuals distributed in training status classifications (weak, regular, and good TS) within the groups G1, G2, and G3. G1 demonstrated a substantially higher frequency of good TS (8 observed vs. 1.89 expected).

Table 6: Observed and Expected Frequencies of Training Status (TS) Classifications (weak TS, regular TS, good TS) Across Physical Exercise Program Groups (G1, G2, and G3).

		TS Classification			
		Weak TS	**Regular TS**	**Good TS**	**Total**
G1	Observed	0	1	8	9
	Expected	5.68	1.42	1.89	9.00
G2	Observed	13	4	0	17
	Expected	10.74	2.68	3.58	17.00
G3	Observed	11	1	0	12
	Expected	7.58	1.89	2.53	12.00
Total	Observed	24	6	8	38
	Expected	24	6	8	38

In contrast, both G2 and G3 displayed an above-expected rate of weak TS (G2: 13 observed vs. 10.74 expected; G3: 11 observed vs. 7.58 expected). These results suggest that generalized activity programs (G2 and G3) may be less effective at improving or maintaining physical fitness than structured exercise programs (G1).

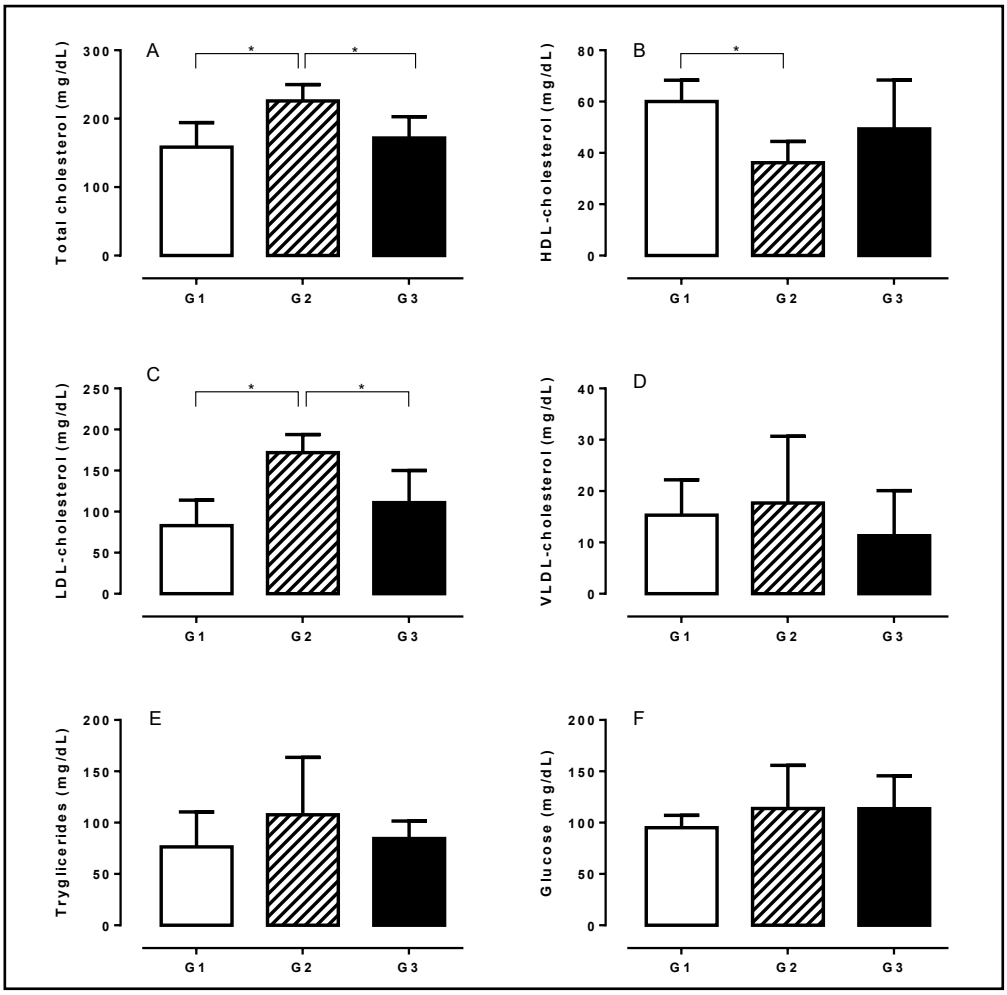

Figure 5. Blood Biomarkers in each group according to the specific program developed - G1 (white bar), G2 (striped bar), G3 (solid black bar). *p < 0.05.

Tables 5 and 6 examined the associations between the exercise programs and vulnerability biomarkers, frailty, and training status. Figures 5, 6 and 7 demonstrate how specific programs influence health biomarkers. Group comparisons for lipid and metabolic biomarkers are shown in Figure 5. Total cholesterol (Figure 5A) was highest in G2 (225.9±24mg/dL) and significantly higher than G1 (158.4±36mg/dL / p<0.001)

and G3 (171.8±31mg/dL / p>0.001). HDL-cholesterol (Figure 5B) was highest in G1 (60.0±8mg/dL), surpassing G2 (36.2±12 mg/dL / p<0.001), with no difference between G1 and G3 (49.4±19mg/dL / p<0.06). LDL-cholesterol (Figure 5C) was also highest in G2 (171.9±22mg/dL) and significantly greater than G1 (83.1±31mg/dL / p<0.000) and G3 (111.0±39mg/dL / p<0.000). VLDL-cholesterol, triglycerides, and glucose (Figures 5D–5F) showed no significant differences among groups. Overall, G2 exhibited an unfavourable lipid profile, while G1 demonstrated healthier metabolic indicators.

Body mass (Figure 6A), BMI (Figure 6B), and body fat percentage (Figure 6C) did not significantly differ across groups, suggesting that differences in program type or socioeconomic background do not strongly affect these body composition variables in this sample. In contrast, the Sarcopenia Index (Figure 6D) was higher in G3 (7.2±1score) compared to G1 (5.7±0.7score / p<0.03). No other differences between-groups were reported for this indicator.

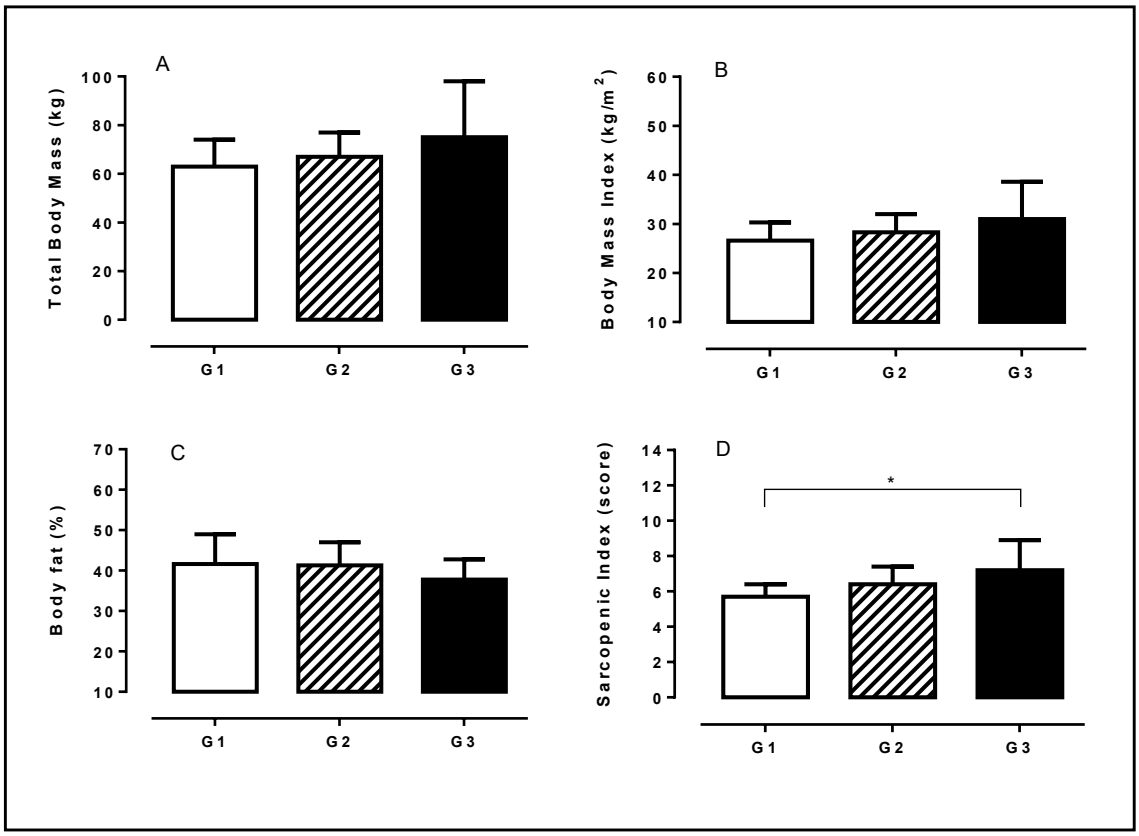

Figure 6. Body composition and Sarcopenia Index according to the specific program developed - G1 (white bar), G2 (striped bar), G3 (solid black bar). *p < 0.05.

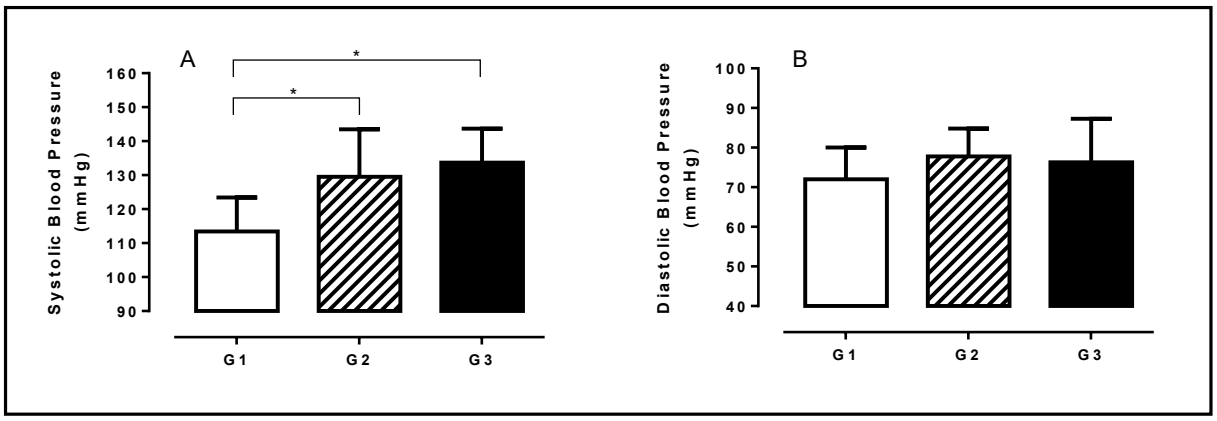

Figure 7. Systolic and diastolic blood pressure according to the specific program developed - G1 (white bar), G2 (striped bar), G3 (solid black bar). *p < 0.05.

Systolic blood pressure differed significantly among programs (Figure 7A). G1 displayed the lowest systolic values (113.4±10 mmHg), suggesting a potential cardioprotective effect of targeted diet and exercise interventions. Both G2 (129.5±14 mmHg, p<0.013) and G3 (133.7±10 mmHg, p<0.003) exhibited higher systolic blood pressure than G1, suggesting that generalized activities may be insufficient to influence cardiovascular regulation meaningfully. Diastolic blood pressure (Figure 7B) showed no significant differences across groups.

7.2 Discussion

The findings of study 3 highlight important relationships between community-based program characteristics and various health biomarkers among middle-aged and older female adults.

G2 participants demonstrated a significantly less favourable lipid profile, with the highest total and LDL cholesterol and the lowest HDL levels among the groups. This pattern suggests that generalized, non-targeted activities may be insufficient to promote cardiometabolic health. In contrast, participants in G1, who engaged in structured exercise combined with dietary guidance, exhibited a healthier lipid profile, reinforcing prior evidence that systematic interventions effectively improve blood lipid regulation and inflammatory markers. This is further supported by a study examining the effects of a 12-week multi-exercise community program on muscle strength and lipid profile in older females, which found that participants had significantly increased muscle index and HDL/Apo-A, reductions in Apo-B, triglycerides, CRP, and waist

circumference, and a lower prevalence of sarcopenia compared to controls (Lim et al., 2024). Similarly, randomized and systematic evidence indicates that combined exercise and nutrition interventions significantly improve skeletal muscle index (SMI), handgrip strength, gait speed, and other sarcopenia-related outcomes in older adults (Yang et al., 2025; Park et al., 2023). Structured aerobic and resistance-based training has also been shown to reduce total cholesterol and LDL while increasing HDL in older and middle-aged adults, alongside improvements in metabolic regulation (Amare et al., 2024; Martins et al., 2010). G3 participants exhibited intermediate values, possibly reflecting partial benefits from general physical activity without the additional support of structured health-guided interventions.

The HOMALS analysis and frequency tables revealed consistent associations between frailty status, training status, and program type. G1 participants were most often robust and displayed the highest prevalence of good training status. In contrast, G2 and G3 participants were more likely to be pre-frail or frail and to have lower training status. These results align with randomized controlled trials in pre-frail and frail community-dwelling older adults, which demonstrate that multidomain lifestyle interventions, combining structured physical training with nutritional and behavioural components, significantly reduce the prevalence of sarcopenia and improve gait speed, lower-limb strength, and physical performance (Lu et al., 2021). In contrast, lifestyle or generalized physical activity interventions without a structured, progressively increasing training stimulus tend to yield smaller or less sustained improvements in muscular fitness, blood pressure, and body composition (Opdenacker et al., 2011). These differences underline the role of structured interventions in maintaining or improving physical function in older adults.

The observed pattern in the sarcopenia index differed from the expected distribution. Based on prevailing literature, participants in G1 would be expected to demonstrate the most favourable muscle health profile, given the combination of structured exercise and dietary guidance, which are typically associated with improved skeletal muscle index and muscle strength (Wu et al., 2021), followed by G2, while G3 would be expected to show the lowest values due to lower program specificity and greater social vulnerability. Contrary to this expectation, G3 presented the highest sarcopenia index, followed by G2, whereas G1 showed the lowest values. One possible explanation is that G1 participants who engaged in structured interventions

for diabetes control may have had more advanced metabolic dysregulation or inflammatory burden at baseline, chronic conditions (such as type 2 diabetes and sarcopenic obesity) that negatively affect muscle mass despite receiving structured exercise (Park et al., 2006; Cruz-Jentoft et al., 2010). Moreover, evidence suggests that, while resistance training improves functional capacity and body composition in individuals with sarcopenic obesity, improvements in metabolic biomarkers are not consistently observed (Polo-Ferrero et al., 2025). A recent network meta-analysis further indicates that the incremental benefit of adding nutritional interventions to exercise is variable and population-dependent, with heterogeneity in muscle mass outcomes across studies (Wu et al., 2021). These factors may partially explain the unexpected SMI pattern observed across groups. Although G3 showed the highest sarcopenia index, all group means remained above established clinical cut-offs for women (<5.67 kg/m²; Cruz-Jentoft et al., 2010), indicating that none of the groups presented overt sarcopenia. These observations highlight the need to review the broader clinical and metabolic context when interpreting muscle health metrics (such as SMI), especially in populations with chronic conditions.

Lower systolic blood pressure in G1 further supports the benefits of target-specific interventions for cardiovascular health management. A systematic review of combined Mediterranean diet and physical activity interventions showed significant reductions in blood pressure and improvements in cardiovascular/metabolic risk markers (e.g. HDL, triglycerides, and insulin sensitivity) in adults (Teixeira et al., 2025). The aforementioned study by Martins et al. (2010) also reports reductions in blood pressure, alongside improvements in lipid profile and body composition, in older adults participating in controlled trials of aerobic and resistance-based programs. No significant differences were observed in diastolic blood pressure; this may suggest that systolic measures may be more sensitive to exercise and diet-based interventions in this population.

7.3 Conclusion

In summary, the results suggest that structured, targeted programs (G1) are more effective at promoting favourable metabolic, cardiovascular, and functional health profiles than generalized activity programs (G2 and G3). Participants in socially vulnerable conditions (G3) did not necessarily exhibit worse outcomes in all

biomarkers, indicating that community engagement itself may present partial benefits. However, evidence consistently indicates that progressive, structured exercise (particularly when combined with dietary guidance) yields more robust improvements in frailty status, muscular fitness, and cardiometabolic health. At the same time, variability across populations and health conditions demonstrates the importance of individualized assessment and cautious interpretation of muscle and metabolic biomarkers.

8. MAIN FINDINGS AND PRACTICAL IMPLICATIONS

This thesis aimed to investigate the role of vulnerability biomarkers in middle-aged and older women engaged in community-based physical exercise programs. Specifically, it examined the independent and combined influence of Growth Differentiation Factor-15 (GDF-15), frailty phenotype, and the General Functional Fitness Index (GFFI) on metabolic, cardiovascular, anthropometric, and functional health parameters. Additionally, it evaluated how different types of exercise programs relate to vulnerability status and health outcomes.

The findings of this thesis highlight that vulnerability in aging cannot be captured by a single marker. Study 1 demonstrated that among the evaluated indicators, the General Functional Fitness Index showed the strongest and most consistent associations with health biomarkers, including lipid profile, blood pressure, and functional performance. Women classified as having good training status showed lower total cholesterol, LDL cholesterol, and triglycerides, as well as lower systolic blood pressure, and higher HDL cholesterol, with superior performance in all functional tests. These findings further suggest that the General Functional Fitness Index may serve as a practical, low-cost clinical screening tool for assessing health status in active older female adults.

The frailty phenotype demonstrated moderate associations, mainly with lipid variables and muscle strength, reflecting vulnerability in biochemical and functional domains. In contrast, the molecular biomarker GDF-15 showed no significant differences in biochemical, hemodynamic, anthropometric, or functional parameters when analysed independently.

Study 2 examined the relationship between GDF-15, GFFI, and frailty phenotype. Although no significant differences were observed across categorical comparisons, logistic regression analyses adjusted for age revealed that both GDF-15 and GFFI significantly predicted frailty status. Weak training status markedly increased the likelihood of being frail or pre-frail, while higher GDF-15 levels were associated with increased frailty probability. These findings support a complementary relationship between molecular and functional vulnerability markers.

Study 3 analysed the influence of different community exercise programs. Participants in a structured exercise program combined with dietary guidance showed better metabolic profiles, lower systolic blood pressure, and a higher prevalence of

robust classification and good training status than those in generalized activity programs. Body composition did not differ significantly between groups.

The findings support a multidimensional model of vulnerability. Among the biomarkers examined, functional fitness (GFFI-derived training status) emerged as the most clinically relevant and sensitive indicator of health status. At the same time, GDF-15 contributed to frailty prediction when analysed in combination with functional measures. Structured exercise programs appear to be more effective in promoting favourable health outcomes in middle-aged and older women.

Several limitations should be considered when interpreting these findings. First, the cross-sectional design does not allow causal conclusions regarding the relationships between vulnerability biomarkers and health indicators. Second, the relatively small sample size of 38 participants may limit the generalizability of the findings. Additionally, the study included only female participants, which may restrict the applicability of the results to broader populations. Finally, the wide age range of participants (54–84 years) may have introduced substantial heterogeneity in biological aging status, functional capacity, and health characteristics, which should be considered when interpreting the results.

Future research should therefore include longitudinal studies to examine how vulnerability biomarkers change over time and how exercise interventions may influence these markers. Integrating another marker of biological aging may also improve the understanding of vulnerability mechanisms in aging populations.

Overall, these findings highlight the importance of maintaining functional capacity and promoting structured exercise programs to support healthy aging.

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